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WINTER COLLEGE ON LASERS, ATOMIC AND MOLECULAR PHYSICS

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INTERACTION BETWEEN LASER RADIATION AND BIOLOGICAL SYSTEMS

F. Hillenkamp
Institut für Biophysik
Universität Frankfurt
Theodor Stern-Kai 7
D 6000 Frankfurt/Main
Fed. Rep. Germany

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and C. A. Sacchi
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Franz Hillenkamp

Institut für Biophysik
Universität Frankfurt
Theodor Stern-Kai 7
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INTRODUCTION

Laser application in biology and medicine is an interdisciplinary field, requiring knowledge in laser-physics and -technology, as well as physical chemistry and biochemistry on the one side and biology as well as the theoretical medical subjects, particularly physiology and histology on the other. Though progress in such fields is usually brought about through a close cooperation among experts of the various subjects involved, it is essential that both those who plan to do research in the field and those who merely want to apply the developed new methods in their own special field have a minimum knowledge of the basic laws and concept of the other subjects. In its first part this introductory chapter will treat the fundamental structure of matter with special reference to organic and living systems as well as the properties of electromagnetic radiation, particularly in the wavelength range from the middle UV ($\lambda > 200$ nm) through the visible into the middle infrared at about 10 µm. In the second part the interaction processes between this electromagnetic radiation and biological systems will be discussed as well as some of the basic reactions of biologic systems to such interactions.

Most of the processes and reactions of biological systems discussed in this chapter are not specific to interaction with optical radiation from laser sources. This reflects the fact that though many of the biomedical applications to be discussed in this volume have been rendered possible only after lasers of suitable wavelengths and of sufficient brightness have become available, the interactions with living matter are almost exclusively such that they could at least in principle be induced by thermal sources as well. Only very few biomedical laser applications have evolved so far that really depend on the unique property of coherence (taken here in the restricted sense of stable phase relationship for amplitude rather than intensity superposition to apply). In all those applications the change in the radiation field is investigated, not that in the biological object (e.g. pattern recognition for cell sorting; doppler-spectroscopy). Likewise there has as yet been no convincing evidence that any nonlinear optical interaction processes, a very important field in modern optics, play a major role in biomedical laser work. The only exception to this principle seems to be the recently developed microsurgery of the anterior segments of the eye in which optical breakdown of transparent, dielectric structures is generated with Q-switched or modelocked laser pulses. A sound knowledge of the results, obtained in more than 50 years of research in photobiology and the basic subjects pertinent to it, is a necessary, though certainly not sufficient prerequisite for successful work in the field of lasers in biology and medicine.

The intention of this chapter is to accumulate the very basic facts of all the subjects involved and the discussion of the various topics will therefore necessarily have to remain incomplete and superficial. A reader will inevitably find the sections pertinent to his own education of little use to him and may on the other hand want a more extended or in depth treatment of the others. As to the latter he is referred to the large number of textbooks on radiation- and quantum physics, physical- and biochemistry, photochemistry and photobiology and perhaps physiology. No special references are given to such books, because of a widely differing selection of available books in each country and language.

1. BASIC ATOMIC- AND RADIATION PHYSICS

The well known Bohr atomic model postulates a positively charged nucleus containing almost the entire mass of the atom, surrounded by a number of negatively charged electrons on well defined circular or elliptical orbits, arranged in shells. The number of electrons equals that of the protons in the nucleus - containing also neutrons equal or somewhat larger in number than the protons - leaving the atom electrically neutral. The chemical and physical behaviour of the atoms, particularly their interaction with other atoms and with optical radiation, is largely determined by the electrons in the outermost shell, the valence electrons. The Bohr atomic model is very useful for a general view of the atomic structure of matter. A real understanding of the interaction between atoms or molecules and radiation can however be reached only if this model is refined or rather reinterpreted in the light of quantum mechanics.

In the quantum mechanical description electrons are not small, solid spheres on fixed orbits, one rather gets spatial probability distributions for the electrons. The solutions of the Schrödinger equation, describing a given atom, render wave functions which can be looked at as amplitude functions. The square of the amplitude functions are then intensities which are related to the electron probability density functions mentioned above. In molecules where two or more atoms interact, the amplitude distribution of valence electrons may - depending on their phase - constructively or destructively interfere, forming new intensity, i.e. probability density functions, similar to the way electromagnetic waves do in interference experiments. Equivalent to the discrete orbits in the Bohr model, only a discrete number of such probability functions can exist as solutions of the Schrödinger equation, specified by a set of quantum numbers n, l and m_l, that can attain integer values only. n is the principal quantum number defining the "electron shell". The atoms most important for the structure of biological matter, have in their ground-, i.e. non excited, state electrons in the first (n = 1 : H) and second (n = 2 : C, O, N) shell. The following discussion will therefore be restricted to the probability density functions of the electrons of these two shells.

For the first shell (n = 1) l and m_l are necessarily 0 and only one allowed spatial distribution, called state, results. It is spherically symmectric about the nucleus. In the second shell (n = 2) l may be either 0 or 1. The state n = 2, l = 0 is spherically symmetric again. For n = 2, l = 1 three states are allowed with m_l equal to -1, 0 or +1. These three distributions are oriented along three mutually perpendicular axis in space (Fig. 1). Historically, optical spectroscopy has been the experimental basis of the quantum mechanical theory of the atom. Because of terms, originally introduced to describe

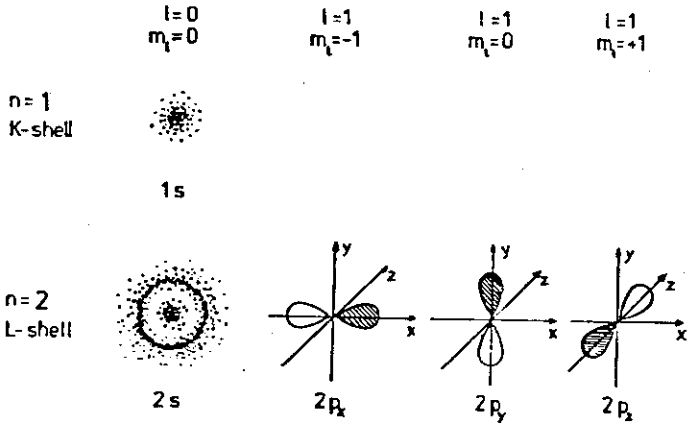

Fig. 1 Graphic representation of some electron probability density functions.

optical spectra, the shells are usually called K-shell (n = 1), L-shell (n = 2), M-shell (n = 3) and so on. States with spherical symmetry (l = 0) are called s-states, those with one symmetry axis (l = 1) p-states, then d- , f-states etc. The latter denomination will be used throughout the further discussion. A graphic representation of the first few probability density functions is given in Fig. 1 along with the various equivalent notations. Strictly, these distributions are only correct for the one electron atom (H), but they can be taken as good approximations for more electron atoms as well.

Electrons can be looked at as small spinning charges that have a magnetic moment, the spin. Two nearly antiparallel orientations of this vector quantity are allowed, specified by the values of the spin quantum numbers s = -1/2 and s = +1/2. The Pauli exclusion principle states that only one electron can occupy each state defined by a given set of the quantum numbers n, l, m_l, and s within a given atom. Because of the two possible spin orientations, a pair of two electrons can therefore occupy each of the probability density states shown in Fig. 1. The same exclusion principle applies to electrons in a molecule that interact with more than one atom, particularly the binding electrons. Atoms with one or more unpaired electrons in their shells have a strong tendency to complement their electronic configuration. They will combine with atoms of their own or another kind to form molecules with both partners sharing a pair of electrons of opposite spin orientation in a common state. Likewise molecules left with one unpaired electron after some external perturbation are known to be very reactive. They are called radicals.

Table 1 gives the electronic configuration of the atoms of the first two periods of the periodic table. The small arrows indicate electrons of a given spin orientation.

A definite energy of the whole atom is associated with every electronic configuration it may have. Though one often speaks about the energy of an electron in a certain state, it is in reality the energy of the whole system that is meant. Atoms, not perturbed by their environment will always occupy the state of lowest total energy. Interaction with the surrounding, e.g. with an electromagnetic field, or through a collision with neighbouring atoms, may stimulate the atom to go into an energetically higher state, i.e. one of the electrons goes into a so far unoccupied state of higher energy, leaving a position in the lower state open. The atom will return to its ground state either spontaneously after a certain time, or stimulated by a suitable external field (laser). All such transitions are associated with the absorption or release of energy amounting to the energy difference between the two states. In the discussion of the interaction between optical radiation and biological objects, the energy exchanged in an individual interaction process is the quantity of particular interest. Instead of drawing the exact probability density functions, one usually only specifies the energy of the

Table 1. Electronic configuration of the elements in the first two periods of the periodic table

element	orbital 1s	2s	$2p_x$	$2p_y$	$2p_z$
H	↑				
He	↑↓				
Li	↑↓	↑			
Be	↑↓	↑↓			
B	↑↓	↑↓	↑		
C	↑↓	↑↓	↑	↑	
N	↑↓	↑↓	↑	↑	↑
O	↑↓	↑↓	↑↓	↑	↑
F	↑↓	↑↓	↑↓	↑↓	↑
Ne	↑↓	↑↓	↑↓	↑↓	↑↓

various states in a much simpler energy level- or Jablonski diagram as shown in Fig. 2. The different states are represented by horizontal lines at different positions along the vertical energy axis. Radiative transitions are indicated by vertical arrows, radiationless transitions via excited vibrational states by vertical waved lines. The probability of transitions, stimulated by an external radiation field, depends linearly on the intensity of that field. The probability for such transitions as well as that for spontaneous relaxation of an excited state furthermore depends strongly on the type of the two states involved,i.e. how strong the coupling between the two is. This coupling is usually expressed as the oscillator strength of the transition. For a first order estimate of a transition probability, one must distinguish between two types of states, defined by the arrangement of spin orientations. If the two electrons in the ground state have antiparallel spin orientation, their two spin moments will cancel each other. Upon excitation into a higher energy state, the electron will usually retain its orientation, leaving the total spin moment zero. States with both spins of the electron pair arranged antiparallel even with one of the electrons in an excited state, are called singlet states S_i, because for transition between such states only single lines are observed spectroscopically. If, particularly through the additional interaction with another atom or molecule, the spin upon transition reverses, the two spins of the electron pair add. The resulting states are called triplet states T_i, because for transition between such states three narrowly spaced lines are observed in emission or absorption spectroscopy. As spin reversal is very unlikely during a transition, S-T or T-S transitions are highly

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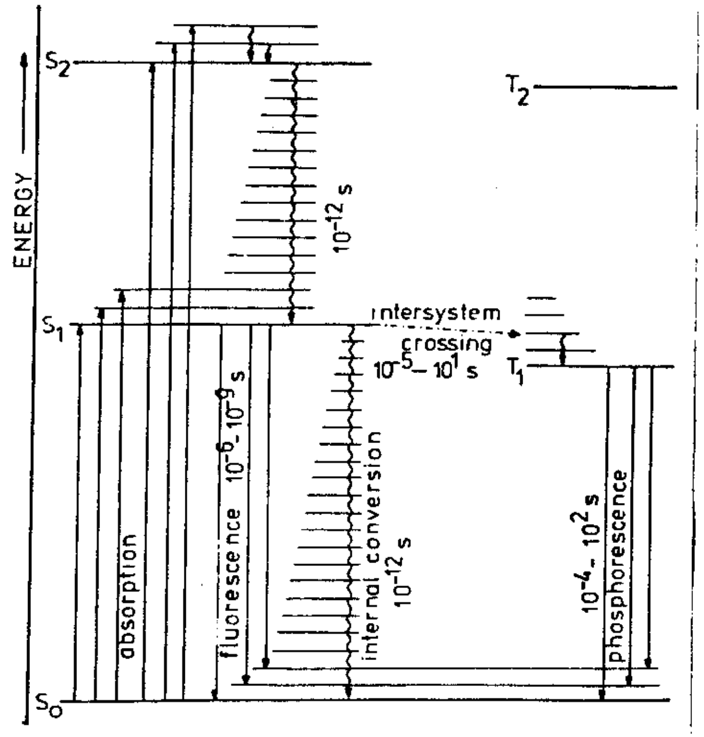


Fig. 2 Schematic energy level diagram of an atom

forbidden in atoms and still rather forbidden in molecules. Other quantum mechanical selection rules for transitions are less strict and will not be discussed here. For allowed transitions in molecules the spatial overlap of the electron probability functions of the two states involved is a good indication of the transition probability. The fact that S-T and T-S transitions are more or less forbidden is of great importance for spectroscopy as well as photochemistry. Molecules almost exclusively have singlet ground states (important exception: O_2). Excitation, e.g. by laser radiation, will then bring them into an excited singlet state. Because of the overlapping vibrational bands (ref$_{12}$Fig. 9), molecules will usually relax within a time of about 10^{-12}s through the so called internal conversion to the first excited singlet state S_1. The energy difference is transferred to the system as heat. Because of the larger energy gap between S_1 and S_o and therefore less overlap of vibrational levels, the electron will remain in S_1 for about $10^{-6} - 10^{-9}$s. Upon a transition from S_1 to S_o, the energy is frequently emitted as fluorescence radiation. Two processes compete with the fluorescence, a radiationless decay via vibrational levels as described above or a transition to the always lower lying T_1 triplet state. This intersystem crossing, though forbidden in principle, has measurable probability because the two states are close in energy. Heavy heteroatoms further enhance this crossing. The transition from the T_1 triplet to the S_o singlet ground state is rather strongly forbidden again. Electrons will therefore remain in this state up to seconds (typically 10^{-3}s). The radiation emitted at a $T_1 - S_o$ transition is called phosphorescence. It is usually very weak because of the low transition probability and competing radiationless desactivation processes. Fluorescence and to a lesser extent phosphorescence, are important methods for the investigations of molecules and their kinetics. The relatively long lifetimes of S_1-states, and much more that of T_1-states, furthermore facilitate a great variety of reactions, leading either to a rearrangement within the molecule itself, even dissociation into two fragments in rare cases, or to reactions with other molecules. Such reactions, otherwise suppressed by high enough energy barriers, can well take place from excited states and are responsible for most of the radiation damage of UV radiation.

In correspondence to discrete energy levels of atoms and molecules, quantum mechanics strictly requires that upon interaction electromagnetic radiation fields exchange energy in discrete amounts only. They are called photons. This energy exchange in discrete amounts, not noticable in the macroscopic world, is of great importance at the atomic level. Atoms and molecules will interact with a radiation field only, if its photon energy equals the energy difference of an allowed transition, usually starting from the ground state. The photon energy E is related to the frequency ν of the electromagnetic radiation through the famous Planck equation

$$E = h \cdot \nu$$

$h = 6.63 \cdot 10^{-34}$Js (Planck's constant). A second useful equation relates the speed of wave propagation (speed of light) to the frequency ν and the wavelength λ of the radiation.

$$c = \lambda \cdot \nu$$

$c = 3 \cdot 10^8 \text{ ms}^{-1}$.

An electromagnectic field can moreover be characterized by the inverse wavelength, called wavenumber, $\tilde{\nu}$. The photon energy is furthermore given as either the energy of one photon, measured in eV (electron volt), or the energy of one mole of photons (one Einstein), measured in kJ mol^{-1} or kcal mol^{-1}. All five quantities are used frequently throughout the pertinent literature. Fig. 3 gives a graphical comparison for quick reference. Fig. 3 also shows the limits of the various regions of interest. For laser applications to biology and medicine, we are interested in optical radiation in the range of 200 nm $\leq \lambda <$ 1 mm. Optical radiation of about $\lambda <$ 200 nm is transmitted in the vacuum only, where vital biological systems cannot exist and is therefore of little interest here. The region of ultraviolet radiation 200 nm $\leq \lambda <$ 400 nm is sometimes divided into the middle UV, with about 200 $\leq \lambda <$ 320 nm and the near UV, with about 320 $\leq \lambda <$ 400 nm. A more exact classification, loosely related to the interaction with biological systems and frequently referred to in safety standards, is

200 nm < λ ≤ 280 nm	ultraviolet: UV-C
280 nm < λ ≤ 315 nm	ultraviolet: UV-B
315 nm < λ ≤ 380 or 400 nm	ultraviolet: UV-A
380 or 400 nm < λ ≤ 760 or 780 nm	visible
760 or 780 nm < λ ≤ 1 mm	infrared (IR)

At least at the moment, radiation of wavelengths longer than about 10 µm (CO_2-laser) is only of little interest, because lasers in this range have only limited output power and biological effects are expected to be thermal as holds for the smaller IR wavelengths.

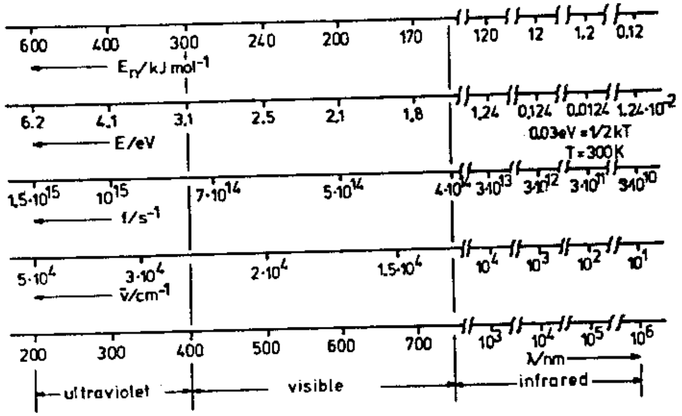


Fig. 3 Scales for photon energy, wavelength, wavenumber and frequency

The most basic experiment in investigating the interaction between optical radiation and matter would be to record the absorption spectrum, i.e. the fraction of the inciden [illegible] as a function of wavelength of the radiation. In the simplest case the expected result is described by Beer's law:

$$I_a = I_o\ (1-10^{-\varepsilon d})$$

I_a = absorbed radiant exposure (absorbed intensity)
I_o = incident radiant exposure
ε = absorptivity
d = path length of the radiation through the sample

For substances dissolved in a solvent at a concentration c_n, ε can further be broken up into

$$\varepsilon = \varepsilon_n \cdot c_n$$

The molar absorptivity, ε_n, is the actual material constant sought. Provided there is no interference from the solvent or other dissolved substances, ε_n is a direct measure of the transition probability (oscillator strength) between two involved states for the substance under investigation. ε_n is therefore determined experimentally and plotted as a function of wavelength. It is most commonly measured in the units $mol^{-1} l \cdot cm^{-1}$. Fig. 4 shows the absorption spectrum of a 10^{-3} $mol \cdot l^{-1}$ solution of benzene in cyclohexane. As one would expect, excitation of the molecule can be achieved over a certain range of photon energies or wavelengths rather than at one energy only, because a number of perturbations widen the energy levels of the various states into energy bands. The strong modulation of the absorption spectrum reflects the vibrational sublevels belonging to the same electronic transition. Such a good resolution of vibrational bands cannot usually be achieved for biological macromolecules.

It must be kept in mind that Beer's law of absorption is not usually followed for absorption of optical radiation in macroscopic volumes of biological tissue because of strong heterogeneity of tissue and a high degree of scattering. Even for microscopic samples the interpretation of results in terms of Beer's law is usually not possible because of the many different absorbers present, the bands of which often overlap strongly. The absorption spectra reported later in this chapter have therefore all been obtained from solutions of pure substances.

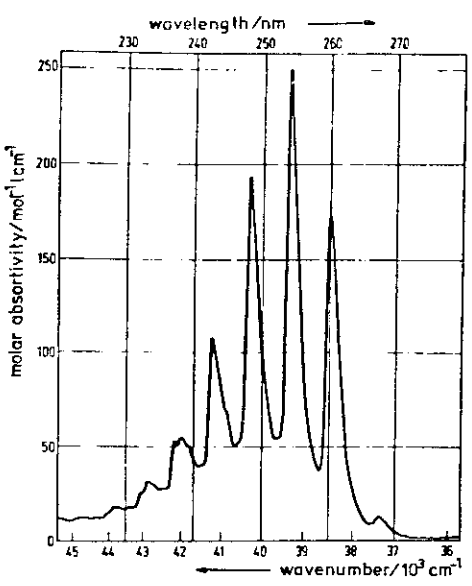


Fig. 4 Absorption spectrum of the $\pi\pi^*$-transition in benzene, dissolved in cyclohexane
$c_n = 10^{-3}$ mol l^{-1}

2. BASIC MOLECULAR PHYSICS

Biomolecules are hydrocarbons, i.e. molecules with hydrogen and carbon atoms as the major atomic constituents. Oxygen, nitrogen, sulfur and phosphorus are the most frequent heteroatoms. Most of the biomolecules are macromolecules with up to some 10^4 atoms and with relative molecular masses of up to 10^6 and a complex spatial structure. The vast majority of bonds between the atoms in the molecules are predominantly covalent,i.e. two atoms share two electrons to form electron pairs of opposite spin orientation. The close proximity of the atoms in molecules (0.1 - 0.2 nm) leads to a change in the probability distribution functions of the valence electrons. The s- and p-states mix to unsymmetric so called sp-hybrid orbitals

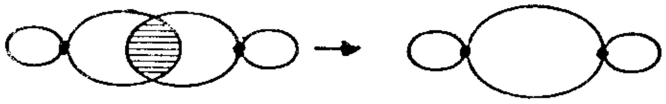


Fig. 5 Formation of a σ molecular orbital from two sp-hybrid atomic orbitals.

as shown in Fig. 5. Such orbitals of two atoms, e.g. two C-atoms, can overlap quite effectively leading to a new molecular orbital (MO) and to a strong interaction. Depending on the relative phases of the electrons wave (amplitude) function in the two overlapping lobes, a strong bond results with the two electrons preferentially in the space between the atom, or a strong antibonding state with the two electrons preferentially in the outer space. Such states with both atoms on the symmetry axis of the MO are called σ-states, the bond a σ-bond. The binding σ-state has lower energy than the antibinding σ^*-state, so the atoms will always bind together, unless excited e.g. by an external radiation field. All the basic bonds in biomolecules are σ-bonds. In addition to hybrid orbitals leading to the σ-bond, valence atoms may have more or less disturbed p-orbitals with symmetry lines in planes perpendicular to the line joining the two atoms. If each of the atoms orbitals has one unpaired electron, a new MO results (Fig. 6). As before, a binding π-state is formed, whereas the antibinding π^*-state is higher in energy and will be populated only after external energy transfer e.g. from a radiation field. Because of symmetry reasons, up to two such π-bonds can form between the two atoms in addition to the σ-bond. The corresponding electron density distribution functions are symmetrical to the two mutually perpendicular planes containing the line joining to two nuclei. They are usually indicated by two lines for the double bond (e.g. C = O) or three lines for the triple bond (e.g. C≡C).

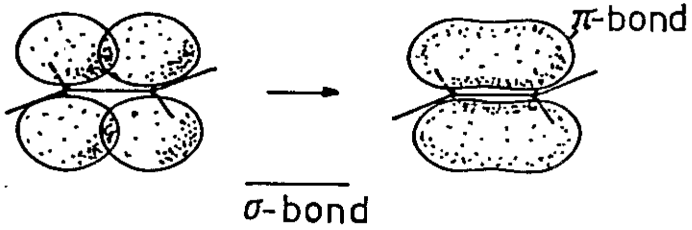


Fig. 6 Formation of a π molecular orbital.

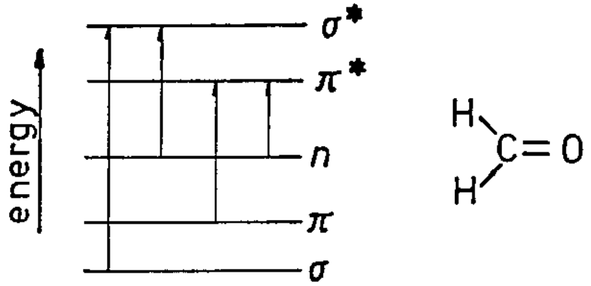


Fig. 7 Energy level diagram of formaldehyde (schematic).

Heteroatoms may in addition have orbitals with two paired electrons (i.e. of opposite spin orientation). Such electrons cannot contribute to a bond, but there may nevertheless be a considerable overlap of their orbitals with binding σ- or π-MO's. Upon interaction e.g. with a radiation field, transitions between the nonbinding n-states and antibinding π^*- or σ^*-states may occur. Fig. 7 shows the energy level diagram of formaldehyde as an example. For the transitions between these states upon excitation by an external electromagnetic field, energy generally increases in the sequence n - π^*, π-π^*, n - σ^*, σ - σ^*. Only n - π^* and π - π^*- in very rare cases n - σ^* - transitions can be excited with radiation in the middle or near ultraviolet and the visible.

Many biomolecules have what is called conjugated double bonds i.e. sequences of alternating single- (σ) and double- (σ+π) bonds. Benzene (Fig. 8) is probably the most famous example for such bonds. In reality, the π-orbitals of such molecules are no longer localized at two definite atoms (Mesomeric states). Polycentric π-orbitals are formed, involving more than two atoms. As a consequence there is more than just one electron pair associated with this group

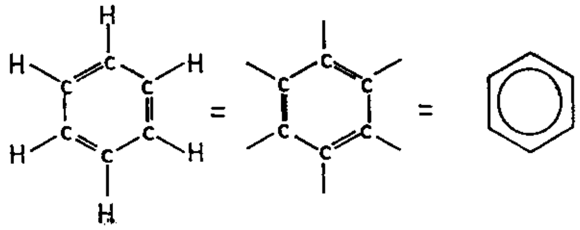


Fig. 8 Mesomeric states of benzene.

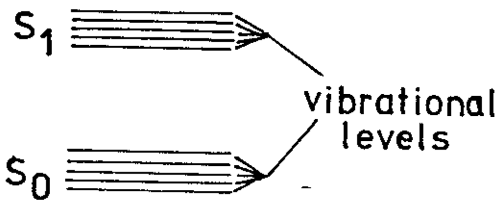


Fig. 9 Vibrational states of molecules (schematic).

of atoms, each of which must have a MO of given and different energy according to the Pauli principle. As a result one finds a number of π- and π^*- states of different energy in molecules with polycentric MO's. The energy gap between the highest occupied π- and the lowest non occupied π^* state can be small enough in such cases to allow for an excitation with light in the near UV or even visible. The majority of biomolecules, absorbing strongly in the visible, make use of this effect. n-, π- and σ-states are singlet-states, the excited π^*- and σ^*-states can be both, singlet- or triplet-states. S-T transitions are relatively allowed if the energy gap is small and/or if a nearby heteroatom leads to a significant spin-orbit coupling.

Molecules cannot only be excited electronically by an external radiation field or e.g. through chemical reactions, they can also go into higher modes of oscillation of the atoms relative to each other and rotation of parts or of the whole molecule. As was found for the electronic states, quantum mechanics requires vibrational and rotational states to be discrete and transitions between such states to be associated with the absorption or emission of a specified amount of energy. Transition energies are much lower than those for electronic excitation. Vibrational transitions can be excited with optical radiation in the near- and middle infrared and are therefore of great importance for the interaction between laser-radiation and living matter. Because of the vibrational modes, all electronic states split up into a number of vibrational substates of different energy. This is shown in principle in Fig. 9. For a large number of molecules irradiated in a volume, energy going into excited vibrational states macroscopically is realized as a temperature rise. Laser excitation of molecule vibrations is therefore the process of prime interest in all biomedical laser applications involving heat production e.g. in laser surgery.

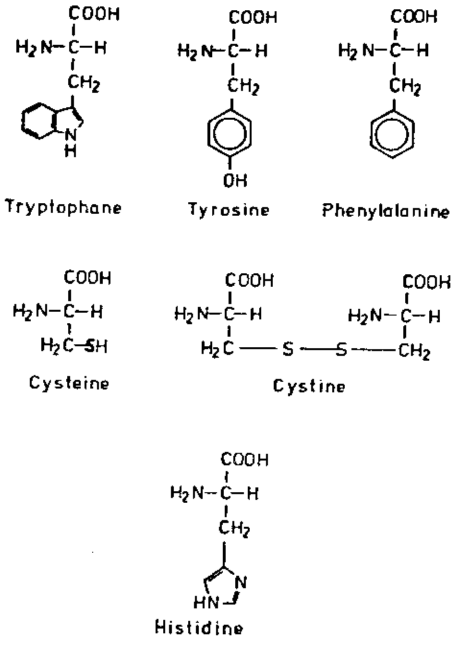


Fig. 10 Amino acids with noticable absorption in the ultraviolet.

3. THE STRUCTURE OF SOME BIOMOLECULES AND THEIR INTERACTION WITH OPTICAL RADIATION

Despite of the seemingly unlimited number of different biomolecules and their amazing adaptability to greatly different functional tasks, their vast majority is constructed from a rather limited number of building blocks, such as amino acids or nucleosides. As the properties of these subunits primarily govern the interaction with optical radiation at least in the visible and ultraviolet, it seems worthwhile to introduce a few of the important ones.

Proteins, with amino acids as their building blocks, are the most abundant biomolecules. The six amino acids most important for the interaction with ultraviolet radiation are shown in Fig. 10.

Fig. 11 shows the molar absorptivity of these amino acids in the UV. It can be very strong indeed. The chromophores i.e. the absorbing centers are the different residues of the amino acids. It is of course not surprising that only amino acids with aromatic residues (polycentric π,π^* -states) and sulfur as heteroatom (n,π^* -transition) have significant absorption in this wavelength range. In addition, histidine has some absorption up to a wavelength of 220 nm due to its imidazole ring. All the other amino acids, particularly the ones with aliphatic rests, exhibit absorption

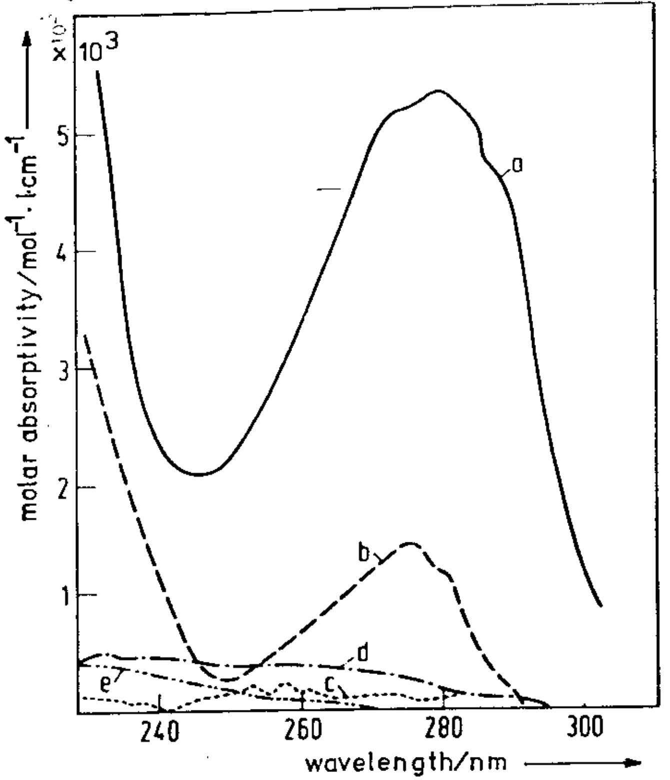


Fig. 11 Wavelength dependence of the molar absorptivity of some amino acids in the ultraviolet
(a) Tryptophane, (b) Tyrosine, (c) Phenylalanine, (d) Cystine, (e) Cysteine
(from: McLaren, A.D. and D. Shugar: Photochemistry of Proteins and Nucleic Acids, Pergamon Press, Oxford 1964)

in the vacuum-UV only ($\lambda < 200$ nm), a region of no interest in the investigation of living systems. As is to be expected from the molecular structure, amino acids show no significant absorption in the near-UV or visible.

A large number of amino acids, linked together by peptide bonds, form the basic structure of polypeptides and proteins. With up to 10^3 amino acid in one such macromolecule, an almost infinite number of combinations is possible, leading to the observed variability. In the native state, one or more polypeptide or protein chains are arranged into structures of high spatial order, their conformation. The α-helix and β-pleated sheets are the most common ones. Collagen, the most abundant structural protein has a very special triple helix structure because of its high content in the amino acids proline and hydroxyproline. The unique structure gives it its mechanical properties discussed in the lecture on vessel coagulation with lasers. The molecular conformation is stabilized mainly through the interaction via hydrogen bonds. Their binding energies are in the range of 0.04-0.4 eV, far below those of the covalent bonds. Excitation of molecules into higher vibrational states, corresponding to a macroscopic rise in temperature, can therefore easily result in breakage of hydrogen bonds.

The wavelength dependent absorption of proteins in the UV and visible is essentially that of the sum of the amino acids they contain. Neither does the peptide bond add substantially to the electronic absorption bonds at $\lambda \gg 200$ nm, nor does the conformation significantly alter those of the amino acids discussed above. As is to be expected, the vibrational states and their transition in the infrared on the other hand very strongly reflect the conformation of a molecule.

Nucleic acids are also polycondensates of high spatial order. The nucleosides as building blocks are molecules consisting of a monosaccharide ring (ribose or deoxyribose) and different bases, derived from either the pyrimidine or purine ring (Fig. 12). A section out of a deoxyribonucleic acid (DNA) is shown in Fig. 13. In DNA only four different nucleosides occur, the bases of which are identified in the figure. A section of ribonucleic acid (RNA) is

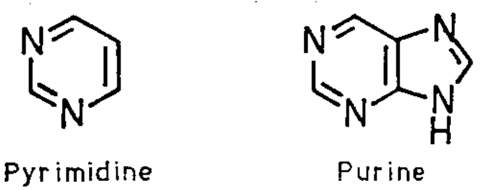


Fig. 12 Purine and Pyrimidine

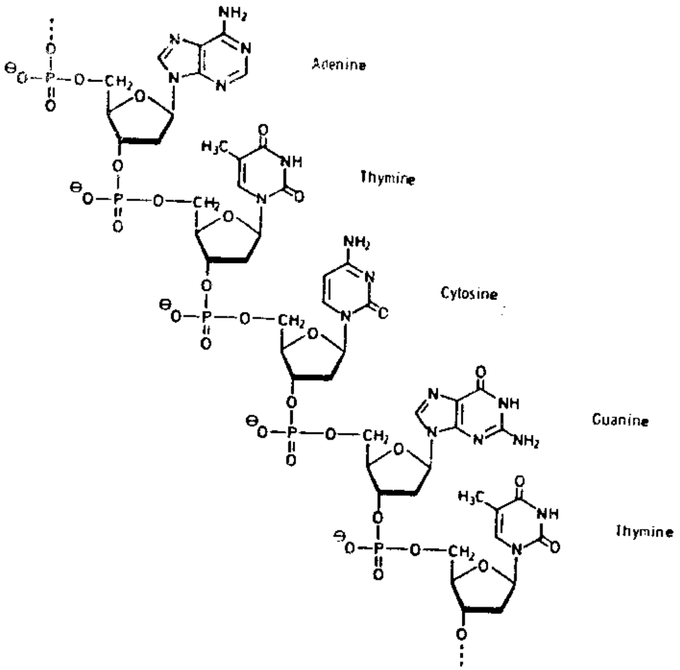


Fig. 13 Section of a deoxyribonucleic acid (DNA).

shown in Fig. 14. RNA has a larger variety in bases than DNA, but four of them are by far the most abundant. Three of them are the same as in DNA, only Thymine is replaced by uracil. Again, the absorption of optical radiation in the wavelength region of interest resides with the aromatic, heterocyclic rings of the five bases. Fig. 15 shows the wavelength dependence of the molar absorptivity of the five bases in the UV. It should be noticed, that the absorption reaches very high peak values with $\varepsilon_{max} > 10^4\ mol^{-1}\cdot l\cdot cm^{-1}$. Neither the ribose and deoxyribose nor the phosphoric acid, linking the nucleosides via two ester-bonds, contribute significantly to the absorption of nucleic acids in the range of $\lambda \gg 200$ nm. Above $\lambda = 320$ nm the absorption of nucleic acids and their constituents is negligible. The famous double helix structure of two corresponding DNA-strands, as well as the various conformational structures

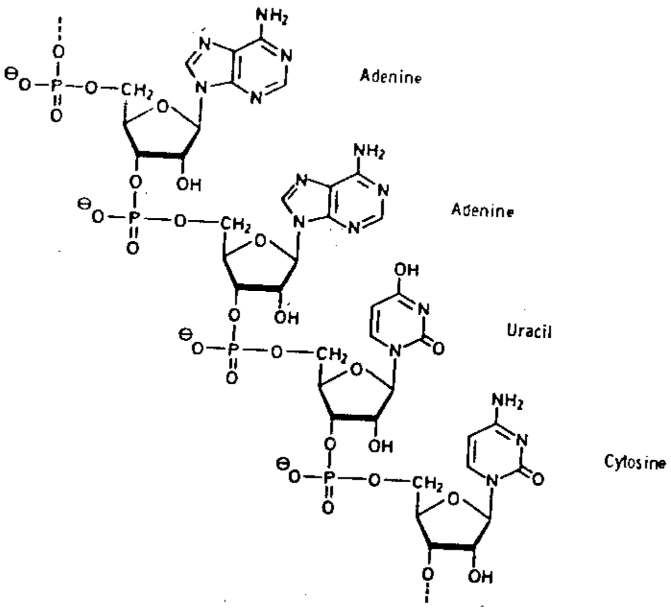


Fig. 14 Section of a ribonucleic acid (RNA).

of RNA are again stabilized by hydrogen bonds. Though these bonds do not qualitatively alter or add to the electronic absorption band in the UV, the close proximity and spatial alignment of bases particularly in DNA results in hypochromism, a quantitatively diminished absorbance as compared to the sum absorption of the bases contained. As in amino acids, the conformational structure of nucleic acids is the determining factor for their vibrational bands and transitions in the infrared. Heating to only moderate temperatures above the physiological ones will lead to breakage of the hydrogen bonds and partial or total loss of the conformational structure.

Certain biochemical groups, particularly those with heteroatoms may exhibit medium (usually n-π^* transitions) or strong (usually π-π^* transitions absorption in the UV. The carbonyl-group of ketones and

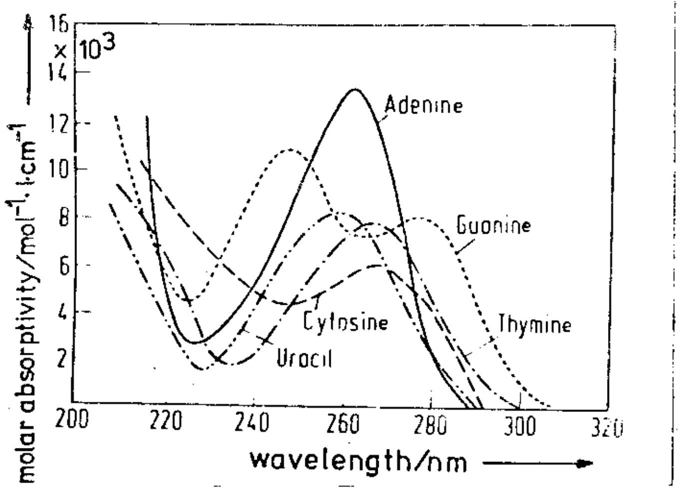
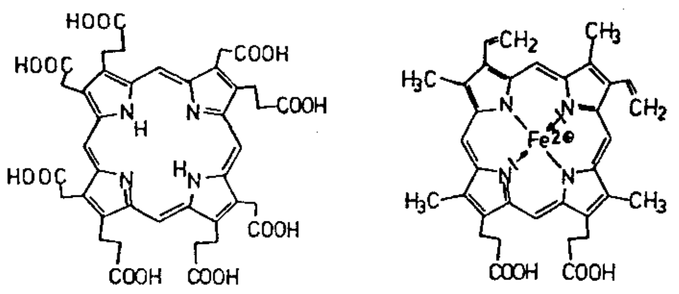


Fig. 15 Wavelength dependence of the molar absorptivity of nucleic acid bases
(from: Davidson J.N. The Biochemistry of the Nucleic Acids, Methuen, London 1965)

aldehydes is one of the most prominent examples as already discussed for the case of formaldehyde. Many such molecules are moreover known to be very efficient as donors or acceptors for triplet state population or depopulation in intermolecular energy transfer processes. They can therefore strongly influence the course of photochemical reactions, even when present in only small concentration. A similar action may be achieved through vital staining of cells or tissue. Dyes are added, the molecules of which specifically bind to or intercalate with biomolecules without significantly disturbing their vital function. Preferentially such dyes are used that absorb in the visible or near-UV and then transfer their excitation energy to the biomolecule. 8-Metoxypsoralen, a dye that intercalates with DNA, is e.g. used in the photochemotherapy of certain skin dieseases, hematoporphyrines are used in laser cancer treatment.

Some of the more important biomolecules, absorbing optical radiation in the visible, will be discussed as the last group. These molecules are proteins with an added prosthetic- i.e. functional group, which in our case contains the chromophore for the absorption of visible light. Several such prosthetic groups are derived from the porphyrine ring shown in Fig. 16. Also shown in the same figure is the heme-molecule that binds to the globin protein to form hemoglobin, the oxygen transporting molecule in red blood cells. Fig. 17 shows the spectral absorption of oxygenated- (Hb) and deoxygenated -(HbO_2) hemoglobin in the visible. When bound to different proteins, the same heme-molecule forms the various cytochromes that were originally found because of their absorption of visible light. Cytochrome c e.g. is an important coenzyme (better cosubstrate) in the respiratory cycle of cells, located mainly in the mitochondria. With the central Fe-ion replaced by a Mg-ion and some changes in the side chains the photosynthetic pigment chlorophyll evolves. All these molecules have a large number of conjugated double bonds that lead to the absorption in the visible. This holds also true for the carotinoid retinal, shown in Fig. 18. When bound to the protein opsin it forms the visual pigment rhodopsin in the photoreceptor cells of the retina. Reduction of the carbonyl group to the alcohol retinol results in the vitamine A, which also absorbs in the visible. Other enzymatic proteins with suitable prosthetic groups such as the flavoproteins also absorb in the visible.



Uroporphyrine III **Heme**

Fig. 16 Porphyrine ring and heme-molecule

Melanine is the brown pigment found in tanned skin, but also in many other organs e.g. in the pigment epithelium of the retina, where it plays an important role for proper vision and in therapeutical photocoagulations of the retina. Its biosynthesis starts out at the amino acid tyrosine and ends at the indolylquinone ring. Within the cell the indolylquinone molecules polimerize (Fig. 19) with the result of chains of conjugated double bonds to render the visible absorption characteristic shown in Fig. 20.

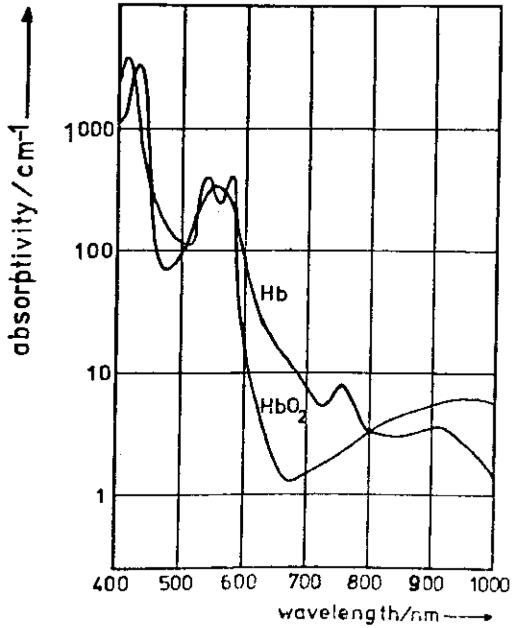


Fig. 17 Spectral absorptivity of oxygenated (HbO_2) and deoxygenated (Hb) Hemoglobin at physiological concentration (150 g l^{-1}) (from: Welsch, M., R. Birngruber, K.-P. Boergen, V.-P. Gabel and F. Hillenkamp. Ber. 75. Zusammenkunft der dtsch. ophthal. Ges. Bergmann Verl. 1978)

In summary, the following rough general rules can be stated:

- for 200 nm $\leq \lambda <$ 315 nm (UV-B and UV-C) biological objects such as cells and tissue will generally absorb optical radiation very strongly. Overlapping absorption bands of many different molecules will usually lead to a rather unspecific excitation of many subsystems.
- for 315 nm $\leq \lambda <$ 400 nm (UV-A) only a limited number of biomolecules show a usually moderate ($\varepsilon_M < 10^2$ mol^{-1}l cm^{-1}) absorption. Interaction with specific molecular systems can often be achieved.
- for 400 nm $\leq \lambda <$ 1000 nm (visible) only very few biomolecules absorb. Human cells and tissue are essentially transparent with the exception of highly melanine pigmented structures and red blood cells which contain a suitable absorber at high concentration.
- for $\lambda >$ 1000 nm (infrared) all biomolecules have specific and strong vibrational absorption bands.
- all tissue is highly scattering throughout the whole range of 200 nm $< \lambda \leq$ 10 µm. It therefore appears translucent to visible light.

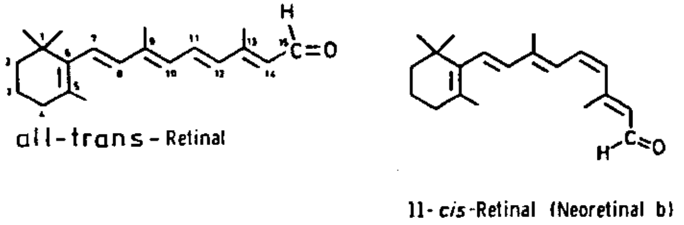


Fig. 18 All-trans- and 11-cis retinal

4. SPECTROSCOPY IN BIOMEDICINE

The interaction between biomolecules and optical radiation can, at least in principle, be used as a sensitive and straightforward spectroscopic tool in the investigation of biological systems and in medical diagnosis. Several properties (monochromaticity, tunability, collimation, short pulses) make lasers ideal sources for many such applications. Several further lectures will deal with specific spectroscopic methods and their application to a number of special problems. Only a very few, general rules will therefore be discussed here.

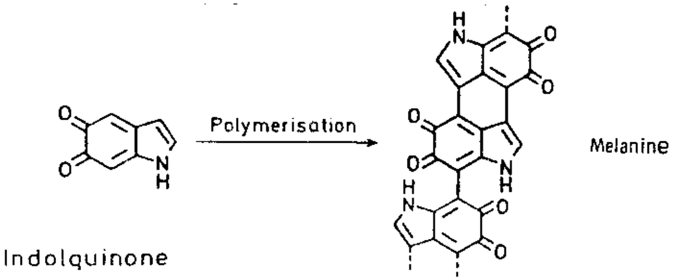


Fig. 19 Indolylquinone and melanine

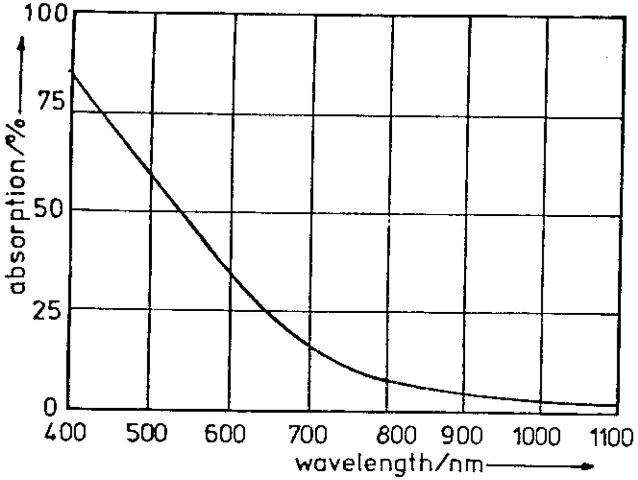


Fig. 20 Spectral absorption of melanine in the visible (from: Gabel, V.-P., R. Birngruber and F. Hillenkamp Ber. 74. Zusammenkunft der dtsch. ophthal. Ges. Bergmann Verl. 1977)

Identification of molecules through the wavelength dependence of their absorption is the simplest form of spectroscopy. Determination of the degree of oxygenation of the hemoglobin in blood through an absorption measurement at a wavelength near 700 nm is a routine method in medical diagnosis - and one of the very few cases in which absorption spectroscopy can be successfully applied. Not only are most of the absorption bands concentrated in the UV below $\lambda = 315$ nm, with strong overlap of the usually rather broad bands, they are moreover caused by the rather unspecific building blocks and depend only very little on the actual structure of the many different macromolecules. Substructure in the spectra due to different vibrational levels is rarely resolved. The number of molecules that could be identified through their absorption spectra in the visible and near-UV is very limited. Moreover, concentration of these molecules (e.g. enzymes) in the cells and tissue very often is too low for an efficient detection. Specific information on the macromolecules and their conformation is contained in the vibrational absorption spectra. Unfortunately living biological systems always contain a high percentage of water (80% in human soft tissue) which also exhibits very strong absorption in the infrared, screening off the bands of interest and rendering absorption spectroscopy in the infrared on vital biological systems more or less obsolete.

Raman spectroscopy could be a very elegant solution to the problem, because it transferes the measurement of infrared vibrational transitions into the visible, thereby circumventing the water absorption. By the very nature of the process, Raman intensities are very weak however. In addition the Raman emission is very often lost in a strong, unspecific background fluorescence in complex biological systems. Only a few successful applications of Raman spectroscopy to real biological systems have been reported so far, but for such applications the method is still in its infancy. A number of physical and technical improvements are possible and Raman spectroscopy may eventually become a very powerful tool in biomedical research and diagnosis.

Practically all biomolecules exhibit optical rotation, i.e. they rotate the plane of linearly polarized light. Measurement of the dispersion (wavelength dependence) of this optical rotation has been a successful analytical method in biochemical research on pure substances in solution. In the very heterogeneous and highly scattering biological objects this method can however be of very limited use only. The same holds true for circular dichroism, a different absorption of waves circularly polarized in opposite senses, exhibited by many biomolecules.

Fluorescence spectroscopy from the lowest excited singlet state after excitation with UV-radiation is another powerful tool. As an analytical method it is superior to absorption spectroscopy, because in fluorescence one has three parameters that carry information about the emitting system: the spectral distribution of the emission itself, its dependence on the excitation wavelength, related to the absorption spectrum in a complex way, and the fluorescence decay time after pulse excitation, yielding information on the lifetime of the S_1 excited state. Moreover, weak fluorescence, e.g. of low concentration samples, can easily be detected with sensitive photodetectors, whereas weak absorption cannot, because it would have to be determined as a very small difference between the two large signals of the incident and transmitted light. In contrast to the absorption spectra, fluorescence spectra, even though they usually originate from the excited states of the various building blocks, carry important information on the macromolecular structure and the molecular environment. Fast intramolecular energy transfer from the absorption site to a fluorescing low energy excited state over considerable distances along the molecule is among the most prominent processes responsible for this fact. Molecular vibrational modes lead to a specific red shift of the emission wavelength, because absorption and emission do not involve the same vibrational sublevels of the electronically excited and the ground state (Fran[illegible]le). In selected cases the fluorescence spectrum of a macromolecule [illegible] even completely different from that of the single building block. This has e.g. been demonstrated for fluorescence spectra of polynucleotides as compared to that of a simple mixture of the single nucleotides. As a severe disadvantage, competing radiationless transitions usually increase drastically, the more complex a molecule gets. Fluorescence of macromolecules will therefore usually be very weak. Vital staining for increased absorption and/or emission is therefore an important technique. In the vital environment of a cell, emission may furthermore be strongly quenched by other molecules present and fluorescence spectra from such objects are a very complex superposition of many different emission centers. So far most of the fluorescence spectroscopy has therefore been done on clean solutions of specific molecules. Phosphorescence from the lowest excited triplet state can usually be detected at very low temperatures only and is therefore not well suited for an investigation of vital systems. The high spectral brightness of laser sources is in many cases a great advantage for a selective and efficient excitation of fluorescence. The exceedingly high concentrations of the radiation flux into very small volumes and short pulses, attainable with lasers, can on the other hand be used only to a rather limited extend. At high irradiances, saturation of transitions occurs and the competing photochemical reactions, discussed in the following section, will lead to a fast degradation of the sample. This is particularly so for all types of optical spectroscopy with the very short picosecond pulses. Early results published with this techniques have therefore often been erraneous and have led to false conclusions.

5. PHOTOCHEMICAL REACTIONS OF BIOMOLECULES

In many of the biomedical laser applications a specific and controlled alteration of the biological system is intended. Such applications range from microirradiation of single cell organelles to macrosurgery on soft tissue and bones. Thermal action after radiation absorption dominates in the majority of such applications. With the advent of powerful lasers in the UV or suitable staining dyes that interact with specific molecular centers in cells and the absorption of which is adapted to a specific laser wavelength photochemical applications have gained considerable interest as well. It seems appropriate therefore to discuss the most important aspects of photchemistry. This field is moreover particularly important in safety considerations.

A reaction is said to be photochemical if it takes place from an, usually electronically, excited state of the molecule that absorbed the photon or to which the energy has been transferred in an identifyable, nonstatistic manner. The overwhelming majority of such reactions can therefore be induced by UV-radiation only, preferentially at wavelengths below 320 nm. The interpretation of such reactions is often difficult, because they may consist of a number of successive steps, often too fast for the intermediate products to be readily identified.

Molecules, excited into higher states will usually relax through internal conversion to the first excited singlet state in times too short for chemical reactions to occur. Some fast, particularly monomolecular reactions start from the first excited singlet state, but the majority involves long lived T_1 triplet states after an intersystem crossing. The latter holds particularly for bi- or polymolecular reactions, where diffusion times must be considered. An important criterion for the likelihood of a photochemical reaction to occur is the change in the electron density distribution function upon excitation and possibly in the spatial arrangement of the molecule as a consequence of it. As a rough rule it can be said that T_1-states are less different from the ground S_0-state than S_1-states. $\pi-\pi^*$ transitions usually induce greater rearrangements than do $n-\pi^*$ transitions. Associated with such changes is often an intramolecular charge transfer resulting in considerable changes in the polarity (dipole moment) of certain groups or the entire molecule. Likewise changes in the pK_a-value may be observed, resulting in protonization or deprotonization of the molecule, a very important step in many biochemical reactions.

A few typical photochemical reations will be discussed as examples. Dissociation of a molecule after excitation is much less frequent than one would expect, though the excitation energy for transitions in the UV is above that of many bonds. The binding energy of a C-C single bond e.g. is only about 3.5 eV, corresponding to a wavelength of about 350 nm. The reason is that the energy, absorbed at the chromophore, is not usually fully transferred to one particular binding site. In addition the dipole forces of the water molecules in hydrated molecules prevent the dissociation products from separating spatially and the bond is reformed, after the excitation energy is lost to the solvent. If dissociation occurs, it is the structure of the molecule that determines the cleavage site, not so much the location of the chromophore. Because of the nature of the covalent bonds, discussed earlier, two radicals with unpaired electrons usually result from cleavage, or a negative and a positive ion. Sometimes excitation leads just to the loss of a hydrated electron, leaving a radical behind. The radicals are very reactive and often responsible for the actual effect, induced in the biological system. Photodissociation of the sulphur bond has e.g. been observed in cystine. This seems to be the dominating photochemical reaction of proteins, excited by UV-radiation. As the cystine-absorption is rather low in the UV, intramolecular energy transfer from amino acids with aromatic residues must be an important factor in such reactions.

Dimerization, the linkage of two equal monomer molecules, is another well documented photochemical reaction. [illegible] that at least in the extensively investigated single cell- and microorganisms the formation of a dimer out of two adjacent thymine bases on the same DNA strand constitutes the dominating biological effect of UV-radiation. Again, intramolecular energy transfer along the DNA to the thymine base, which has the lowest lying T_1-state of all bases, enhances the reaction. As explained before, biological action must not necessarily mean a change in chemical composition. The two thymine bases remain essentially unchanged, but with the altered spatial structure, the DNA loses its ability to unfold and transcribe its information at this site. Thymine dimerization can also very effectively be induced with radiation in the wavelength range between 300 and 315 nm in the presence of sensitizing molecules with a carbonyl-group e.g. benzophenone. This is a good example for intermolecular energy transfer processes that preferentially occur between triplet excited molecules.

Photoinduced isomerization has been demonstrated for many systems. Isomers are molecules of equal chemical composition, but different symmetry of parts of the molecule or residues with respect to a specific molecular plane, defined e.g. by double bonds or ring structures. Associated with the primary reaction in vision is the most famous naturally occurring photoinduced cis-trans isomerization. In its unexposed form, the 11-cis retinal binds to the opsin protein (see also fig. 18). In this form, the aliphatic chains are both on the same side (cis) of the double bond between the carbon atoms 11 and 12. After excitation with visible light the molecule relaxes into the all-trans configuration that does not bind to the protein. The transduction of the information then takes place via very fast changes in the linking Schiff base and in the conformation of the protein. The details of these processes that finally lead to the electrical excitation of the receptor membrane are still under investigation.

Photochemical substitution, oxydation and reduction are other reactions that can occur. Photoionization is only rarely observed, because ionization energies of all but a few alkaline elements are too high to be supplied by a single UV-radiation photon. In observed cases the missing energy difference is supplied by the solvated electron.

In summary it can be said that photochemical reactions dominate biological effects of radiation below about 320 nm in wavelength. At least in cases, where a physiological function is related to absorption in the visible or near-UV, photochemically induced damage must be considered even at radiation levels or exposure situations only slightly above normal. In a living biological system a variety of different and competing photoinduced reactions are usually observed that make interpretation of results and therapeutic use difficult.

The treatment of cancer with lasers, emitting visible radiation (Argon, Kryton) after vital staining with hematoporphyrines is a good example. Neither has the active dye and it's exact chemical structure been fully identified yet, nor is it known, to which subcellular structures it binds and what the details of it's photochemical action are. Experimental results seem to indicate that one of the most important aspects is the prolonged retention time of the dye in malignant cells relative to benign ones and that molecular oxygen is important in the reaction because of it's triplet ground state (photodynamic action). It is well documented that excessive irradiation with UV-radiation significantly increase the occurrence rate of skin cancer, most probably through alteration in the genetic apparatus of cells. Great care should therefore be taken when using UV-emitting lasers for biomedical applications.

6. THERMAL CHANGES IN CELLS AND TISSUE

For thermal reactions to occur, the energy, absorbed from the radiation field, must first be randomized within a large ensemble of molecules through statistical processes. The energy may be supplied through excitation into electronically excited states, followed by a complete relaxation into vibrational modes (internal conversion e.g. in hemoglobine) or directly into the vibrational bands by infrared radiation. After the randomization, occupation density of the various vibrational states as function of their energy will follow the well known Boltzmann distribution. The macroscopic parameter of this distribution is the absolute temperature T. It should be kept in mind that the border between photochemical and thermal effects is not an absolute one. Both types of reaction may lead to the same final product (but must not necessarily do so) and are therefore often difficult to separate from each other. Photochemical reactions from vibrationally highly excited ("hot") ground states have also been postulated. Most important, both types of reaction will usually enhance each other.

As was explained earlier, a moderate rise in temperature on the molecular level predominantly effects a breakage of hydrogen and other van de Waal's bonds that stabilize the conformation of the molecule. This is called a denaturation of the molecules, because the majority of biochemical reactions require a sort of key to keyhole fit in the spatial structure of the molecules for the reactions to take place. Denaturation therefore necessarily means loss of vital function. It is interesting to note that such loss of conformation can be seen directly through the change in optical scattering, as is well known e.g. for egg white. Energetically the molecule can reach the low final denaturated state 3 from the vital state 1 only by surpassing an intermediate state 2, higher in energy, as shown in Fig. 21.

It has been shown experimentally that such denaturation reactions are described to a good approximation by the mathematical formalism that holds for the kinetics of first order (involving one reaction partner only) chemical reactions. The reaction rate of the denaturation is then given as:

$$\frac{d\Omega}{dt} = P \exp(-E_a/RT)$$

with P as the velocity constant and E_a the activation energy. R is the universal gas constant. E_a and P must be determined experimentally for any given molecule. Order of magnitude values are e.g. $P = 10^{70}\ s^{-1}$ and $E_a = 4 \cdot 10^5 Jmol^{-1}$ for liver tissue. The very large value of P reflects the dramatic increase in entropy for such reactions through the loss of spatial order. The rate process model of thermal denaturation predicts that the complete temperature - time history governs the degree of denaturation, not just the peak temperature reached and experiments indeed support this prediction. The details of such thermal denaturation processes will be discussed in one of the seminars. In contrast at least to the general principles of photochemical reactions, thermal denaturation takes place even in non excited systems at room- or body temperature. A rise in temperature only increases the reaction rate, the increase becoming very drastic however at certain specific temperatures, because temperature appears in an exponential function.

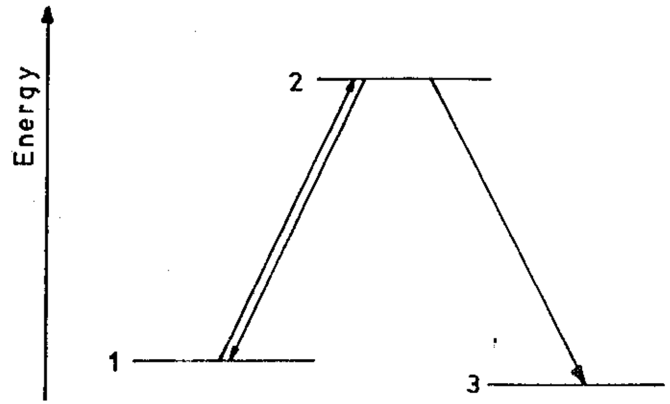


Fig. 21 Energy levels and transition in thermal denaturation

Thermal effects in biomedical laser applications usually involve the irradiation and denaturation of complex, macroscopic volumes. The effects are monitored through the macroscopic measurements of the failure of certain sensitive subsystems such as enzyme systems or the vital function of single cells or whole tissue volumes. These methods of histochemistry and microscopic- or electronmicroscopic histology will be discussed in a further lecture. It should be kept in mind however that values thusly determined e.g. for a special type of tissue, as given above for liver tissue, must necessarily represent the overall reactions of the whole complex system and cannot usually be related to a single of the many involved molecular species.

A method commonly used in attempts to relate an observed overall reaction to the absorption of a specific chromophore or the known wavelength dependence of a certain reaction is the recording of an action spectrum. For such an action spectrum the incident energy E (λ) is recorded, necessary to obtain the effect of interest, e.g. a 50% survival rate of cells in a cell culture. Because the absorption spectrum of the suspected chromophore reflects the ratio of absorbed to incident quanta, the energy E (λ) has to be scaled by multiplying it by λ for the two wavelength dependences to be comparable qualitatively.

$$\frac{E(\lambda)\cdot\lambda}{E(\lambda_o)\lambda_o} = \frac{A(\lambda_c)}{A(\lambda)}$$

For the judgement of macroscopic thermal effects in laser irradiated tissue, a few more physical processes habe to be discussed. The distribution of absorbed energy within an irradiated volume is governed both by the scattering properties of the tissue and the absorptivity of it at the specific laser wavelength used. As explained before, with the exception of blood, most tissues are essentially non absorbing in the visible. Penetration of radiation into the tissue can therefore be deep, with irradiance (Wcm^{-2}) or radiant exposure (Jcm^{-2}) however decreasing rapidly because of scattering. A theoretical analysis based on the theory of Rayleigh- and Mie-scattering is difficult, because of the strong heterogeneity of tissue and large variations between different types of tissue. Often a number of layers of tissue with different scattering properties are penetrated as e.g. in the laser irradiation of the walls of stomach or bladder. Only rather recently have systematic experiments been started to measure the scattering of laser radiation in various types of tissue. In favourable cases, e.g. for the pigment epithelium of the retina, where the light absorption is confined to a cellular monolayer, Beer's law can be applied as a good approximation. The penetration depth ($I_t/I_o = 1/e$) of light into blood vessels, or blood layers covering tissue, stays below [illegible] 1 mm throughout the entire visible spectrum, decreasing to less than 0.1 mm at the hemoglobine absorption bands around 550 nm and below 450 nm. At the two major emission wavelengths of the argon laser (488 and 514 nm), frequently used for coagulation and even cutting, penetration depth is about 0.2 mm. Both, absorptivity and scattering determine the penetration of visible light into blood. When using argon lasers for surgical purposes, the irradiation field therefore always has to be clear of blood, if mere superficial coagulation of the blood is to be avoided. For lasers emitting in the infrared, most frequently used in a great variety of surgical applications, the radiation is absorbed mainly by the water within the tissue. For the neodymium laser, emitting in a wavelength of $\lambda = 1.06$ µm, used preferentially for coagulation purposes, absorptivity of H_2O is below 1 cm^{-1}. Penetration depth is several centimeters, as demonstrated by the 40% transmission of radiation at this wavelength through the refractive media of the eye. For this wavelength the penetration of radiation into tissue will be limited by scattering to typically some millimeters. CO_2-lasers, emitting at a wavelength of 10.6 µm are mainly used for cutting of tissue and bones and sealing of blood vessels up to about 1 mm in diameter. At this wavelength, the absorptivity of H_2O is about 700 cm^{-1}. As a result, CO_2-laser radiation will penetrate only a few cell layers of the tissue and scattering will be of minor influence only.

The volume of the affected tissue will be influenced by heat conduction and possibly -convection through flowing blood as well. The thermal parameters, specific heat, heat conductivity and heat diffusivity, of tissue are equal to that of water, but again heterogeneity, particularly the blood vessel supply within the affected volume, limit theoretical predictions. Heat conduction is moreover strongly influenced by the geometry of the beam and the irradiated volume and the time course of the irradiation. A seminar will deal with the problems of heat diffusion within tissue.

Thermal denaturation of biomolecules will be the dominating heat effect up to temperatures of 100°C. The evaporation of the tissue water will act as sort of a buffer and limit the average temperature to this value until all the water has been evaporated. There are strong indications however that even within this regime of operation much higher temperatures can be reached locally. After all the water is lost, the tissue structure will desintegrate, but carbonization of the organic material may occur under continued irradiation or at the thermal hot spots mentioned before. The full extent of the damage into the tissue can be judged after a time delay of about one day only, because of biological secondary reactions to sensitive, vitally important subsystems within the cells e.g. of particular enzymes, following thermal damage. In water free structures such as bone, temperature will go far beyond 100°C e.g. when cut with a CO_2 laser. Often strong emission of incandescent light from the irradiation site is observed in such cases, indicating temperatures well in excess of 1000°C.

7. EFFECTS OF VERY SHORT PULSES

Pulsed exposure, if suitably timed, would have the great advantage of avoiding or limiting heat conduction and confining the action to the absorption center or it's immediate surrounding. It can nevertheless be stated as a general rule that pulses of duration below about 1 ms are not well suited for therapeutical medical applications, because of difficult to predict and control sideeffects.

So far, the only demonstrated exception is the laser microsurgery of the anterior segments of the eye, used e.g. for disruption of physiological (lens capsule) or pathological membranes or other inhomogeneities in the transparent media. In such applications pulses of ns- (Q-switched-) or ps- duration (mode locked lasers) are focused to small spots to achieve irradiances above about 10^8 Wcm^{-2}. At these irradiances optical breakdown through avalanche-processes of free electrons or multiphoton absorption (depending on the wavelength used) occurs. A plasma results in the focus that contains enough energy to produce the desired action most probably through a mechano-acoustic effect. Care must be taken to create a dense enough plasma in short enough a time, to shield the underlying, very sensitive retina from all but a minute fraction of the laser pulse.

Irradiation with short pulses of sufficient energy will even without optical breakdown lead to a steep temperature rise in the tissue. The induced, fast adiabatic expansion will then create pressure waves (acoustic transients) that have been shown to have peak pressures of several hundred atmospheres. Such transients can propagate quite well in tissue and, depending on the wave geometry can cause damage even in places a distance away from the irradiation site. Such damage is expected to occur preferentially in highly ordered structures such as the christae of mitochondria or the discs in the segment ends of photoreceptors and has in fact been found there after irradiation of the retina with Q-switsched laser pulses of about 20 ns duration. At least some of the damage, observed after irradiation with sub-µs pulses is certainly thermo-mechanical in nature rather than caused by nonlinear optical effects as sometimes suspected in the literature. Mechanical damage of the described type is unspecified and difficult to control. As a general rule, short pulses should therefore be avoided in therapeutic laser applications. Depending on the irradiation parameters as well as the optical and thermal parameters of the irradiated tissue, the safe lower limit will be between about 1 ms for small spots in highly absorbing cases (like minimal spot retinal irradiation) and about 1 µs for the less extreme situations. The upper limit for efficient exposure times is of course set by excessive energy loss from the irradiated volume through heat conduction.

CONCLUSIONS

The interaction mechanisms, discussed in this lecture, are all not laser specific and most of them have been seen to occur and investigated with thermal light sources as well. They can, at least in principle, explain the overwhelming majority of phenomena observed in biomedical laser applications. A few other effects, e.g. wound healing and various types of what is often called biostimulation with low level laser irradiation at various wavelengths do not seem to fit into the discussed frame. This does however not mean that new, exotic interaction processes have to be postulated, based e.g. on the coherency of laser light. An investigation into the basic mechanisms of these so far unexplainable phenomena could be very interesting and fruitful, should the phenomena themselves be proven beyond scientific doubt. Anyone, who endeavours into this field, should however do it with the full knowledge and proper appreciation of the interactions already known to exist and with a scrutiny and care comparable to that employed in the investigations that have led us to our present state of knowledge.