INTERNATIONAL ATOMIC ENERGY AGENCY
UNITED NATIONS EDUCATIONAL, SCIENTIFIC AND CULTURAL ORGANIZATION

INTERNATIONAL CENTRE FOR THEORETICAL PHYSICS
34100 TRIESTE (ITALY) - P.O B. 586 - MIRAMARE - STRADA COSTIERA 11 - TELEPHONES: 224281/2/3/4/5 6
CABLE: CENTRATOM - TELEX 460392-1

SMR/100 - 63

WINTER COLLEGE ON LASERS, ATOMIC AND MOLECULAR PHYSICS

(24 January - 25 March 1983)

Applications of Flash Spectroscopy to Molecules of Biological Relevance.

T.G. TRUSCOTT

Department of Chemistry
Paisley College of Technology
Paisley
Renfrewshire, PA1 2BE
Scotland
U.K.

YEARLY REVIEW

APPLICATIONS OF FLASH SPECTROSCOPY TO MOLECULES OF BIOLOGICAL RELEVANCE

This review concerns work published during 1980 and 1981 in which flash photolysis has been used to study several types of biological molecules. No attempt is made to review *in vivo* studies such as those concerned with photosynthesis, vision and bacteriorhodopsin; useful annual reviews and conference reports for some aspects of these subjects have recently been published [36, 106]. However, some work concerned with model systems where the environment may be more relevant to the biological *in vivo* situation than in a homogenous solution are considered. In addition, while the review does not deal comprehensively with the application of pulse radiolysis to biological molecules a few examples of this technique will be discussed particularly where the results are directly relevant to those obtained by flash photolysis.

Many of the photophysical properties of 8-methoxypsoralen (8-MOP)*, the most important drug of the group of furocoumarins used in the phototherapy of psoriasis (PUVA), have been previously elucidated [78]. More recently attention has turned to the adducts of psoralens with thymine and to psoralens (furocoumarins) which only can form monoadducts with DNA such as 3-carbethoxypsoralen (3-CPs). Bensasson *et al.* [15] have used laser flash photolysis (353 nm excitation) to study the 4',5' photoadduct of psoralen with thymine. The triplet absorption spectrum of this adduct shows a rather broad maxima at ~500 nm with triplet extinction coefficient (ϵ_T) ~8500 $dm^3mol^{-1}cm^{-1}$, which decays by first-order kinetics with rate constants of 4.0 and 9.4 × 10^4 s^{-1} in water and methanol respectively. The quantum yield of $S_1 \rightarrow T_1$ intersystem crossing (Φ_T) was estimated to be ~0.08 in both solvents. Of particular relevance to the possible involvement of triplet monoadduct in DNA cross-link formation is the reactivity of the adduct triplet with thymine, and a rate constant of 1.2 × 10^8 $dm^3mol^{-1}s^{-1}$ was reported for this reaction, a somewhat higher rate constant (2.4 × 10^9 $dm^3mol^{-1}s^{-1}$) being obtained for the adduct triplet reaction with tryptophan. In both cases the quenching reaction was shown to be an electron transfer process of the type $^3Adduct + T \rightarrow Adduct^- + T^+$, where T is thymine or tryptophan, rather than double bond addition which, in the case of thymine, would have led to a thymine–psoralen–thymine diadduct. Ronfard-Haret *et al.* [90] have reported the major photophysical properties of 3-CPs using both laser flash photolysis (353 nm) and pulse radiolysis. The triplet lifetime was found to be dependent on the ground state concentration with intrinsic first-order rate constants in the range ~10^4 10^5 s^{-1} for several solvent systems, the triplet plus ground state reaction rate constant being ~10^9 $dm^3mol^{-1}s^{-1}$ for homogeneous solutions. The Φ_T values reported were 0.3, 0.44 and ≥0.35 in benzene, ethanol and water respectively and, as such, are markedly higher than those reported earlier for 8-MOP and psoralen. In addition these workers report the 3-CPs triplet state reactivities towards a number of substrates of biological interest including tryptophan, tyrosine (electron transfer) and oxygen (energy transfer). Also, the (dark) binding of 3-CPs to DNA was investigated using the pulse radiolysis technique, the value of the complexing constant (K) being ~5 × 10^3. Pulse radiolysis has also been used [10] to obtain values of K with DNA in aqueous solution for 8-MOP, psoralen, khellin, angelicin, 5,7-dimethoxycoumarin, 5-MOP and 4' aminomethyl-4,5',8 trimethylpsoralen, the values obtained (K × 10^{-3}) being ~12, 25, 16, 28, > 80, 7 and 3 respectively. Guiotto *et al.* [43] have reported some photophysical data of 4,5' dimethylangelicin (I), 4'-aminomethyl-4,5'-dimethylangelicin (II) and the water-soluble derivative 4'-N,N-dimethylaminoethoxymethyl-4,5'-dimethylangelicin (III). The latter compound shows high photobinding to the macromolecule and is of potential therapeutic interest. The values of Φ_T obtained in methanol by 265 nm laser flash photolysis were rather low, being 0.063, 0.028 and 0.059 for I, II and III respectively and may indicate that the large differences between the photobiological activities of the three furocoumarins do not arise from intrinsic differences in their photophysical characteristics. The photophysical properties of 4'-aminomethyl-4,5',8-trimethylpsoralen (often called aminomethyl psoralen) have been studied [92] using laser flash photolysis (353 nm). This furocourmarin is of interest because of its high solubility in water and exceptional affinity for DNA. It was reported that, in water, the lowest triplet, formed with Φ_T ~0.2, has an intrinsic lifetime of 100 μs and is quenched by the ground state with a rate constant of ~10^9 $dm^3mol^{-1}s^{-1}$ and by thymine with a rate constant of 2 × 10^9 $dm^3mol^{-1}s^{-1}$. Addition of DNA caused the disappearance of the triplet absorption and also extinguishes the fluorescence emission. It seems likely from this work that the first excited singlet state is rapidly deactivated in the presence of DNA and could be the precursor of the first pyrimidine photoadduct. The effect of DNA on the triplet yields and lifetimes and also on the fluorescence emission of psoralen and 8-MOP has been reported by Beaumont *et al.* [11]. Of particular interest was that the yield of 8-MOP triplet when bound to DNA was similar to that for 8-MOP in the absence of DNA while for psoralen it was concluded that the effect of DNA binding was to reduce the triplet yield to a very low value or possibly reduce the lifetime of the triplet to <50 ns. Since both these furocoumarins show similar skin photosensitising abilities it seems likely, once again, that the triplet state is not the precursor of DNA-monoadduct formation. Almost certainly the complexation data for furocoumarins to DNA recently obtained together with the photochemical data which are becoming increasingly available will be an essential feature in establishing the molecular basis for skin photosensitisation by furocoumarins.

The study of the photophysical properties of amino acids and peptides has continued using laser flash photolysis. In addition the role of organic disulphide linkages, their formation and destruction have been investigated because of their relevance to the structure and function of enzymes. Dissociative electron attachment ($RSSR^- \rightarrow RS^\cdot + RS^-$) is an important process and flash photolysis has been used to obtain the rate constants for electron transfer from the radical anions of aromatic compounds to diaryl disulphides [102]. It was found that electron-withdrawing substituents increased the rates of reaction and vice versa reflecting the electron-acceptor abilities of the S–S bonds. Morine and Kuntz [71] have used flash photolysis to study both the S–S and C–S bond cleavage in the photolysis of disulphides such as penicillamine disulphide. A comparison of the transient absorption spectra suggests that C–S bond breakage can be a primary photolytic process. This process was found to become more important as the resulting carbon centred radical is stabilised by increasing alkyl substitution or resonance interaction with an aromatic system. The perthiyl radical ($RSS^\cdot$) product is characterised by λ_{max} ~380 nm, ϵ (380 nm) ~1700 $dm^3mol^{-1}cm^{-1}$ and decays by second-order kinetics with k ~3.7 × 10^8 dm^3 $mol^{-1}s^{-1}$ in water. Particular interest in the photochemistry of amino acids has concerned tryptophan and indole and also the electron transfer reactions involving tryptophan and tyrosyl radicals in peptides and proteins. Klein *et al.* [56] reported a comparative study of the nitrous oxide quenching and the effect of solvent (water and cyclohexane) and temperature (60–1.5°C) on the excited triplet and singlet states of indole. In cyclohexane Φ_T is ≃0.43 and is more or less temperature independent, while in water the Φ_T value varied from 0.07 at 60°C to 0.43 at 1.5°C. It was concluded that the indole first excited singlet decays in water at 1.5°C mainly by the same pathways as in hexane, i.e. fluorescence and triplet formation. However a difference appears in a third deactivation pathway which is N–H bond dissociation in cyclohexane but photoionisation in water. It is suggested that the mechanism of the photoionisation involving either the fluorescent state or a prefluorescent state with a photoionisation threshold [40] remains an open question. The multiple pathways of tryptophan photoionisation have also been discussed by several other groups [18, 20, 42]. In addition Pyault *et al.* [86] report that in oxygen free aqueous solutions Φ_i values for indole and tryptophan are constant within the first absorption band which excludes a threshold process. In the second band Φ_i increases indicating that this process competes with intersystem crossing to the triplet state. In a picosecond laser flash photolysis study of aqueous solutions of tryptophan and indole Mialocq *et al.* [68] found that the electron was formed during the 20 ps pulse. It seems that for the monophotonic photoionisation the electron originates from the unrelaxed first singlet state and not the flurorescent state. Mialocq *et al.* [67] have also shown that the photoionisation of phenol, using a 265 nm 27 ps laser, occurs with high efficiency and that this is best explained by a consecutive two photon process, the second photon being absorbed by the excited singlet state S_1. The effect of nucleotides and polynucleotides on the photophysics and photochemistry of tryptophan has been investigated in aqueous solutions with 265 nm laser flash photolysis by Le Doan [31]. The tryptophan triplet state and the hydrated electron were found to be quenched near the diffusion rate by the nucleotides. It seems possible that such studies could lead to a better understanding of the interaction site between proteins and nucleic acids. A series of papers have concerned electron transfer between tryptophan and tyrosine in peptides and proteins using both the laser flash photolysis technique [95] and the pulse radiolysis technique [84, 85]. Thus 265 nm laser excitation of oxygen-saturated neutral aqueous solutions of tryptophyl-tyrosine, tyrosyl-tryptophan and the diketopiperazine derivative C-tryptophyl-tyrosine showed electron transfer from tyrosine to electron-deficient tryptophan with rates ~1–2 × 10^5 s^{-1}. The transfer rates in the proteins β-lactoglobulin and α-chymotrypsin were 2.6 × 10^4 s^{-1} and 4 × 10^3 s^{-1} respectively. Using the pulse radiolysis technique the indole group was oxidised with radicals such as $N_3^\cdot$ or $Br_2^{\cdot-}$ and the subsequent electron transfer then monitored. Thus for a series of peptides of the type tryptophyl-(glycyl)$_n$-tyrosine electron transfer rates of 7.3, 5.1 and 2.4 × 10^4 s^{-1} were reported for $n = 0$, 1 and 2 respectively. The low activation energy of the process suggested an electron-tunnelling mechanism. The consequence of these observations for the so-called 'selective radical-probe technique' in which selective oxidising radicals such as $Br_2^{\cdot-}$ are used to

Abbreviations used: BR, bilirubin; 3-CPs, 3-carbethoxypsoralen; Chl, chlorophyll; cyt *c*, cytochrome *c*; DMTH, N,N', dimethylthymine; DMU, N,N' dimethyluracil; ϵ_T, extinction coefficient; FMN, flavin mononucleotide; Hb, haemoglobin; Mb, myoglobin; 8-MOP, 8-methoxypsoralen; NAD, nicotinamide adenine dinucleotide; ϕ_T, quantum yield of intersystem crossing.

probe the functional sites of proteins should clearly be investigated.

The effect of ultraviolet and ionising radiation on nucleic acids is closely linked to mutagenisis, carcinogenisis and radiotherapy. While earlier work [91] using laser flash photolysis has allowed the measurement of a number of properties of the triplet excited states of pyrimidines, their nucleosides and nucleotides there have been relatively few reports of studies by flash photolysis of DNA bases during the period covered by this review. However Arce *et al.* [6] have studied purine in acetonitrile and in water at various pH values. The transients produced in water were identified as the triplet state (with bands at 300, 310 and 390 nm), the hydrated electron and the radical cation and anion of purine. In acetonitrile no photoionisation was detected but the shape of the triplet spectrum was similar to that in water. Many of the photophysical properties of thymine and uracil and some nucleosides and nucleotides are well established but in order to further understand the excited state ordering in pyrimidine bases Becker *et al.* have studied both by fluoresecence [12] and laser flash photolysis [13] N,N' dimethylthymine (DMTH) and N,N' dimethyluracil (DMU). The Φ_T for DMU and DMTH in acetonitrile were reported as 0.02 and <0.04 (transient undetected) respectively, so that the Φ_T values of N,N' dimethyl substituted molecules are at least 10-fold less than Φ_T of the unsubstituted molecules in acetonitrile and rather similar to the Φ_T values for the unsubstituted molecules in water (H-bonding) solvents. These results were taken as further confirmation of the state orderings deduced by emission studies [12].

Considerable work has continued on the role of flavins in the oxido–reduction of cytochrome *c* and other components of the mitochondrial electron transport chain such as nicotinamide adenine dinucleotide (NAD). Much of the interest in flavins derives from their postulated role as 'crossover' points from one-electron to two-electron reaction mechanisms in biological redox systems. In the presence of electron donors triplet excited flavin may be either one-electron reduced to semiquinone or two-electron reduced via a non-radical pathway [48]. When cyt *c* is also present in such systems the reduction of cyt *c* can result. Salet *et al.* [93] have used laser flash photolysis (353 nm) and pulse radiolysis to measure the kinetics of reduction of cyt *c* by the semi-reduced form of flavins. Thus flavin mononucleotide radicals ($FMNH^{\cdot}$), generated by excitation of FMN in the presence of electron donors such as histidine, guanosine monophophate and EDTA, were found to reduce cyt *c* with rate constants near 5×10^7 $dm^3mol^{-1}s^{-1}$. No quenching was observed ($<2 \times 10^7$ $dm^3mol^{-1}s^{-1}$) in the presence of cyt *c* with no electron donor even though cyt *c* is known to contain histidine suggesting that histidine residues in cyt *c* are not directly accessible to excited FMN molecules. Salet *et al.* also report that ^3FMN is quenched by ground state molecules ($kq \sim 1.6 \times 10^8$ $dm^3mol^{-1}s^{-1}$) and estimate a first-order rate constant by extrapolation to 'zero' ground state concentration of $\sim 5 \times 10^4$ s^{-1}. These data suggest that cyt *c* reduction by flavins may involve not only reaction of fully reduced $FMNH_2$ with cyt *c* but also the involvement of $FMNH^{\cdot}$. Ground-state oxygen may also play a role when present in sufficient quantities by reacting with $FMNH^{\cdot}$ to give $O_2^{\cdot-}$ which in turn can reduce cyt *c*. In addition it has been shown [80, 81] that single electron transfer from several NADH analogues to singlet excited oxygen can occur, ciliciently yielding $O_2^{\cdot-}$. Ahmad *et al.* [1] used laser flash photolysis (436 nm) to determine the rate constants for the reduction of horse heart cyt *c* by both semireduced ($FH^{\cdot}$) and fully reduced (FH^-) forms of various flavin analogues. It was found for example, that substitution in the dimethylbenzene ring of the flavin caused appreciable changes in the rate constant whereas substitution at the N-10 position did not. The overall results can be accounted for by assuming that a collision leading to reaction between flavin and cyt *c* requires an orientation that positions the aromatic ring-N-5 region of the flavin toward the haem crevice and the N-10 pyrimidine ring away from it. The quenching of flavin triplet states by aliphatic α-substituted acetic acids [2] such as pyruvate ($kq \sim 1.4 \times 10^7$ $dm^3mol^{-1}s^{-1}$), glyoxylate ($kq \sim 1.3 \times 10^6$ $dm^3mol^{-1}s^{-1}$) and lactate ($kq \sim 5.5 \times 10^5$ $dm^3mol^{-1}s^{-1}$) has shown that the triplet quenching occurs virtually exclusively by a one-electron transfer mechanism, and extends earlier work showing the same mechanism, when the α-substituent is an aromatic group [46, 75]. This work seems to support the contention that the flavin sensitised photodecarboxyalation of such acid proceeds via a radical mechanism rather than via a two-electron mechanism [57]. Two examples are now established of two-electron reduction of flavin triplet states, these involve reactions involving CN^- and BH_3CN^- [48, 104, 105]. This work together with interesting studies on 5-deazaflavin [38, 104, 105] will not be discussed here since they have been recently covered in a previous Yearly Review [72]. A study of the mechanism of the flavin sensitised photooxidation of ascorbic acid using laser flash photolysis (347 nm) has been reported by Heelis *et al.* [47] and has also been reviewed by Müller.

Ledbetter *et al.* [61] reported that the N_2 laser (337 nm) can be used to induce the key intermediate of pyridoxal 5'-phosphate (vitamin B_6) catalysis of transamination without the presence of an enzyme. Recently Ledbetter *et al.* [62] have extended this work to obtain the lifetime and activation energy of the *p*-quinoid intermediate (characteristic absorption band ~ 495 nm) of transamination. From the dependence of lifetime on temperature the Arrhenius activation energy (*Ea*) for proton transfer was found to lie in the range 4.2–8.4 kJ mol^{-1} for various amino acid side group substituents. Good correlations of *Ea* with the amino acid side chain group substituent constants

[10] were obtained and the *Ea* values demonstrated that native substrates have a very labile proton.

The naturally occurring pteridines include lumazines and pterins. The latter compounds are found throughout the plant and animal kingdoms where they occur in very small concentrations. Recently Chahidi *et al.* [23] and Momzikoff and Santus [69] have used laser flash photolysis (353 nm) and fluorescence and phosphoresence to determine the photophysical parameters of 2-amino-4(3H) pteridinone (pterin) and 2-amino-4-hydroxy-6(1',2'-dihydroxypropyl)-pteridine (L-biopterin) and to study the photosensitising properties with substates such as tryptophan. At pH 9.2 two transient triplet absorptions are detected ($\tau_1 \sim 0.3$ μs, $\tau_2 \sim 2.3$ μs) which are associated with a triplet deprotonation process ($pK_T \sim 9.5$–10). The longer lived triplet is characterised by $\Phi_T \sim 0.2$ and ϵ_T (550 nm) ~ 2000 $dm^3mol^{-1}cm^{-1}$. It reacts with solvent forming the semi-reduced pterin with a quantum yield of ~ 0.06. The photosensitising properties with respect to tryptophan, histidine, guanosine and several other substrates are thought to imply singlet oxygen formation and/or direct interaction of the pterin triplet with the substrates.

Carotenoids have been much studied by both flash photolysis and pulse radiolysis mainly because of their roles in photosynthesis and because of their use as drugs (for example, β-carotene in the treatment of erythropoietic protoporphyria). Retinyl polynes are also of interest both because of their link to the visual chromophores and also because of their use as drugs. Many of the photophysical properties of carotenoids have been well established for several years. However the interaction of carotenoids with singlet oxygen has continued to be studied and Rodgers and Bates [89] have extended the earlier studies of Wilkinson and Ho [109] in which the mechanism of the efficient quenching of O_2^* ($^1\Delta_g$) by carotenoids was shown to involve electronic energy transfer producing the carotenoid triplet:

$$O_2^* (^1\Delta_g) + Car \rightarrow O_2 (^3\Sigma_g) + {}^3Car^*$$

In general Rodgers and Bates found that for the five carotenoids they studied the reaction rate constants lie in the range 2.2–17.8×10^9 $dm^3mol^{-1}s^{-1}$ and thus, like those carotenoids studied by Wilkinson and coworkers, must be regarded as efficient energy acceptors where they can undergo collisions with singlet oxygen. In this situation the carotenoid triplet is produced thus diverting the electronic energy of singlet oxygen into states which decay via physical channels so removing the potentially damaging chemical reactions of singlet oxygen in biological systems. The variation in the second-order rate constants measured by Rodgers and Bates (e.g. zeaxanthin 2.8×10^9 $dm^3mol^{-1}s^{-1}$, β-carotene 13.8×10^9 $dm^3mol^{-1}s^{-1}$ and canthaxanthin 17.8×10^9 $dm^3mol^{-1}s^{-1}$) are interpreted in terms of the different S_0–T_1 energy gaps of the carotenoids with respect to that of the singlet oxygen donor, however this explanation does not fit all the data reported. In addition Lindig and Rodgers [63] have reported the rate parameters for the quenching of singlet oxygen by several water-soluble and lipid-soluble substrates in aqueous and micellar systems including several amino acids, an analogue of α-tocophenol (vitamin E), sodium azide and also β-carotene. For several quenchers the rate constants reported are comparable to the values found in homogeneous solution and where significant differences were found these were rationalised according to the individual case. However a particularly unexpected result concerned β-carotene which, in micellar dispersion, did not react at all with singlet oxygen. This may well be due to aggregation of the carotenoid in water-rich mixtures and clearly shows that the indiscriminate use of β-carotene as a singlet oxygen quencher in mainly aqueous media is questionable. Progress in our understanding of interfacial electron transfer involving radical ions of β-carotene (and diphenythexatriene) has been made by Almgren and Thomas [4] using both laser flash photolysis (347 nm) and pulse radiolysis. The latter technique was used to study the radical ions of β-carotene in micellar solutions. In non-micellar solutions such as Triton X-100 the radical anion is produced, while in the presence of CTAB the radical cation is also formed. Similar trends were detected for β-carotene in vesicles. The yield of carotene radical anion itself also increases on the addition of CTAB to Triton X-100 micellar solutions and, also, it was noted that some side reaction of the e_{aq}^- occurs on the addition of CTAB (presumably with micelle-bound impurities of the Triton X-100). The increased yield of radical anion is interpreted by Almgren and Thomas as showing that the reaction sites for the electron are located in the outer mantle region of the micelle. The increased positive charge associated with the CTAB can then be envisaged as attracting the electrons closer to the micelle where the carotene is located. The yield of carotene radical anion was also increased on addition of biphenyl to a Triton X-100 micellar solution. The results show that electron transfer from biphenyl radical to β-carotene occurs as a strictly intramicellar process. Furthermore the kinetic data show that the half-life of the growth of carotene radical is 3–5 μs whereas the half-life for the decay of the biphenyl radical when no carotene is present is ~ 10 μs. This means that the rate constants for both electron transfer and deactivation are both about 10^5 s^{-1} and, perhaps rather surprisingly, that the intramicellar electron transfer process is slow. Almgren and Thomas suggest that this may indicate different solubilisation sites for carotene and the biphenyl radical anion in the non-ionic micelle. Other recent studies of carotenoid triplets have shown that the triplet–triplet annihilation rate constant for β-carotene is $\sim 3.6 \times 10^9$ $dm^3mol^{-1}s^{-1}$ [53]. Also Kuzmin *et al.* [58] have studied the quenching of the retinal triplet state by a nitrosyl radical and interpreted their results in terms of isomerisation occurring in non-relaxed

triplets or that triplets observed spectroscopically decay by way of a single triplet state which has a small electronic energy gap to the ground state. The isomerisation of retinal triplet isomers have also been recently studied using resonance Raman spectroscopy [107, 108]. Much of the recent work on carotenoids has concerned resonance Raman studies of carotenes and fluorescence and flash photolysis study of carotenoporphyrins. Typical examples of the time resolved resonance Raman studies of the all-*trans*-β-carotene triplet state and the triplet states of several other C-40 carotenoids are given by Jensen *et al.* [52] and Jensen and Wilbrandt [54]. The β-carotene spectrum is discussed in detail and it is concluded that the C=C double bond order is decreased and that the molecule may be substantially twisted at the 15,15' bond in the triplet state. Also, resonance Raman studies on the picosecond time scale have led Dallinger *et al.* [29] to conclude that the de-excitation lifetime of β-carotene singlet state is $\leqslant 1$ ps. This result appears to require close contiguity on the part of energy transferring partners as in singlet–singlet energy transfer to chlorophyll from carotenoid accessory pigments in the photosynthetic antenna [87]. The flash photolysis (and fluorescence) studies of carotenoporphyrins have concerned the mimicing of the antenna and photoprotective carotenoid functions of carotenoids in photosynthesis by studies of triplet (and singlet) energy transfer in the synthetic carotenoprophyrin. Thus Bensasson *et al.* [16] have used laser flash photolysis (353 nm) to show that, after excitation, for those molecules where folding allows close interaction of the carotenoid and porphyrin π-electron system, the carotenoid triplet appears within the time resolution of the equipment (~ 30 ns) and the porphyrin triplet cannot be detected. However, where such a conformation is precluded, the porphyrin triplet is first observed and then decays with a concomitant (relatively slow) growing in of the carotenoid triplet. Similar conclusions for singlet–singlet energy transfer had been previously reported using fluorescence techniques [30, 70]. These results clearly illustrate that neither the protective role or the antenna function of carotenoids is achieved simply by placing a carotenoid in the proximity of the porphyrin and that a specific conformation is also required. Clearly such conclusions may also be of importance in the design of synthetic solar energy conversion systems.

The pyrrole pigments such as the chlorophylls, photosynthetic antenna pigments including phycobiliproteins, metalloporphyrins as model systems, the porphyrins related to haem and myoglobin, the bile pigments and those used in phototherapy such as haematoporphyrin constitute an enormous subject area with very many studies by flash spectroscopy. It is beyond the scope of the review to cover this vast literature and instead typical references are quoted for these individual topics together with a few comments reflecting some of the major themes of the work.

One electron reduction of quinones (Q) by photoexcited chlorophylls has been extensively studied and recent work has concerned heterogeneous systems so as to understand the environmental effects on chlorophyll photoelectron transfer. Typical work [17, 24–26, 49, 50] has concerned lipid bilayer systems, cellulose acetate films and aqueous micellar solutions. It is found that quinone quenching of chlorophyll (Chl) within a lipid bilayer is a diffusional process resulting in the formation of radical ion species. Efficient separation of these radicals is greatly enhanced by electron transfer from the primary $Q^{\cdot-}$ to another Q molecule at the bilayer–water interface and that this leads to only a relatively slow rate of reverse electron transfer. Such a combination of rates could, of course, be valuable with respect to energy storage systems. In addition to chlorophylls another chlorin which has been studied by both conventional (μs) and laser flash photolysis is bonellin [66], a physiologically active pigment present in the marine echuroid *Bonellia viridis*. The singlet excited state was shown to have an energy level of ~ 187 kJ mol^{-1}, a lifetime of ~ 6.3 ns and fluorescence quantum yields of 0.07 and 0.2 at 298 and 77K respectively. The lowest triplet energy level was estimated at ~ 180 kJ mol^{-1} and the $S_1 \rightarrow T_1$ equantum yield to be ~ 0.85. The triplet was quenched by oxygen and by benzoquinone with second-order rate constants of $\sim 1.7 \times 10^8$ dm^{3}mol^{-1}s^{-1} and $\sim 5.2 \times 10^9$ dm^3 mol^{-1}s^{-1} respectively. The latter value may reflect an electron transfer process. Matthews *et al.* [66] also showed that copper bonellin has a markedly shorter triplet lifetime (< 20 ns) which may account for its lack of photodynamic action. Bacteriochlorophyll (a dihydroporphyrin) has also been studied both by laser flash photolysis [51] and resonance Raman spectroscopy [28]. The photophysical properties of bacteriochlorophyll *a* such as Φ_t and Φ_f are substantially different (lower) than for Chl *a*. There have been many studies by flash photolysis and pulse radiolysis of model systems related to photosynthesis and/or to solar energy research such as those involving metalloporphyrins [8, 45, 55, 82, 100] and porphyrin–quinone and porphyrin–porphyrin covalently linked systems [65, 73, 74, 76, 79]. The results show the importance of orientation on the rate of electron transfer and also suggest a reason for the apparent absence of quinones as primary electron acceptors in photosynthesis despite their use as secondary acceptors.

In the blue-green algae the antenna proteins consist of the phycobiliproteins, these molecules contain an open chain tetrapyrrole covalently bound to an apoprotein. The absorbed energy follows a pathway from c-phycoerythrin to Chl *a* as determined for example by Porter *et al.* [83]. Recent fluorescence [110] and picosecond laser flash photolysis [33] studies are interpreted in terms of the occurrence of singlet–singlet annhilation intramolecularly among the several phycobilin chromophores within the individual phycobiliprotein molecules in solution.

The active centre of haemoglobin (Hb), which carries oxygen from the lungs to the tissue, and myoglobin (Mb), which stores oxygen in red muscle and transports it to mitochondria, is protohaem and it is the ferrous iron of protohaem which binds the ligand oxygen. Haemoglobin is an allosteric protein which exhibits cooperativity in the binding of oxygen, unlike myoblobin. There have been many studies of Hb and Mb by flash photolysis, (for example [14, 27, 34, 35, 44, 64, 77, 88]) and also by time resolved resonance Raman spectroscopy, (for example [97, 99, 103]). In addition there have also been several studies of protohaem and related iron-porphyrins as models systems, for example [5, 9, 19, 22, 98].

The major theme of the studies by flash photolysis concern the binding of CO to protohaem alone, and CO and oxygen to Mb and Hb. For protohaem, the rates of rebinding of CO as a function of temperature, determined by laser flash photolysis, are interpreted in terms of several barriers impeding the approach of the ligand to the haem iron. At low temperatures (< 15 K) the theoretical rebinding rate is much slower than the experimental values, and this can be explaining in terms of tunnelling. For Mb and the individual subunits of Hb, similar conclusions have been reached. The ligand dissociation from MbO_2, MbCO; HbO_2 and HbCO occurs in the picosecond time scale, and for HbO_2 and HbCO the dissociation results in the formation of a species (Hb*) with a broadened Hb-like transient spectrum The characteristic stable deoxy-Hb is formed in 5 μs with a first-order rate constant of $\sim 0.5 \times 10^6$ s^{-1}. Time resolved resonance Raman spectroscopy indicates that the Hb-like photointermediate (Hb*) formed after deligation is a high spin Fe(II) haem with the iron atom in the porphyrin plane.

Several time phases of ligand rebinding to haemoglobin have been reported, including an ultra-fast geminate recombination and a biphasic recombination in the micro- and millisecond time range. The ultra-fast process being understood in terms of random-walk diffusion of the ligand in the protein.

As well as Hb there are two other classes of oxygen-carrying proteins, haemocyanins and haemerythrins and Alberding *et al.* [3] have extended the application of flash photolysis to haemerythrin. These workers have shown that the dioxygen–iron bond in this protein is photosensitive and that the recombination process is strongly dependent on solvent viscosity.

Laser flash photolysis has also been used to study porphyrins such as protoporphyrin which cause skin photosensitivity, these arising from a heritable error of haem metabolism. The photophysical parameters have been reported for protoporphyrin IX and its dimethyl ester [94] and for several other related porphyrin esters [21]. In addition Lambert *et al.* [59] have reported comparative studies on haematoporphyrin and the so-called 'haematoporphyrin derivative' which is proving useful in the phototherapy of malignant tumours [32]. In general the triplet yields, lifetimes and energy levels [39] fall into a rather narrow range so that it seems likely that the differences in photosensitising effects amongst the porphyrins studied cannot readily be attributed to different parameters of the triplet states.

Bilirubin (BR) is the product of haem catabolism in humans and the study of the excited states of BR continue to be investigated because of their relevance to the treatment of hyperbilirubinemia in the newborn by phototherapy. Thus Sloper and Truscott [96] used nanosecond laser flash photolysis (347 nm) to investigate the excited states of BR in a variety of environments. Quantum yields for BR triplet formation were shown to be < 0.005 in several solvents and to be ~ 0.01 in benzene. Although the yields of triplet formation were found to be very low the photoisomerisation process was shown to be fast in the solvents studied and also when BR was bound to its highest affinity site on human serum albumin. Greene *et al.* [41] used picosecond laser flash photolysis to study BR excited states. Transient absorption spectra, recorded after excitation of BR bound to HSA with a 0.5 ps pulse of 305 nm light, decay with a lifetime of ~ 19 ps at 22°C and ~ 35 ps at 2°C. Similar data was reported for BR in chloroform. Using this and other data the authors conclude that configurational isomerisation of BR is the predominant non-radiative pathway that competes with pigment fluorescence and that photoisomerisation proceeds via a short-lived, partially twisted excited-singlet state intermediate. The results also imply that BR molecules remain relatively unhindered with respect to photoisomerisation when bound to HSA at its highest-affinity site.

Department of Chemistry T. G. TRUSCOTT
Paisley College
High Street
Paisley PA1 2BE, Scotland, UK

REFERENCES

1. Ahmad, I., M. A. Cusanovich and G. Tollin (1981) *Proc. Natl. Acad. Sci. USA* **78**, 6724–6728.
2. Ahmad, I. and G. Tollin (1981) *Photochem. Photobiol.* **34**, 441–445.
3. Alberding, N., D. Lavalette and R. H. Austin (1981) *Proc. Natl. Acad. Sci. USA* **73**, 2307–2309.
4. Almgren, M. and J. K. Thomas (1980) *Photochem. Photobiol.* **31**, 329–335.
5. Andrews, J. R. and R. Hochstrasser (1980) *J. Am. Chem. Soc.* **102**, 3110–3114.
6. Arce, R., L. A. Jimenez, V. Rivera and C. Torres (1980) *Photochem. Photobiol.* **32**, 91–95.
7. Atkinson, G. H., J. B. Pallix, T. B. Freedman, D. A. Gilmore and R. Wilbrandt (1981) *J. Am. Chem. Soc.* **103**, 5069–5072.
8. Ballard, S. G. and D. C. Mauzerall (1980) *J. Chem. Phys.* **72**, 933–947.
9. Bartocci, C., F. Scandola, A. Ferri and V. Carassiti (1980) *J. Am. Chem. Soc.* **102**, 7067–7072.

10. Beaumont, P. C., E. J. Land, S. Navaratnam, B. J. Parsons and G. O. Phillips (1980) *Biochim. Biophys. Acta* **608**, 182–189.
11. Beaumont, P. C., B. J. Parsons, S. Navaratnam, G. O. Phillips and J. C. Allen (1980) *Biochim. Biophys. Acta* **608**, 259–265.
12. Becker, R. S. and G. Kogan (1980) *Photochem. Photobiol.* **31**, 5–13.
13. Becker, R. S., R. V. Bensasson and C. Salet (1981) *Photochem. Photobiol.* **33**, 115–116.
14. Beece, D., L. Eisenstein, H. Frauenfelder, D. Good, M. C. Marden, L. Reinisch, A. H. Reynolds, L. B. Sorenson and Y. T. Yue (1980) *Biochemistry* **19**, 5147–5159.
15. Bensasson, R. V., C. Salet, E. J. Land and F. A. P. Rushton (1980) *Photochem. Photobiol.* **31**, 129–133.
16. Bensasson, R. V., E. J. Land, A. L. Moore, R. L. Gouch, G. Dirks, T. A. Moore and D. Gust (1981) *Nature* **290**, 329–332.
17. Bera, S. C., S. Nehari, J. M. Levy, N. Periasamy and H. Linschitz (1980) *3rd Int. Conf. on Photochemical Conversion and Storage of Solar Energy*, August 1980, 15–16.
18. Bernas, A. (1981) *Am. Soc. Photobiol.* (9th Ann. Meet.) 95–97 (Abstr.).
19. Bizet, C., P. Morliere, D. Brault, O. Delgada, M. Bazin and R. Santus (1981) *Photochem. Photobiol.* **34**, 315–321.
20. Blum, A., A. M. Brendzel and L. I. Grossweiner (1981) *Am. Soc. Photobiol.* (9th Ann. Meet.) 106–107 (Abstr.).
21. Bonnett, R., A. A. Charalambides, E. J. Land, R. S. Sinclair, D. Tait and T. G. Truscott (1980) *J. Chem. Soc. Faraday I* **76**, 852–859.
22. Brault, D., C. Bizet, P. Morliere, M. Rougee, E. J. Land, R. Santus and A. J. Swallow (1980) *J. Am. Chem. Soc.* **102**, 1015–1020.
23. Chahidi, C., M. Aubailly, A. Momzikoff, M. Bazin and R. Santus (1981) *Photochem. Photobiol.* **33**, 641–649.
24. Chauvet, J.-P., R. Viovy, R. Santus and E. J. Land (1981) *J. Phys. Chem.* **85**, 3449–3456.
25. Cheddar, G., F. Castelli and G. Tollin (1980) *Photochem. Photobiol.* **32**, 71–78.
26. Cheddar, G., J. K. Hurley and G. Tollin (1980) *3rd Int. Conf. on Photochemical Conversion and Storage of Solar Energy*, August 1980, 13–14.
27. Chernoff, D. A., R. M. Hochstrasser and A. W. Steele (1980) *Proc. Natl. Acad. Sci. USA* **77**, 5606–5610.
28. Cotton, T. M., K. D. Parks and R. P. Van Duyne (1980) *J. Am. Chem. Soc.* **102**, 6399–6407.
29. Dallinger, R. F., W. H. Woodruff and M. A. J. Rodgers (1981) *Photochem. Photobiol.* **33**, 275–277.
30. Dirks, G., A. L. Moore, T. A. Moore and D. Gust (1980) *Photochem. Photobiol.* **32**, 277–280.
31. Le Doan, T., J. J. Toulme, R. Santus and C. Hélène (1981) *Photochem. Photobiol.* **34**, 309–313.
32. Dougherty, T. J., G. Lawrence, J. H. Kaufman, D. Boyle, K. R. Weishaupt and A. Goldfarb (1979) *J. Natl. Cancer, Inst.* **62**, 231.
33. Doukas, A. G., V. Stefoncic, J. Buchert, R. R. Alfano and B. A. Zilinskas (1981) *Photochem. Photobiol.* **34**, 505–510.
34. Duddell, D. A., R. J. Morris and J. T. Richards (1980) *Biochim. Biophys. Acta* **621**, 1–8.
35. Duddell, D. A., R. J. Morris, N. J. Muttucumaru and J. T. Richards (1980) *Photochem. Photobiol.* **31**, 479–484.
36. Ebrey, T. G. and T. Yoshizawa (1981) *Photochem. Photobiol.* **33**, 417–601.
37. Farmilo, A. and F. Wilkinson (1973) *Photochem. Photobiol.* **18**, 447–450.
38. Goldberg, M., I. Pecht, H. E. A. Kramer, R. Traber and P. Hemmerich (1981) *Biochim. Biophys. Acta* **673**, 570–593.
39. Gouterman, M. and G. E. Khalid (1974) *J. Mol. Spectrosc.* **53**, 88–100.
40. Grand, D., A. Bernas and E. Amouyal (1979) *Chem. Phys.* **44**, 73–79.
41. Green, B. J., A. A. Lamola and C. V. Shank (1981) *Proc. Natl. Acad. Sci. USA* **78**, 2008–2012.
42. Grossweiner, L. I. (1981) *Am. Soc. Photobiol.* (9th Ann. Meet.) 84–85 (Abstr.).
43. Guiotto, A., P. Rodighiero, G. Pastorini, F. Bordin, F. Baccichetti, F. Carlassare, D. Vedaldi, F. Dall' Acqua and E. J. Land (1981) *Il Farmaco* **36**, 536–550.
44. Guzzo, A. V., J. A. Hutchinson and L. J. Noe (1981) *J. Photochem.* **17**, 35 (Abstr.).
45. Harel, Y. and J. Manassen (1980) *Photochem. Photobiol.* **31**, 451–458.
46. Heelis, P. F. and G. O. Phillips (1979) *Photobiochem. Photobiophys.* **1**, 63–70.
47. Heelis, P. F., B. J. Parsons, G. O. Phillips and J. F. McKellar (1981) *Photochem. Photobiol.* **33**, 7–13.
48. Hemmerich, P., W. R. Knappe, H. E. A. Kramer and R. Traber (1980) *Eur. J. Biochem.* **104**, 511–520.
49. Hurley, J. K., F. Castelli and G. Tollin (1981) *Photochem. Photobiol.* **34**, 623–631.
50. Hurley, J. K., F. Castelli and G. Tollin (1980) *Photochem. Photobiol.* **32**, 79–86.
51. Janzen, A. F., J. R. Bolton and J. S. Connolly (1980) *3rd Int. Conf. on Photochemical Conversion and Storage of Solar Energy*, August 1980, 103–105 (Abstr.).
52. Jensen, N.-H., R. Wilbrandt, P. B. Pagsberg, A. H. Sillesen and K. B. Hansen (1980) *J. Am. Chem. Soc.* **102**, 7441–7444.
53. Jensen, N.-H., R. Wilbrandt and P. B. Pagsberg (1980) *Photochem. Photobiol.* **32**, 719–725.
54. Jensen, N.-H. and R. Wilbrandt (1981) *J. Photochem.* **17**, 37 (Abstr.).
55. Kalyanasundaram, K. and M. Grätzel (1980) *Helv. Chim. Acta.* **63**, 478–485.
56. Klein, R., I. Tatischeff, M. Bazin and R. Santus (1981) *J. Phys. Chem.* **85**, 670–677.
57. Knappe, W. R. and P. Hemmerich (1976) *Justus Liebigs Ann. Chem.* 2037–2057.
58. Kuzmin, V. A., D. S. Kliger and G. S. Hammond (1980) *Photochem. Photobiol.* **31**, 607–609.
59. Lambert, C., R. S. Sinclair and T. G. Truscott (1981) *Am. Soc. Photobiol.* (9th Ann. Meet.) 112 (Abstr.).
60. Land, E. J. and T. G. Truscott (1979) *Photochem. Photobiol.* **29**, 861–866.
61. Ledbetter, J. W., H. W. Askins and R. S. Hartman (1979) *J. Am. Chem. Soc.* **101**, 4284–4289.
62. Ledbetter, J. W., J. M. Hanckel and T. J. Cornish (1981) *Photochem. Photobiol.* **34**, 115–118.
63. Lindig, B. A. and M. A. J. Rodgers (1981) *Photochem. Photobiol.* **33**, 627–634.
64. Lindqvist, L., F. Tfibel, M. P. Fontaine, B. Alpert and J. C. Andre (1981) *J. Photochem.* **17**, 37 (Abstr.).
65. Loach, P. A., J. L. Y. Kong and K. G. Spears (1981) *Am. Soc. Photobiol.* (9th Ann. Meet.) 59–60 (Abstr.).
66. Matthews, J. L., S. E. Braslavsky and P. Camilleri (1980) *Photochem. Photobiol.* **32**, 733–738.
67. Mialocq, J. C., J. Sutton and P. Goujon (1980) *J. Chem. Phys.* **72**, 6338–6345.
68. Mialocq, J. C., E. Amouyal, A. Bernas and D. Grand (1981) *J. Photochem.* **17**, 132 (Abstr.).
69. Momzikoff, A. and R. Santus (1981) *C. R. Acad. Sci. Paris* **293**, 15–18.
70. Moore, A. L., G. Dirks, D. Gust and T. A. Moore (1980) *Photochem. Photobiol.* **32**, 691–695.
71. Morine, G. H. and R. R. Kuntz (1981) *Photochem. Photobiol.* **31**, 1–5.
72. Müller, F. (1981) *Photochem. Photobiol.* **34**, 753–759.

73. Netzel, T. L., I. Fujitta, C.-B. Wang and J. Fajer (1980) *3rd Int. Conf. on Photochemical Conversion and Storage of Solar Energy*, August 1980, 53–54.
74. Netzel, T. L., M. A. Bergkamp, C.-K. Chang and J. Dalton (1981) *J. Photochem.* **17**, 451–460.
75. Novak, M., A. Miller, T. C. Bruice and G. Tollin (1980) *J. Am. Chem. Soc.* **102**, 1465–1467.
76. Overfield, R. E., K. J. Kaufmann and M. R. Wasielewski (1980) *3rd Int. Conf. on Photochemical Conversion and Storage of Solar Energy*, August 1980, 41–43.
77. Parkhurst, L. J. (1981) *J. Photochem.* **17**, 36 (Abstr.).
78. Parsons, B. J. (1980) *Photochem. Photobiol.* **32**, 813–821.
79. Pellin, M. J., M. R. Wasielewski and K. J. Kaufmann (1980) *J. Am. Chem. Soc.* **102**, 1868–1873.
80. Peters, G. and M. A. J. Rodgers (1980) *Biochem. Biophys. Res. Commun.* **96**, 770–776.
81. Peters, G. and M. A. J. Rodgers (1981) *Biochim. Biophys. Acta* **637**, 43–52.
82. Pileni, M.-P., A. M. Braun and M. Grätzel (1980) *Photochem. Photobiol.* **31**, 423–427.
83. Porter, G., C. J. Tredwell, C. F. W. Searle and J. Barber (1978) *Biochim. Biophys. Acta* **501**, 232–245.
84. Prütz, W. A., J. Butler, E. J. Land and A. J. Swallow (1980) *Biochem. Biophys. Res. Commun.* **96**, 408–414.
85. Prütz, W. A., E. J. Land and R. W. Sloper (1981) *J. Chem. Soc. Faraday I* **77**, 281–292.
86. Pyault, C., D. Gerard and G. Laustriat (1981) *J. Photochem.* **17**, 35 (Abstr.).
87. Razi Naqvi, K. (1980) *Photochem. Photobiol.* **31**, 523–524.
88. Reynolds, A. H., S. D. Rand and P. M. Rentzepis (1981) *Proc. Natl. Acad. Sci. USA* **78**, 2292–2296.
89. Rodgers, M. A. J. and A. L. Bates (1980) *Photochem. Photobiol.* **31**, 533–537.
90. Ronfard-Haret, J. C., D. Averbeck, R. V. Bensasson, E. Bisagni and E. J. Land (1982) *Photochem. Photobiol.* **35**, 479–489.
91. Salet, C., R. Bensasson and R. S. Becker (1979) *Photochem. Photobiol.* **30**, 325–329.
92. Salet, C., T. M. De Sa E Melo, R. V. Bensasson and E. J. Land (1980) *Biochim. Biophys. Acta* **607**, 379–383.
93. Salet, C., E. J. Land and R. Santus (1981) *Photochem. Photobiol.* **33**, 753–755.
94. Sinclair, R. S., D. Tait and T. G. Truscott (1980) *J. Chem. Soc. Faraday I* **76**, 417–425.
95. Sloper, R. W. and E. J. Land (1980) *Photochem. Photobiol.* **32**, 687–689.
96. Sloper, R. W. and T. G. Truscott (1980) *Photochem. Photobiol.* **31**, 445–450.
97. Spiro, T. G. and J. Terner (1981) *J. Photochem.* **17**, 65 (Abstr.).
98. Stanford, M. A. and B. M. Hoffman (1981) *J. Am. Chem. Soc.* **103**, 4104–4113.
99. Strong, J. D., J. M. Burke, P. Daly, P. Wright and T. G. Spiro (1980) *J. Am. Chem. Soc.* **102**, 5815–5819.
100. Straub, K. D., P. M. Rentzepis and D. Huppert (1981) *J. Photochem.* **17**, 419–425.
101. Taft, R. W. (1953) *J. Am. Chem. Soc.* **75**, 4231–4238.
102. Tagaya, H., T. Aruga, O. Ito and M. Matsuda (1981) *J. Am. Chem. Soc.* **103**, 5484–5489.
103. Terner, J. T. G. Spiro, M. Nagumo, M. F. Nicol and M. A. El-Sayed (1980) *J. Am. Chem. Soc.* **102**, 3238–3239.
104. Traber, R., H. E. A. Kramer, W. R. Knappe and P. Hemmerich (1981) *Photochem. Photobiol.* **33**, 807–814.
105. Traber, R., E. Vogelmann, S. Schreiner, T. Werner and H. E. A. Kramer (1981) *Photochem. Photobiol.* **33**, 41–48.
106. Vermaas, W. F. J. and Govindjee (1981) *Photochem. Photobiol.* **34**, 775–793.
107. Wilbrandt, R. and N.-H. Jensen (1981) *J. Photochem.* **17**, 64 (Abstr.).
108. Wilbrandt, R. and N.-H. Jensen (1981) *J. Am. Chem. Soc.* **103**, 1036–1041.
109. Wilkinson, F. and W.-T. Ho (1978) *Spectrosc. Lett.* **11**, 455–463.
110. Wong, D., F. Pellegrino, R. R. Alfano and B. A. Zilinskas (1981) *Photochem. Photobiol.* **33**, 651–662.