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COLLEGE ON BIOPHYSICS: FROM MOLECULAR GENETICS TO STRUCTURAL BIOLOGY (1 - 12 October 2001)

POSTER PRESENTATION LIST

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Hepatitis B Surface Antigen Aggregates: Their Molecular Pattern and Subcellular Localization in the Transformed *Pichia Pastoris* Cells

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Microdiversity of Leptospirillum Ferrooxidans Isolates Recovered from Uranium Wastes and their Interaction with U(VI)

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To What Degree the Aminoacid Content Determines the Structural Class of Protein

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NMR identification of native like residual structures in HIV-I protease tethered heterodimer in 6 M guanidine hydrochloride

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Understanding protein folding requires complete characterization of all the states of the protein present along the folding pathways. For this purpose NMR has a significant advantage over other techniques because of the great detail it can unravel with regard to 'structure' and dynamics of proteins. We have obtained complete backbone resonance assignment of a HIV-1 protease tethered dimer mutant in the 'unfolded state' using a combination of two new triple resonance 3D experiments, HNN and HN(C)N and other standard triple resonance experiments, namely, HNCA, HN(CO)CA, CBCANH and CBCA(CO)NH. The new experiments use the good ^{15}N resonance dispersion present in unfolded proteins. Analyses of chemical shifts revealed the presence of three stretches of conserved native like β sheet structures. Unusually shifted ^{15}N and amide proton chemical shifts of residues adjacent to some prolines and tryptophans also indicate presence of some structural clements in HIV1-TD. These results are important from the point of view of understanding protein folding mechanism.

Title of the poster

STRUCTURE-ACTIVITY RELATIONSHIP SUDY OF SOME ANTI-HIV NUCLEOSIDE ANALOGS AND ANTIBACTERIAL FLUOROQUINOLONE DERIVATIVES: A MOLECULAR ELECTROSTATIC POTENTIAL MAPPING STUDY.

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COLLEGE ON BIOPHYSICS: FROM MOLECULAR

GENETICS TO STRUCTURAL BIOLOGY (1 - 12 October 2001)

Effect of additives on the structure and hydration of proteins.

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Sugars and polyols are often used to enhance the stability of proteins. In addition to its fundamental importance, the stabilisation process has received considerable attention in recent years on account of its practical utility in terms of preservation, practically in relation to food industry. Thermodynamic and physico-chemical studies indicated that the stabilising effect of these compounds is achieved by the modulation of the solvent structure around the protein molecule. It is generally believed that the stabilisation of the protein conformation is achieved not by the specific binding of the additives, but by their preferential exclusion from the protein surface and the consequent preferential hydration of the protein.

In order to elucidate the effect of the stabilising additives on the structure of protein and the associated ordered water molecules in the hydration shell, the crystal structures of tetragonal lysozyme grown in the presence of sucrose, sorbitol and trehalose have been refined. Also refined are the structures of orthorhombic and monoclinic lysozyme grown under conditions in which tetragonal lysozyme is normally grown.

A comparison of the two sets of structures with the structure of native tetragonal lysozyme reveals that the effect of the additives on the structure of the protein molecule is less than that of the normal minor changes associated with differences in molecular packing. The additives do not even affect the level of hydration as indicated by the numbers of ordered water molecules associated to the protein. Still more surprisingly, they do not cause any significant reorganisation of water molecules in the hydration shell. Thus it appears that the cause of the stabilising effect of the additives need to be sought outside the immediate neighbourhood of the protein molecule. Among the three additives, only one interacts with the protein in a coherent manner. A sucrose molecule binds at the binding groove of the enzyme molecule.

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TITLE:
EFFECT OF HYDROCORTISONE ON THE INHIBITORY ACTION OF PURINES AT THE NEUROMUSCULAR JUNCTION

ABSTRACT;
The effect of hydrocortisone on the presynaptic action of purines was studied at the neuromuscular junction of the frog under two-electrode voltage clamp conditions. Daily administration of hydrocortisone (100 mg/kg into lymphatic system) increased initially and depressed later the amplitude of multiquantal end-plate currents evoked by motor nerve stimulation. Iniatial facilitatory phase of hormone action was accompanied by removal of presynaptic action of ATP. On the later phase (in 2 weeks of hydrocortisone administration) the inhibitory action of ATP was restored once again. Counteraction of ATP effect was reproduced under superfusion of the isolated muscle by physiological solution containing hydrocortisone, indicating non-genomic action of hormone onto presynaptic P2 receptor. Independently of acute or chronic administration of hydrocortisone the presynaptic action of another purine adenosine was preserved. We suggest that prevention of inhibitory action of ATP might be one component of facilitatory acute stress reaction while such inhibitory feedback action is missing under chronic stress process.

Thermodynamic Database for Proteins and Mutants (ProTherm): Features and Applications for Predicting Protein Mutant Stability

M. Michael Gromiha, J. An, H. Uedaira, S. Selvaraj, P. Prabakaran, H. Kono[1], M. Oobatake[2] and Akinori Sarai

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Thermodynamic database for proteins are important for understanding the mechanism of protein folding and stability. We have developed an electronically accessible database, ProTherm [1,2] which includes more than 9000 data for several thermodynamic parameters, experimental details, structural, functional and literature information. A WWW interface enables users to search data based on various conditions with different sorting options for outputs and links with other sequence, structural, functional and literature databases. The mutation sites and surrounding residues are automatically mapped on the structure and can be directly viewed through 3DinSight developed in our laboratory. The ProTherm is freely available at http://www.rtc.riken.go.jp/jouhou/protherm/protherm.html.

Using the database, we have analyzed the important factors influencing the stability of proteins upon buried, partially buried and surface mutations [3]. A simple model has been proposed to predict the stability of protein mutants and observed a good agreement between experimental and computed stabilities, which will be useful for protein engineering experiments.

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DENATURATION STUDY OF HUMAN PLASMA LDL: EPR AND FLUORESCENCE SPECTROSCOPY

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Human plasma low density lipoproteins (LDL) are supramolecular lipid – protein assemblies stabilized by noncovalent interactions (Hevonoja et al. 2000). Since crystallographic methods have not determined the complete structure of these complexes, numerous spectroscopic methods are used to elucidate their structure. The role of cystein residues in the apolipoprotein B100 (on the LDL particle) structure and particularly their involvement in correct folding and assembly of lipoprotein particle are essential in the structure - function relationship of these complexes (Tran et al. 1998). The aim of this study was to determine the accessibility of free cysteins to different agents, their role in the domain structure determination and conservation in the protein family.
The stability of LDL was probed using denaturants with different capability to influence the conformational stability of apoB. The following denaturing agents have been used: urea, guanidine hydrochloride, sodium dodecyl sulphate and dithiothreitol. The accessible free thiol groups were followed as a function of the concentration of the denaturant and the time of denaturation using biochemical assay (Ellman 1959).
EPR spectra of the selective thiol spin labeled LDL were measured (Kveder et al. 2000).
Native tryptophan fluorescence, fluorescence quenching (with iodide and cesium ions) and anisotropy measurements were performed on native and denatured LDL.
Fluorescence spectroscopy together with the results of the EPR study reflect the domain structure of the apoB and are related to the proposed 3D structural model of the LDL particle (Chauhan et al. 1998, Orlova et al. 1999).

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COLLEGE ON BIOPHYSICS:
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(1 - 12 October 2001)

Structural basis of the differential pore-forming activity in membranes of the isoforms of Sticholysin from *Stichodactyla helianthus*.

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ABSTRACT

Sticholysins I and II are two highly hemolytic polypeptides purified from the Caribbean Sea anemone *Stichodactyla helianthus*. Their high sequence homology (93%) indicates that they correspond to isoforms of the same hemolysin. The spectroscopic measurements show a close similarity in the secondary structure content, conformation and stability of both toxins. Exposure of the toxins to high pHs (> 11), a free radical source (AAPH), urea or temperature produce permanent changes in the toxin that lead to a significant loss of HA. It is significant to note that this loss of hemolytic

activity occurs when other indicators, probably with the only exception of near-UV CD spectra, barely detect changes in the protein structure. This emphasizes the sensitivity of the protein function to changes in the macromolecule conformation. The most noticeable difference between both toxins is the considerably higher activity of St II, both measured in terms of erythrocyte internal K^+ exit or hemolysis; which is related to enthalpic factors. This difference is not due to an incomplete association of St I to the membrane. We consider then that the different pore forming capacity of both toxins in erythrocytes can be explained in terms of the difference in charge of the N-terminal fragment, than can considerably reduce the St I insertion rate in the membrane probably due to the negatively charged outer leaflet of the red blood cell, without a significant reduction of its capacity to bind to the cell membrane. This electrostatic effect, together with a slightly more relaxed structure in St II, could explain the higher pore forming capacity of St II in the red blood cell membrane.

Thermal denaturation of barnase and binase complexes with Asp35Ala, Asp39Ala and Glu76Ala barstars: dependence of RNase melting temperature on free energy of complex formation

Vladimir A. Mitkevich, Y.S. Ermoluk, A.A. Schulga, V.M. Lobachov, C.N. Pace, G.I. Yakovlev, M.P. Kirpichnikov, A.A. Makarov (Moscow & College Station)

Abstract:

We have studed the effect of restriction of conformational mobility of particular residues in the RNase active center as well as of their complete immobilization on the heat stability of the protein globule. Thermodynamics of melting of the binase and barnase complexes with their natural inhibitor barstar and its single mutants in which the most important residues Asp35, Asp39, and Glu76 interacting with positively charged residues of the RNase active center are replaced with Ala. It was shown that the mutations do not cause structural alterations in the protein. Denaturation temperatures of the mutants are much higher than that of barstar. Mutations in barstar lead to an increase in the dissociation constant of its complex with RNases up to five orders of magnitude in the series: barstar A (Cys40,82Ala barstar), E76A, D35A, and D39A mutants. Melting of complexes consists of two transitions, the first of which indicates melting of the enzyme after complex dissociation, whereas the other corresponds to melting of free inhibitor. Linear relationship was found between melting temperature of complexes and logarithm of their dissociation constants: better binding results in increased melting temperature. Melting of the RNase weakened complexes with mutant barstars follows a different scheme that with barstar. It was shown that barstar achieves strong binding to barnase at the expense of its own stability.

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OCTYL GLUCOSIDE INDUCED FORMATION OF THE MOLTEN GLOBULE-LIKE STATE OF GLUTAMATE DEHYDROGENASE

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Abstract:

Interaction between n-octyl-β-D-glucopyranoside (octyl glucoside) and bovine liver glutamate dehydrogenase (GDH) was studied by using techniques such as equilibrium dialysis, UV-spectrophotometry, circular dichroism (CD), fluorescence energy transfer, extrinsic fluorescence and differential scanning calorimetry (DSC) in 50 mM sodium phosphate buffer solution (pH 7.6). The Equilibrium dialysis experiment shows a higher binding of octyl glucoside to GDH that induces the enzyme inhibition up to 80% in 20 mM octyl glucoside solution. CD study indicates that whereas some of GDH tertiary structure has lost as the result of the interaction between octyl glucoside and GDH, but it has acquired some secondary structure. The Measurement of binding of hydrophobic fluorescent probe, 1-anilino-naphthalene-8-sulfonate (ANS), to GDH reveals that the binding of ANS to GDH is increased in the presence of octyl glucoside, and this finding may be interpreted in the terms of increment of surface hydrophobic patch(es) of GDH because of its binding to octyl glucoside. Fluorescence energy transfer studies also mark more binding of the reduced coenzyme (NADH) to GDH and also the Lineweaver-Burk plots (with respect to NADH) indicate the existence of substrate inhibition in the presence of octyl glucoside. DSC studies also suggest increment of GDH flexibility as well as acquiring some structure due to interaction with cited detergent. These observations are aimed to explain the formation of the molten globule-like structure of GDH, which is induced by a non-ionic detergent like octyl glucoside.

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DNA STRUCTURAL TRANSITIONS INDUCED AT LOW pH

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Binding of H^+ to DNA, leads reversibly, to alternative DNA structures, especially to disrupted AT-basepairs and to Hoogsteen syn dG:dCH+ base pairs, which exist under physiological conditions in purified and chromosomal DNA. Besides, irreversible changes occur in DNA, when at low pH, upon further protonation, the disruption of not only AT- but also of protonated Hoogsteen GC-base pairs takes place. In a Hoogsteen base pair, protonation of cytosine is accompanied by a conformational change of the guanine from the C2'-endo-anti to C2'-endo-syn. This enables hydrogen bonding between N(7) of guanine and cytosine N(3). Studies are required to elucidate whether the equilibrium between protonated and non-protonated GC-base pairs can be influenced by chemical parameters.

In this work, a confocal Raman microspectrometer was used to investigate the influence of low concentrations of magnesium ions on low pH-induced DNA structural changes. Based on the observation that the midpoint of transition of Watson-Crick GC-base pairs to protonated GC-base pairs lies around pH 3 (analyzing the 681 cm^{-1} line), measurements were carried out on calf-thymus DNA at neutral pH and at pH 3. Effects of low concentrations of magnesium ions upon protonation mechanism of opening AT- and changing the protonation of GC-base pairs in DNA are discussed.

Our Raman spectra show that low concentrations of magnesium ions, protect DNA against protonation of cytosine (line at 1262 cm^{-1}). Low salt concentrations do not protect adenine and guanine N(7) against binding of H^+ (characteristic lines at 1304 cm^{-1} and at 1488 cm^{-1}, respectively). The possible presence of Hoogsteen basepairs in DNA samples is considered.

This work was supported in part by a CEC grant under contract nr. ERB-CIPA-CT-92-2223.

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OVEREXPRESSION, PURIFICATION *in vitro* REFOLDING AND SELF-ASSEMBLY OF THE 11S GLOBULIN FROM AMARANTH SEED IN *Escherichia coli*

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Amaranth (*Amaranthus hypochondriacus*) has been identified as a nutraceutical food. The 11S storage globulin from amaranth seed called amarantin, identified in our laboratory, has a high nutritional-functional value with an outstanding content of essential amino acids. The expression of its cDNA in a heterologous system would allow for the rapid production of large quantities of homogeneous protein, permitting to carry out its molecular-functional characterization with the objective of redesigning amarantin by protein engineering. Here, we report the expression of amarantin cDNA in *E. coli* as well as its immunodetection, purification, *in vitro* refolding and self-assembly. Thus, expression *E. coli* strain BL21 (DE-3) transformed with vector pT7-AMAR, which contains amarantin cDNA without signal peptide, was grown on LB broth treated with carbenicillin; protein expression was induced with 0.4 mM IPTG. A good level of expression of recombinant amarantin with a molecular weight of 59 kDa was obtained. Then the expressed protein was purified using anion-exchange chromatography and reversed phase HPLC; it was also refolded *in vitro* by two methods: dialysis and rapid dilution, whose results were identical. The self-assembly assays of transgenic amarantin, which were carried out by gel filtration chromatography and sucrose density gradient ultracentrifugation, showed that it mostly self-assembled into 7S trimers with molecular weight around 180 kDa. Finally, both transgenic and native amarantins exhibited similar electrophoretic, immunochemical and surface hydrophobicity properties.

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Zia Azam Rana (Faisalabad, Pakistan)

Development of polyclonal antibodies from the invitro expressed AV1 (Coat Protein) and AC1 (Replication associated) protein of CLCuV:

ABSTRACT
Cotton leaf curl virus is an important causative agent of cotton curl disease. It caused serious losses to cotton crop (*G. hirsutum*) a backbone of economy of Pakistan.
Plant viruses identification using antibodies are important diagnostic tool but they are limited because of no availability of antisera. The present studies were carried out to develop polyclonal antibodies against the coat (AV1) and replication associated (AC1) of cotton leaf curl virus.
Antisera are usually raised by injecting purified virus particles in laboratory animals but regarding Gemini viruses the major limitations are the low concentration of virus particles in the plants and their fragility after purification. To tackle this situation the coat (AV1) and replication associated (AC1) genes of cotton leaf curl virus were cloned in the bacterial expression vector pET 32a for over expression of their proteins. Purified proteins were then injected intramuscularly into female albino rabbits and animals after screening were bled to collect antiserum. The antibodies raised against AV1 were of good titer and detected the virus particles in infected plants. The antibodies produced can be used for various immunological tests like ELISA, western blotting and immuno-gold labeling for the detection of cotton leaf curl virus in infected plants and particularly the identification of host plant species for Gemini viruses. The present studies will pave the way to investigate the virus movement in plant cells and have a better understanding of systematic infections and long distance movement of viruses in plants.

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How interactions between orbitals contribute to molecular properties

Abstract: The contribution of stereoelectronic interactions to several molecular properties has been examined using *ab initio* calculations and Natural Bond Orbital (NBO) analysis. For this task, a method based on the NBO formalism has been devised: a NBO localization was performed, followed by the deletion of selected off-diagonal Fock matrix ($\hat{F}$) elements written in the NBO basis, $\langle \sigma_m | \hat{F} | \sigma_n^* \rangle$ and $\langle \sigma_n^* | \hat{F} | \sigma_m \rangle$ representative of the interaction between selected NBOs σ_m and σ_n^*. The density matrix was then recalculated in the AO basis using the modified Fock matrix. With this modified density matrix molecular properties are recalculated and compared with the values obtained without deletions. The method was applied to study several model properties, like atomic charges, NMR shielding tensors and coupling constants.

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Hepatitis B surface antigen aggregates: their molecular pattern and subcellular localization in the transformed *Pichia pastoris* cells

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Abstract

Intensive work on the physico-chemical characterization of hepatitis B surface antigen (HBsAg) has provided a new perception of this complex structure. The individuality of HBsAg that distinguishes it from other proteins as a class resides in its supramolecular architecture. Indeed, HBsAg is a multi-component structure comprised of three proximally-identical envelope proteins (S, M and L) and host lipids. All these components are highly integrated to provide the biological function of HBsAg as a whole, as well as to make it extremely resistant to proteolytic degradation. In the gene-transformed *Pichia pastoris* cells, HBsAg is formed by expression of single, non-glycosilated S protein which multiplies, forms oligomers and self-assembles into lipid-containing particles. In this work, the appearance of the synthesized S protein in the transformed *P. pastoris* cells was detectable by immunoelectron microscopy since 25 h of fermentation, whereas completely assembled HBsAg particles were visualized by transmission electron microscopy after 60 h of fermentation. HBsAg particles were tightly associated to cellular membranes and transported within the cytoplasm in small vesicles. Upon high expression levels, the particles were delivered to vacuoles, probably for degradation. However, given the high resistance to proteolytic degradation, HBsAg particles accumulated in vacuoles undergoing aggregation. The large multi-particle clusters were visualized in a limited population of the highly vacuolized cells taken at final hours of the fermentation process (>100 h). These aggregates were mainly detectable within the central vacuole and in the periplasmic space of the cell. Hence, the observed intracellular aggregation of HBsAg may be considered as a consequence of its accumulation in vacuoles. MDA levels, an indicator of lipid peroxidation, measured in the yeast supernatants at 110 h of fermentation (2.98 ± 0.2 nM/mg protein) were significantly higher than in the cell samples taken at 100 h of fermentation (2.03 ± 0.3 nM/mg protein). On the other hand, HBsAg aggregates purified from the yeast extract by size exclusion chromatography exhibited lower phospholipid content, as compared to the non-aggregated material, supporting the involvement of HBsAg lipids in the aggregation phenomenon. In conclusion, the intracellular aggregation of HBsAg, confirmed here by electron microscopy, is considered as a consequence of accumulation and adaptation of wholly-assembled particles to the aging cellular environment, being lipid peroxidation a critical mediator of this conversion.

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MICRODIVERSITY OF *LEPTOSPIRILLUM FERROOXIDANS* ISOLATES RECOVERED FROM URANIUM WASTES AND THEIR INTERACTION WITH U(VI)

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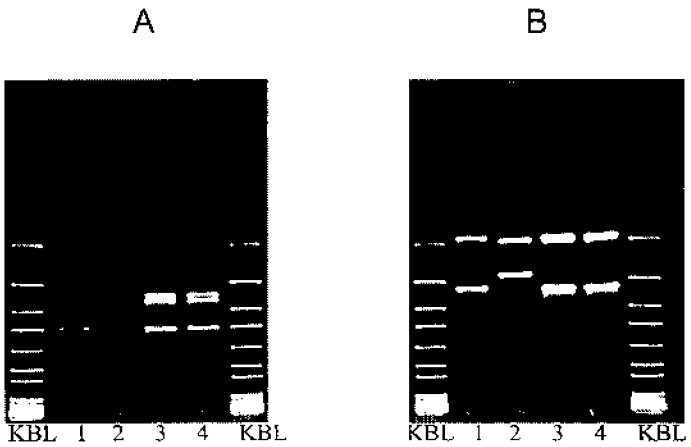
A large number of acidophilic chemolithoautotrophic iron oxidizing bacterial isolates recovered from two uranium mining waste piles was classified as *Leptospirillum ferrooxidans*. For this purpose a combination of classical and molecular microbiological methods was applied. The letter involved Amplified Ribosomal DNA Restriction Endonucleases analysis (ARDREA) and 16S rDNA sequence analysis. In the ARDREA two sets of primers were involved: one universal -$16S_{7f}$ and $16S_{1406r}$, and one species specific -$16S_{176f}$ and $16S_{679r}$ (De-Wulf-Durand *et al*,.1997, Tzvetkova *et al.*, 1999). In both cases using endonuclease *Rsa*I, two groups were discriminated among the isolates studied. These groups correspond to the recently characterized *Rsa*I RFLP types I and II of *L. ferrooxidans*, which include the type strain of the species *L. ferrooxidans* DSM 2705^T (type I) and the strains *L. ferrooxidans* DSM 2391 and Lf30A (type II); (Fig.1 and Tzvetkova *et. al.*, 1999).

An additional extensive comparative sequence analysis of the variable region 3 of a series of natural isolates and the deposited sequences of this region in the EMBL allowed to distinguish seven *L. ferrooxidans* groups. Each group possesses a specific signature in the helix 18 of the variable region 3 (Fig.2). These signatures discriminate particular types of the species and possibly reflect the genetic adaptations of different parts of the natural *L. ferrooxidans* populations to different concentrations of heavy metals and other components of their natural environment. The variability of the helix 18 influences target sites of several frequently cutting endonucleases and allows discrimination of some of the above mentioned groups.

The interaction of some *L. ferrooxidans* natural isolates with uranium was studied at different concentrations of the metal and at two pH values. Results demonstrating

accumulation of U(VI) at unusually low pH values are presented and discussed in the poster



1. *L. ferrooxidans* B1-K4
2. *L. ferrooxidans* B1-K3
3. *L. ferrooxidans* JG-3
4. *L. ferrooxidans* JG-24

Fig. 1. ARDREA- of several natural *L. ferrooxidans* isolates

A) 16S *Rsa*I –ARDREA with universal primers 7f and 1406r.

B) 16S *Rsa*I –ARDREA with *L. ferrooxidans* species specific primers 176f and 679r.

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                                    ⇩                                   ⇩
E.coli positions                    452                                 486
L.f.Z2[DSM2705^T](M79441)           AAAAGGGATATCGAA A-AA    C--NGAT-GACGGTA  GroupI
L.f str.L15(X86776)                 AA AGGGATATCGAA A-AA    C--CGAT-GACGGTA

L.sp. isol. LfLa(M79384)            AAAGGCGCGTCAGAA A G-    -TTCGAT-GACGGTA  GroupII
TzTL.f B1-K4                        AAA GCGCGTCAGAA A G-    -TTCGAT-GACGGTA
TzTL.f JG-3                         AAA GGGCGTCAGAA A G-    -TTCGAT-GACGGTA
TzTL.f JG-24                        AAA GCGCGTCAGAA A G-    -TTCGAT-GACGGTA

TzTL.f JG-7                         AAA GGGCGTCAGAA A G-  A C--CGAT-GACGGTA  GroupIII
TzTL.f B1-K3                        AAA GGGCGTCAGAA A G-  A C--CGAT-GACGGTA

Unind. Cl. OS7 (X86773)             AA GC    GAA AAG  - C--CGAT-GACGGTA  GroupIV

Unind. Cl. OS17 (X86772)            AAA GGAT TCAGAA A G - -AT-TTCGAT-GACGGTA  GroupV
L.f. DSM2391 (AJ237903)             AAA GGAT TCAGAA A G - -AT-TTCGAT-GACGGTA
L.f is.EPA15Clpss38(X91230)         AAA GGAT TCAGAA A G - -AT-TTCGAT-GACGGTS

L.f is EPA15Clpss14(X91228)         AA GAGTGTCCGAA A GG-C-AG-TCCGAT-GACGGTA  GroupVI
L.f. isol. LA (AJ237902)            AA GAGTGTCCGAA A GG-C-AG-TCCGAT-GACGGTA
```

```
Uncnd. Cl. OS4 (X86770)        AA  GGGGGCCTGAA AAGGTC-A C--CGAT-GACGGTA  GroupVII
Lf. 30A (X72852)               AA  GCGGGCCTGAATAAGGTC-A C--CGAT-GACGGTA
```

Restriction endonuclease	Target site
Hae III	GGCC
Cfo I	[illegible]
EcoR V	GATATCGA
Taq I	TCGA
Ava II	[illegible]

Fig. 2. Variability in helix 18 (V3) and target sites for the frequently cutting enzymes *Hae*III, *Cfo*I, *EcoR*V, *Taq*I, *Ava*II.

References

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2. Tzvetkova, Tz. *et.al.,*1999. Recovery and characterization of *Leptospirillum ferrooxidans* in soil samples of two uranium mining waste piles. *FZR*-**285,** 58.

COLLEGE ON BIOPHYSICS:
FROM MOLECULAR GENETICS TO STRUCTURAL BIOLOGY
Trieste, 1 - 12 October 2001

To what degree the aminoacid content determines the structural class of protein.

VP Valuev

There is a notion that the majority of proteins can be split into four large structural classes, determined by predominance of the elements of secondary structure: alpha–helical, beta-structural, with alternating alpha-helices and beta-sheets and with separate alpha-helical and beta-structural domains. This notion follows both from structural and functional classifications (Murzin et al.,1995; Holm and Sander, 1996) and from the physical considerations (Chothia, 1984). It is also suggested, that the fold of a protein is determined greatly by its amino acid content (Shakhnovich and Gutin, 1993). We tried to distinguish the structural classes starting from their amino acid content, extending the previous works, and to establish the relation between the quality of discrimination and the structural properties of a given protein. The dataset we used was the representative set of proteins in SCOP classification, which shared less than 40% homology. This dataset was split into training and test sets. The recognition method was based on neural networks. The best performance reached on test set was about 70% sensitivity and 75% selectivity for α proteins, 75% sensitivity and 78% selectivity for β proteins, 76% sensitivity and 67% selectivity for α/β proteins, and the α+β class was recognised very poorly, and its merging with α/β class gave no improvement, which suggests that this class is somewhat artificial (in accordance with CATH classification). Linear methods we applied to the same task failed to yield good discrimination.