2998-Pos Board B45

Functionally Significant Collective Motions in Horseradish Peroxidase

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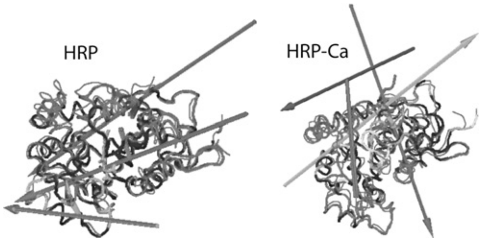
Horseradish peroxidase C is a class III peroxidase whose structure is stabilised by the presence of two endogenous calcium atoms. The effect of calcium depletion on protein conformational dynamics was investigated by performing 20-ns molecular dynamics simulations on explicitely solvated hrpc and Ca-depleted hrpc models. Cross-correlation analysis identified correlated motions that are perturbed in the absence of calcium.The trajectories were also analyzed using the essential dynamics method to describe the conformational space sampled by the respective models. Principal components analysis was used to decompose the covariance matrix extracted from the simulations and reconstruct the trajectories along the principal coordinates representative of functionally important collective motions. The results indicate that the motion of the native species is defined by a few preferred directions identified by the first four eigenvectors. The eigenvectors are significantly sampled, suggesting that, on average, large motions involving different subdomains of the protein occur. The analysis reveals that the calcium depletion affects the most important components of the hrpc motions and modify the overall dynamics in regions where functionally significant residues are located -notably in the heme pocket.



2999-Pos Board B46

Characterization of alpha/3_{10}-Helical Peptide Conformational Equilibria by ^{1}H NMR H/D Exchange Measurements

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Peptides composed exclusively of alpha,alpha-dialkyated amino acid residues, such as the alpha, alpha-dimethylglycine residue (aib), are known to adopt 3_{10} helices in solution. The 3_{10} helix is characterized by an i-to-i+3 intramolecular hydrogen-bonding pattern as opposed to the i-to-i+4 hydrogen-bonding pattern of the more familiar alpha-helix. Recent work in other laboratories has concluded that the alpha-helix may be the preferred helical structure for homooligomers of some alpha, alpha-dialkylated amino acid residues, such as alpha-methyl-alpha-ethylglycine, under certain conditions of solvent and C-terminal protection. In order to understand more deeply the helical preferences of short oligomers of aib, we have performed ^{1}H NMR-based hydrogen/deuterium exchange studies, using methanol solvent, to characterize the alpha/3_{10} helix equilibrium in a series of short oligomers (n = 4, 6) of aib residues. We have compared oligomers of aib C-terminated in tert-butyl ester and the tert-butyl ester of beta-alanine. In methanol solvent, the $\beta\epsilon\tau\alpha$-alanine-based C-protecting group appears to allow for a greater percentage of α-helix than the tert-butyl ester.

3000-Pos Board B47

Isotope-edited Infrared Spectroscopy of 3-10 Helical Peptides

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Infrared spectroscopy (IR) is a valuable tool for probing the conformation and dynamics of peptides. The amide I band is particularly sensitive to the strength and position of the hydrogen bonds that define secondary structure. Site-specific structural information can be obtained by sequential, systematic isotope labeling of the backbone (1). In this study, isotope-edited infrared spectroscopy was applied for the elucidation of residue level structural information of model 3-10 hexameric peptides. A series of peptides comprised of four aminoisobutyric acid (Aib) and two α-methyl valine residues were prepared to include two 13C labels at the N-terminus, middle and C-terminus positions (2). The IR spectra were measured in CDCl3 solution. Far-ultraviolet circular dichroism spectra in 2, 2, 2- trifluoroethanol confirmed 3-10 helical structure for all peptides. The spectral features of the 12C and 13C amide I depend on the position of the isotope label and the nature of the buffer. These differences are discussed in terms of the x-ray structure of the peptides and the details of the 3-10 helical conformation.

1. Decatur, S.M. (2006) Accounts of Chemical Research 39:169-175.
2. Maekawa, H.; Toniolo, C.; Broxterman, Q.B.; Ge, N.H. (2007) J. Phys. Chem B. 111, 3222-3235.

3001-Pos Board B48

Structural characterization of two peptides corresponding to Helix 4 of Apolipoprotein E

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Apolipoprotein E (ApoE) is a ligand for the low density lipoprotein (LDL) receptor. The amino-terminal domain of ApoE is a 4-helix bundle and helix 4 is involved in LDL receptor binding. This region associates with lipids and undergoes structural changes which allow a key arginine residue, Arg_{172} to participate in receptor binding. To characterize this region and to study lipid-induced conformational changes we developed 2 synthetic peptides: one corresponding to residues 128-164 (ApoE-164) and the other corresponding to residues 128-183 (ApoE-183). Secondary structure prediction indicates amphipathic alpha-helical structure for both peptides. Circular dichroism spectroscopy (CD) studies show that ApoE-164 exhibits concentration-dependent self association whereas ApoE-183 does not. To study the effect of lipids on the two peptides we used three detergents: 1,2-diheptanoyl-sn-glycero-3-phosphocholine (DHPC), dodecyl phosphocholine(DPC), and octyl-beta-d-glucopyranoside(BetaOG). CD shows that both peptides exhibit an increase in alpha-helix content at the critical micelle concentration (CMC) for all three detergents. The alpha-helix content for both peptides in the absence of detergent is about 30%. Upon the addition of DHPC, DPC or BetaOG there is a 30-40% increase in alpha-helicity at the CMC. This approach is directed towards understanding (i) how lipids induce a conformational change in the 165-183 region, and (ii) how the relative orientation of Arg_{172} is changed such that it contributes to LDL receptor binding.

3002-Pos Board B49

Prediction of Protein Loop Structures Using a Local Move Monte Carlo Approach and a Grid-Based Force Field

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We have developed an improved local move Monte Carlo loop sampling approach for loop predictions. The method generates loop conformations based on simple moves for the torsion angles of side chains and local moves for backbone of loops. To reduce the computational costs for energy evaluations, we developed a grid-based force field to represent the protein environment and solvation effect. Simulated annealing has been used to enhance the efficiency of the local move MC loop sampling and identify low-energy loop conformations. The prediction quality is evaluated on a set of protein loops with known crystal structure that has been previously used by others to test different loop prediction methods. The results show that this approach can reproduce the experimental results with the RMSD within 1.8 Å for all the test cases. The local move MC loop prediction approach developed here could be useful for improvement of the quality the loop regions in homology models, flexible protein-ligand and protein-protein docking studies.

3003-Pos Board B50

Specificity Of The Helical Conformation Induced By 2, 2, 2, Trifluoroethanol

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The specificity of helix-induction in the 15-mers of poly-L-glutamic acid and poly-L-lysine by 2,2,2-trifluoroethanol (TFE) was investigated by circular dichroism (CD), NMR, and FTIR at three pH values: 2, 7, and 13. TFE was observed to promote the induction of helical conformation in poly-L-glutamic acid at pH 2.0, and pH 7.0. Similarly, TFE induces helical conformation in poly-L-lysine at pH 7.0 and pH 13. At pH 7.0, the helical conformation was induced in both poly-L-glutamic acid and poly-L-lysine only at higher concentrations of TFE (> 70 % v/v). At lower concentrations of the fluorinated alcohol, very little or no effect was observed in the backbone conformation of the homopolypeptides. ^{1}H-^{15}N HSQC spectra (obtained at ^{15}N natural abundance, at pH 7.0) of the homopolypeptides showed that profound conformational changes occur in the backbone of the polypeptide chains in higher concentrations of TFE. Analysis of the 2D NMR data in conjunction with those obtained using Far UV CD and FTIR revealed that the helix conformation induced at higher concentrations of TFE is non-specific. Additionally, the role of the charged amino and carboxyl groups at the ends of the peptides were studied to determine if these groups affect helix induction by TFE. 15-mers of poly-L-glutamic acid and poly-L-lysine were synthesized from amide resins and their C-terminals were acetylated. The protected poly-L-glutamic acid and poly-L-lysine were titrated with TFE at pH 2, 7 and 13, and the secondary structures were determined using CD: no significant difference was found when compared to the non-protected homopolypeptides. The results obtained in this study clearly question the validity of structures of short peptides characterized in high concentrations of organic solvents.

3004-Pos Board B51

Kinetics of Film Formation of Poly-L-Proline at High Temperatures

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At room temperature and in aqueous solution, poly-L-proline predominantly adopts a relatively open left-handed 3_1-helix termed polyproline II (PPII). The corresponding far UV electronic circular dichroism (ECD) spectrum exhibits a couplet with a pronounced minimum at ~ 204 nm, and a much less intense maximum at ~ 228 nm. When poly-L-proline is incubated in a quartz cell for two hours at temperatures above 60°C, a gradual decay of the PPII signal is observed. It is replaced by a spectrum which displays a strong negative signal at ~220 nm (?), thus being indicative of a right handed conformation. This PPII signal decay is thought to be caused by the formation of a film on the inner surface of the cell. After removal of the poly-L-proline solution from the cell, the ECD was measured on both the liquid and cell individually. The ECD of the removed liquid portion resembles a typical PPII spectrum, while that of the cell results in the aforementioned spectrum, with a significant minimum at ~220 nm, and a minor minimum at ~204 nm. The rate of film formation depends strongly on the incubation temperature. Bi-exponential rise and decay modeled the disappearance of the PPII signal in aqueous solution and growth of the film, respectively. The respective relaxation constants were determined to be 10^{-3} s^{-1} and 10^{-4} s^{-1} for the PPII signal disappearance and film formation, respectively, at T= 65C ? (Laura?). The analysis of the kinetics measured at different temperatures between 60° and 75° C reveals a non- Arrhenius behavior. A further structural characterization of the obtained poly-L-proline film and a thermodynamic analysis are currently being carried out in our laboratory.

3005-Pos Board B52

Thermophilic protein structure adaptation examined with Burial Depth and Travel Depth

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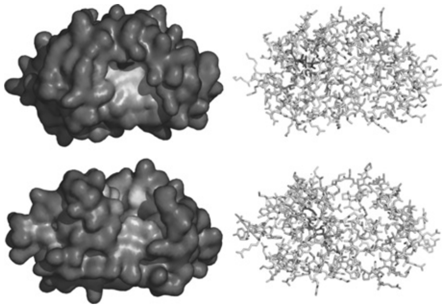
Organisms evolved at extreme temperatures (above 80° C) have constraints on their protein structures. These constraints result in differences in residue utilization and overall structure. By studying thermophile/mesophile pairs of homologous structures, we have examined these differences.

Geometric measures, specifically Burial Depth (distance from the molecular surface to each atom) and Travel Depth (distance from the convex hull to the molecular surface that avoids the protein interior), along with common metrics like packing are used to gain insight into the constraints experienced by thermophiles.

Our results show that extreme thermophiles show significant trends towards becoming more ''ball-like''. Their mean Travel Depth is less than their mesophilic counterparts, indicating smaller, less numerous and less deep pockets. Their mean Burial Depth is higher indicating that they bury more surface area and are more compact. This can be tracked on the individual residue level, for instance Alanine becomes more significantly buried under thermophilic conditions, and charged residues become less buried.



Shown is an example pair with the thermophile at top. At left are Travel Depth surfaces, at right Burial Depth. Note the fewer, smaller pockets and the deeper core in the thermophile.

3006-Pos Board B53

Small Conformational Changes Detected For Short Lived Transient Species During A Photoreaction

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he focus of the present study is firstly to investigate the origin of global conformational change during the photoreaction. Secondly, how such changes are transduced and spread to all over the protein molecule was investigated by studying the photoreaction of the site directed mutants. The diffusion of pG and pB of E46Q mutant showed the small difference, suggesting that the absence of negative charge at 46 position is responsible for the lack of partial unfolding in structure of PYP upon pB formation. The role of N-terminal region was further studied by replacing phenylalanine by alanine at position 6 (F6A). The diffusion constant of pB of F6A was not very different from that of pG, which is explained by a small conformational change in N-terminal tail upon pB formation. Previously, creation of a disulfide bond between position 6 (Phe6) and 121 (Phe121) showed the restricted movements of N-termius and less structural fluctuation upon pB formation. These results argued the more ordered and folded structure of pB of mutants lacking Phe6

3007-Pos Board B54

Probing the Dynamics and Conformations of Free and Ligand-bound Gamma-synuclein

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The synucleins are a family of natively unstructured, soluble proteins consisting of α-, β-, and γ -synuclein. They are primarily expressed in neurons, though they differ in their localization. Relatively little is known about the physiological roles of any of the synucleins, although the most studied of the group, α-synuclein, has been linked to Parkinson's disease. γ-synuclein was first identified in breast cancer cells, and was later found to be overly expressed in several other types of cancer, including ovarian cancer and retinoblastoma. Furthermore, subsequent studies found that γ-synuclein overexpression in breast carcinomas interferes with the activity of commonly used chemotherapeutic drugs, such as taxol and nocodazole. In the current study, we use a combination of single molecule Förster resonance energy transfer (FRET) and fluorescence correlation spectroscopy (FCS) to examine the conformations and dynamics of γ-synuclein alone and bound to potential ligands. Our findings will not only help understand the structural properties of a disordered protein, but will also lead to a better understanding of the mechanisms through which γ-synuclein alters the efficacy of antitumor drugs.

3008-Pos Board B55

Conformational Motion of Biological Macromolecules

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The beauty of biological macromolecules as a dancer in solution can be seen if we manage to understand graceful movements of the dancers. Biological macromolecules are dynamic machines so for proper functionality they need changing of shape, dancing through each other and non-contacting communicating to execute their ''understanding'' on distances. However there is no single theory to explain encountered experimental data and increase our understanding on the conformational motion. The problem is a suggestion of non-equilibrium nature of the macromolecules. Does it mean that non-equilibrium system like biological macromolecules can act as the dancer and can function properly? Does it mean that there is some rule that non-equilibrium macromolecules follow? Can the rule be generalized for any kind of non-equilibrium systems or not? My attempt to give a mathematical definition of the problems and point to their solution will be presented. Let consider bonded *n* point and correspond to the system some geometric figure with $V=n$ vertexes, virtual F,E faces and edges correspondingly. The conformational motion of the system is a changing the shape of the figure so we have two possibilities 1) the Euler Characteristic of the figure is changing in time as well as F,E changes; and 2) the Euler Characteristic of the figure is constant but F,E changes. Set of all possible {F,E} gives us the group with undefined operator f where f is operator which transfers elements of the set in each other $f{:}(F_i,E_j)\in\{F,E\}\rightarrow(F_g,E_k)$. The tusk could be solved if we manage to find the operator f. The value of the f can be immediately found by adding some physical conditions to the operator. The physical conditions and the equation of conformational motion will be presented at