2998-Pos Board B45

Functionally Significant Collective Motions in Horseradish Peroxidase

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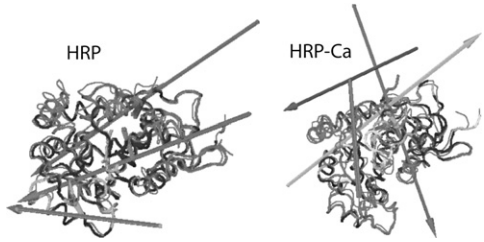
Horseradish peroxidase C is a class III peroxidase whose structure is stabilised by the presence of two endogenous calcium atoms. The effect of calcium depletion on protein conformational dynamics was investigated by performing 20-ns molecular dynamics simulations on explicitely solvated hrpc and Ca-depleted 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 βεταα-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