theory of conformational motion which gives possibility to be written time evolution equation of the entropy. Up together, taking into account previously presented equation of conformational motion and non-equilibrium entropy definition, solves hydrophobic-hydrophilic interactions problem. Preliminary calculations show excellent matching with experimental data. Complete picture of hydrophobic-hydrophilic interactions and time evolution equation of the entropy will be presented.

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In Silico Study of the Inhibition of Taq Polymerase by Fullerol C60(oh)20

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Polymerases are enzyme proteins catalyzing the polymerization of nucleic acids by the addition of nucleotides to a substrate ss-DNA (or RNA) against a template ss-DNA, making the correct Watson-Crick base pairs. *Taq*DNA polymerase (*taq*pol), a thermostable polymerase from the bacterium *thermus aquaticus*, is widely used in polymerase chain reactions (PCR) - a popular technique for the amplification of a DNA sample. Recent studies have reported that fullerol C60(OH)*n*, a water-soluabe fullerene derivative, can inhibit PCR [1, 2]. The experimental evidence suggests that fullerol inhibits PCR by interacting with the enzyme *taq* polymerase [2]. Since polymerases in all organisms use the same mechanism for the polymerization of nucleotides, fullerol may inhibit other polymerases and thus affect DNA duplication in cells.

We have used molecular dynamics (MD) and molecular docking to understand the mechanism of *taq* polymerase inhibition. The crystal structure of polymerizing domain (Klentaq) of the *taq* polymerase is obtained from [3]. Molecular docking is used to find the binding sites of fullerol on Klentaq (Autodock 4.2 [4]). MD simulation are started from the conformations obtained from docking studies. By a detailed comparison of the simulations of the protein in the presence of fullerol with simulations in the absence of fullerol, insight into the mechanism of inhibition is obtained.

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232-Pos

Electrostatic Contribution to the Transition States Binding Free Energy Using Simplified Coarse Grained Model

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Simplified coarse-grained (CG) models (of the type originally developed by Levitt and Warshel (1)) can provide an effective way of evaluating the free energies of explicit protein models (2). Here we use such a reference potential strategy in evaluating the transition state electrostatic binding free energies calculations, which are essential for the rational enzyme design. The method is examined and demonstrated in studies of the effects of mutation on the transition state binding free energies of the esterase catalytic substrates (*p*-nitrophenyl acetate, *1*-and *2*-naphthyl acetates) of human carbonic anhydrase II (hCAII) and dienelactone hydrolase (DLH). These have been computed using empirical valence bond (EVB) approach in MOLARIS program (3) with and without the use of the CG model as a reference potential. The calculated electrostatic contributions to the transition state binding free energies reproduce the experimental trends (4, 5). This indicates that the method should provide a powerful tool for exploring the esterase activities of hCAII and DLH for understanding the promiscuity of these enzymes.

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Hydration Analysis on Atp Hydrolysis by Microwave Dielectric Spectroscopy

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Recent theoretical works reveiled that the hydration energies of nucleotides and inporganic phosphates are one of important factors in the ATP hydrolysis energy. Here we have measured the hydration states of the reactants and the products of ATP hydrolysis reaction by precesion microwave dielectric relaxation spectroscopy. The complex dielectric spectra of hydrated solutes, such as sodium salts of ATP^{4-}, ADP^{3-}, HPO_4^{2-}, were measured. We detected constrained water and hyper-mobile water around nucleotides and phosphates. Hydration change upon the neutralization reaction of phosphate, $H_2PO_4^- + OH^- \diamond HPO_4^{2-} + H_2O$, was investigated by measuring high-resolution complex dielectric spectra of mono- and di-sodium phosphate solutions at 20°C to understand the hydration effect on the thermodynamics of phosphate buffer reaction. From each solution spectrum the dielectric spectrum of a spherical volume containing each hydrated solute in water was derived based on a suspension theory. Each spectrum was decomposed into a bulk water ($f_{cw} \sim 17$ GHz) component and two Debye dispersion components, assigned as constrained water ($f_{c2} \sim 6.4\text{GHz} < f_{cw}$) and hyper-mobile water ($f_{c1} \sim 19.5\text{GHz} > f_{cw}$), respectively. The dielectric dispersion of hyper-mobile water was about five times stronger than the constrained one. The strengths of these two Debye dispersions decreased by 20% (ΔN_2 7) for the constrained water number and by 10% (ΔN_1 15) for the hyper-mobile water number upon the neutralization reaction, while those decrements were compensated by an increase of dispersion strength of bulk water. It is thought that the entropy changes corresponding to the number increases of constrained water and hyper-mobile water molecules are negative and positive, respectively. So the present result provides us a physical explanation of the small effect of hydration change on the total entropy change upon the reaction.

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Thermodynamic Studies on the Cataract-Associated Mutant, E107a, of Human Gamma-D Crystallin: Molecular Basis for Cataract Formation

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The E107A mutation in human gammaD-crystallin (HGD) is associated with nuclear cataract. The concentration of crystallins in the lens is extremely high, approaching that within protein crystals. The composition in the lens nucleus, i.e. the central core, is such that the alpha- and gamma crystallin weight fractions are comparable. In earlier studies we showed that mutations in HGD dramatically compromised protein solubility, while maintaining the overall protein structure. In contrast, in the E107A mutation neither the structure (circular dichroism and tryptophan fluorescence emission) and stability (thermal denaturation), nor the solubility are affected significantly. However, as expected, the mutation raises the pI by ~1 pH unit, i.e. from 7.4 to 8.4. The increase in pI suggested a change in the interaction of E107A with alpha crystallin, which is negatively charged at neutral pH. To determine changes in such interaction, we compared the liquid-liquid phase boundaries in binary mixtures containing either HGD or E107A, and alpha crystallin. Our preliminary studies show that while the phase-separation temperatures of mixtures in the two cases (i.e. HGD+alpha and E107A+alpha) remain comparable, the nature of the paired compositions of the two phases in equilibrium are distinct. In particular, the tie-line slopes are altered in the direction predicted to correspond to increased alpha-gamma attraction, on the basis of molecular dynamics simulation and thermodynamic perturbation theory (1). Thus, it appears that increased attractive interactions between the E107A mutant of HGD and alpha crystallin could destabilize the crystallin mixture in the lens nucleus, and lead to increased light scattering and cataract.

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Urea Facilitates the Translocation of Single-Stranded DNA and RNA Through the α-Hemolysin Nanopore

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The staphylococcal α-hemolysin (αHL) protein nanopore is under investigation as a fast, cheap detector for nucleic acid analysis and sequencing. Although the discrimination of all four bases of DNA by the αHL pore has been demonstrated, the analysis of single-stranded DNAs and RNAs containing secondary structure mediated by base pairing is prevented because these nucleic acids cannot be translocated through the pore. Here, we show that a structured 95-nucleotide single-stranded DNA and its RNA equivalent are translocated through the αHL pore in the presence of 4 M urea, a concentration that denatures the secondary structure of the polynucleotides. The αHL pore is functional even in 7 M urea and therefore it is easily stable enough for the analysis of challenging DNA and RNA species.