HIGH DENSITY PACKING OF POLYPEPTIDE CHAINS BY COMPUTER CALCULATIONS

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Protein structures in single crystals show a remarkably high packing density of about 0.75, which corresponds to the maximal packing density of identical spheres. To study the restrictions imposed on the spatial structure by this high density condition, a distance geometry algorithm was used to pack polypeptide chains of 10-20 amino acid residues into spheres of predefined volume. The densely packed structures obtained in different computer runs for the same packing density were compared. The influence of the amino acid sequence on the maximal packing density was also investigated. In order to investigate the consequences of the use of simplified representations of the peptides, calculations were done for molecular structures where all the individual atoms were considered as well as for a simplified residual representation. The residual representation was applied to study the packing properties of possible nucleation cores in small globular proteins, i.e. regular secondary structures or hydrophobic domains.

THEORETICAL STUDY ON THE KINETICS OF PROTEIN FOLDING AND UNFOLDING BY COMPUTER SIMULATION. H. Taketomi, F. Kano and N. Gō, Computer Ctr., Kyushu University, Fac. of Liberal Arts, Showa University and Dept. of Physics, Fac. of Science, Kyushu University, Fukuoka 812 Japan.

The strongly idealized lattice model of proteins is a powerful tool for investigating not only the equilibrium aspects of protein folding and unfolding but also the kinetic ones. We generated the time process of the lattice proteins by computer simulation and analysed it by the method of time-correlation functions. By this simplified model analysis, we could investigate a) the prototype of the kinetic characteristics of proteins, b) the relations between the kinetic properties and the three types of interactions (long-range, short-range, hydrophobic), c) the effect of the amino acid substitutions on the kinetic behavior of proteins. The results are as follows; 1) the time-correlation functions are approximately described by two phases (fast mode and slow mode). 2) the slow mode reflects the overall folding and unfolding process. 3) the relaxation times of the fast mode are temperature insensitive, while the ones of the slow mode show a peak near the transition temperature. 4) Arrhenius plot (the temperature dependence of rate constants) shows linear dependence in the case without hydrophobic interactions. 5) the slight unfavorable substitutions of amino acids cause the transition slow.

POLYMERIZATION OF THE NINTH COMPONENT OF COMPLEMENT. J. Tschopp, H.J. Müller-Eberhard and E.R. Podack. Research Institute of Scripps Clinic, La Jolla, Calif. USA

The interaction of C8 and C9 with the membrane attack complex (MAC) of complement is the critical event that leads to membrane perturbation and cytolysis. Previous observations indicated that multiple C9 molecules are bound by a single C5b-8 complex. However, in free solution only one molecule of C9 can maximally bind to one molecule of C8 as evidenced by analytical ultracentrifugation. Incubation of isolated C9 with 0.6 M guanidine HCl, 0.1 M octylglucoside or 1.5% sodium desoxycholate at 37° resulted in the formation of C9 polymers with sedimentation coefficients ranging from 8S to 33S. As measured by light scattering, C9 polymerization was a slow, temperature sensitive reaction requiring 2 hr at 37° for completion. Below 20° no polymerization was observed. C9 polymerization was accompanied by increased binding of ANS, suggesting the exposure of hydrophobic sites on C9 polymers. Upon electron microscopic analysis, C9 polymers consisted of extended and often curved strands of varying length and 40 Å to 65 Å width. Some C9 polymers resembled the circular ultrastructural complement lesion on membranes. A structural model for C9 polymers will be proposed. It is postulated that C9 polymerization during MAC assembly may constitute the molecular basis for C5b-9 dimerization and C9 mediated cytolysis.

MONTE CARLO SIMULATION STUDY OF FLUCTUATIONS IN BPTI

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Small amplitude thermal fluctuations in a small protein, basic pancreatic trypsin inhibitor (BPTI) is studied from the point of view of the conformational energy. Only dihedral angles are treated as variables. In order to see shape of the energy surface near the energy minimim a second derivative matrix at the minimum point is calculated and diagonalized. The energy actually calculated is found to be approximated well by that calculated from the second derivative along most directions of eigenvectors. This fact implies that these eigenvectors are good collective dynamical variables (modes) for the description of the internal motion of proteins. In order to study the energy surface beyond the harmonic approximation the thermal equilibrium of this molecule is simulated by a Monte Carlo method. This simulation is methodologically new in two points; 1) the modes mentioned above are chosen as independent variables, 2) each step of simulation is taken anisotropic in the multidimensional conformational space in such a way that all modes are equilibrated in the same time range. Because of these properties this method of simulation is powerful to attain rapid thermal equilibration. In records of this simulation new types of thermal fluctuations of unharmonic nature are observed.

A MONTE CARLO STUDY OF AN EQUILIBRIUM FOLDING PROCESS IN AN IDEALIZED MODEL OF BOVINE TRYPSIN INHIBITOR. S. Miyazawa and R. L. Jernigan, Laboratory of Theoretical Biology, DCBD, N. C. I., N. I. H., Bethesda, MD 20205, USA

The protein is regarded as a self-avoiding chain which consists only of C^{α} and C^{β} atoms. The backbone dihedral angles are permitted to take discrete values at every 10 degrees. Intra-residue interactions consist of one term taken from the statistical distributions of (ϕ , ψ) observed in 20 proteins and another term to favor the native conformation of each residue. Inter-residue interactions are simplified by assuming there is an attractive energy only between close contacts in the native structure. Each conformation generated randomly with a strong bias toward the native conformation is classified according to its conformational energy. Estimated conformational entropies show characteristics of a two-state transition which can be attributed to long range interactions. From the order of appearance of contact regions while decreasing the conformational energy, folding pathways are proposed. Nuclei which appear at a beta turn and in the alpha helix grow to form the native pair of interacting beta strands and the alpha helix. The formation of the hydrogen bonded beta strand pair appears to represent the state of highest free energy on the pathway. Following the appearance of this beta sheet, native contacts appear either toward the amino or carboxyl terminus; these results indicate the presence of alternative folding pathways.

INTERMEDIATES IN THE FOLDING OF RIBONUCLEASE A. A.L.Fink, R.G.Biringer, B.Lustig and B. Painter. Division of Natural Sciences, University of California, Santa Cruz, CA 95064, USA.

The folding process of ribonuclease A was investigated using methanol-based cryosolvents. Increasing concentrations of methanol result in thermal denaturation occurring at lower temperatures and with decreased cooperativity. Both un- and re-folding have been studied with uv, fluorescence, c.d. and NMR in the -30 to 75°C range. Unambiguous evidence for partially-folded intermediates is revealed. For example, refolding at -20°C exhibits multiphasic changes in absorbance corresponding to Tyr burial. ^{1}H-NMR reveals native-like and partially-folded states in both unfolding and refolding. Analysis of the His resonances, in conjunction with Tyr burial/exposure, permitted determination of some of the details of the folding pathway. The native conformation is very similar in aqueous and 50%methanol solutions. The kinetics of refolding at 0°C are also very similar in aqueous and 50% methanol. The partially-folded intermediates are destabilized by guanidine hydrochloride.

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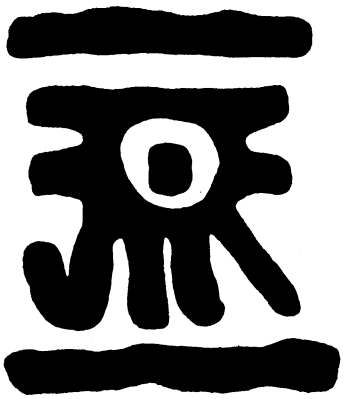

ABSTRACTS

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