S–A4–01

PATHWAYS OF PROTEIN FOLDING

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The pathways of folding of two small proteins, chymotrypsin inhibitor 2 (CI2), and barnase, have been analysed in detail from the denatured state to the final folded structure at the level of individual residues. The single module of CI2 folds via a novel mechanism termed nucleation-condensation, in which a largely unstructured denatured state condenses around a nucleus that is in the process of being formed in the transition state. The multi-modular barnase involves as its rate-determining step the consolidation and docking of modules of structure. Because we know their folding pathways in detail we have been able to use barnase and CI2 to work out a mechanism for the molecular chaperone GroEL.

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GroEL AND ITS LIGANDED STATES

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Whereas the sequence of a polypeptide dictates its native conformation, the folding pathway can present false minima that interfere with the ultimate expression of a functional gene product.

The bacterial chaperonin, GroEL, is a 14-subunit (60-kD each) double toroidal assembly that assists the folding of proteins in conjunction with a 7-subunit (10 kD each) complex, GroES, and the hydrolysis of Mg^{2+}-ATP. GroEL/GroES are essential components of *E. coli* and homologues are found in all eubacteria, archaea and eukaryotic cytoplasmic inclusions. Analogous proteins operate in the cytosol of eukaryotes. Chaperonins function by binding (and, probably, unfolding) nonnative proteins in the central cavity of the cylindrical double toroid preventing further misfolding and/or aggregation, and then releasing them in a form that permits refolding.

The structures of GroEL and its various liganded states will be presented in an effort to provide a structural context for what is known about their role in the folding process and as a basis for the design of genetic and biochemical experiments that further our understanding of the chaperonin-assisted folding mechanism.

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NONLOCAL INTERACTIONS IN THE UNFOLDED STATES OF GLOBULAR PROTEINS.

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The unfolded state, the starting point of the folding pathways is a collection of multiple conformations, but to what extent is what we consider unfolded really unfolded? In order to test this question we studied the conformations and dynamics of the unfolded, partially folded and native states of globular proteins, using Bovine pancreatic trypsin inhibitor (BPTI), RNase-A. Time resolved dynamic nonradiative excitation energy transfer (ET) measurements combined with protein engineering were used to determine the intramolecular segmental end-to-end distance (EED) distributions and intra-molecular diffusion rates in site specifically labeled derivatives of these proteins. The results show that in the partial folding conditions of low GuHCl concentration, reduced BPTI is in a compact state, but in this state the polypeptide chain is not in a condensed statistical coil conformation. These results show that stabilization of long range loops by specific non local interactions (NLIs) may facilitate folding into a native like topology.

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ANTIBODY STRUCTURE, PREDICTION AND REDESIGN

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Purpose: So far the difficulty of predicting the structure of the H3 loop of antibodies has represented the main limitation in modelling the complete antigen binding site. We carefully analyzed all available structures of immuno-globulins searching for rules relating the loop conformation to its amino acid sequence .
Methods: The analysis was performed with the program PINQ on atomic coordinates from PDB.
Results: The H3 residues next to the framework region (torso) can only assume very few distinct main-chain conformations, determined by the amino acid sequence of this region. In some of these, the remaining part of the loop (tip) is a standard hairpin. In the other cases, database searching tecnhiques often allow to predict the entire loop structure.
Conclusions: The identification of key-residues determining the mainchain conformation of the torso of the H3 loop led us to identify "canonical structures" for this region; for a subset of these, canonical structures for the whole loop can be defined. This opens the road to the prediction of the complete antigen-binding site.