Structure and Function of PEP-Dependent Sugar Transport Systems

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S–A1–01

THE *THERMOPLASMA* PROTEASOME AND ITS IMPLICATIONS FOR THE STRUCTURE AND FUNCTION OF EUKARYOTIC PROTEASOMES

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Proteasomes are high molecular weight proteinases implicated in the degradation of misfolded proteins and of short-lived regulatory protein via the ubiquitin pathway. The 20S proteasome is an ATP-independent proteinase degrading only unfolded proteins. The peptides generated fall into a narrow size range suggesting that a molecular ruler is an intrinsic feature of the proteasome. The 20S proteasome forms the catalytic core of the ATP-dependent 26S proteasome in which several regulatory subunits associate with it. The 20S core complex is made of four seven-subunit rings stacked together to form a barrel-shaped complex. In the proteasome from *Thermoplasma acidophilum*, which has taken a pivotal role in elucidating the proteasome structure and catalytic mechanism, the α-subunits form the two outer and the ß-subunits the inner rings. Eukaryotic proteasomes are formed by 14 different subunits which are related to either the α- or ß-subunits of the *Thermoplasma acidophilum* proteasome. The active site is formed by the N-terminal Threonine residue of the ß-subunit and is located in the innermost cavity of the 20S barrel. Access to this proteolytic compartment is controlled by a narrow polypeptide channel formed by the α-subunits which allows only unfolded proteins to enter. The autocatalytic activation of the ß-subunit which requires the removal of a pro-sequence is tightly linked to the assembly of the complex and requires the presence of α-subunits which act as ß-subunit specific chaperones. Moreover, the α–subunits carry the nuclear location signals required for the translocation between the cytoplasm and the nucleus.

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Intercalation, DNA Kinking and Transcription Control: Structures of Protein/DNA Complexes

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Biological processes involved in the control and regulation of transcription are dependent on protein-induced distortions in DNA structure that enhance the recruitment of proteins to their specific DNA targets. This function is often accomplished by accessory factors that bind sequence specifically and locally bend or kink the DNA. The recent determination of the three-dimensional structures of several protein-DNA complexes, involving proteins that perform such architectural tasks, brings to light a common theme of side chain intercalation as a mechanism capable of driving the deformation of the DNA helix. The protein scaffolds orienting the intercalating side chain (or side chains) are structurally diverse, presently comprising four distinct topologies that can accomplish the same task. The intercalating side chain (or side chains), however, is exclusively hydrophobic. Intercalation can either kink or bend the DNA, unstacking one or more adjacent base pairs and locally unwinding the DNA over as much as a full turn of a helix. Despite these distortions, the return to B-DNA helical parameters generally occurs within the adjacent half-turns of DNA.

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STRUCTURE and FUNCTION of PEP-DEPENDENT SUGAR TRANSPORT SYSTEMS

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The phosphoenolpyruvate-dependent phosphotransferase system (PTS) allows bacteria to take up nutrients from the environment. It is composed of the general proteins Enzyme I (E_I) and HPr, and the carbohydrate specific Enzyme II (E_{II}). Enzyme II usually consists of two cytoplasmic domains, IIA and IIB, and a transmembrane domain, IIC. The phosphoryl group of PEP is transferred, via E_I and HPr, to the IIA domain of E_{II}. Subsequently, the IIA domain phosphorylates IIB on a cysteine residue. The phosphorylated IIB activates the actual translocator IIC and phosphorylates the carbohydrate upon release in the cytoplasm.
Apart from carbohydrate translocation, the different PTS-proteins have been implicated in regulation of various other cellular processes, such as the sigma54-dependent transcription of nitrogen metabolic genes in Gram-negative bacteria, and chemotaxis in enteric bacteria.
To understand the atomic basis of these processes X-ray structures have been determined of the IIB enzyme of the cellobiose specific EII from *E. coli*, and of $IIA^{nitrogen}$, a regulatory protein that negatively regulates the transcription of genes involved in nitrogen metabolism.

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FREE ENERGY OF ATOM PAIR INTERACTIONS IN PROTEINS

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Purpose: The questions of how and why proteins fold to unique structures and how they maintain their native folds are controversial issues. In particular, the role of H-bonds in protein folding has remained controversial.

Methods: Radial distribution functions of atom pair interactions are derived from a library of protein structures. The distribution functions are transformed to Helmholtz free energies resulting in potentials of mean force of atom pair interactions.

Results: Potentials for peptide H-bonds have a large energy barrier separating a narrow energy valley at H-bond contact from large distances and the free energy balance of H-bond formation is close to zero.

Conclusions: H-bond formation opposes folding to compact states, but once formed H-bonds act as kinetic traps and a network of such bonds keeps polypeptide chains in a precise spatial configuration. On the other hand H-bonds do not contribute to the thermodynamic stability of native folds.