

Nucleotide / Protein Interaction: Energetic and Structural Features

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Adenosine triphosphate (ATP) acts as a major source for performing electrical, osmotic or mechanical work in cellular systems. It is therefore not surprising that several active transport systems are driven by ATP. Typical examples are Na^+, K^+ -ATPase or Ca^{2+} -ATPase, where cations are transported against existing concentration gradients across the corresponding cell membranes. These ion pumps selectively catalyze the hydrolysis of ATP and convert the resulting energy to the energy requiring, uphill cation translocation process, which is mediated somehow by conformational properties of the protein. It is generally assumed that either the transfer of the γ -phosphate group of ATP to an amino acid side chain or already the selective binding of ATP induces a conformational rearrangement. In the case of Na^+, K^+ -ATPase, for example, high- and low-affinity binding sites have postulated for ATP although the stoichiometry aspects concerning nucleotide binding have not yet been elucidated in a satisfactory manner.

We report here the results of calorimetric titrations of ADP and ATP to Na^+, K^+ -ATPase and Ca^{2+} -ATPase in media of different ionic composition under the conditions of very slow triphosphate hydrolysis. In particular, the effect of alkaline earth cations on nucleotide binding is considered. The resulting stoichiometric coefficients are correlated with the values obtained for high-affinity inhibitor binding, determined by titration calorimetry, too.

The results of the calorimetric studies are interpreted on the basis of a recent X-ray structure analysis of Ca^{2+} -ATPase. The large entropic contributions obtained are related to protein/water interactions in the transmembrane part of the protein and are considered as expression of a coupling between structure domains in different parts of the protein.