Short communication

Hypothetical Structure of the ATP-binding Site of $(Na^+ + K^+)$-ATPase

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In recent years numerous controversial data have accumulated about the structure and properties of the ATP-binding site of $(Na^+ + K^+)$-ATPase. This study attempts eliminating the present discrepancies and proposes using the method of molecular graphics a structural arrangement meeting all chemical criteria of interaction of the ATP molecule with the active site of the enzyme including its free accessibility.

Incubation of $(Na^+ + K^+)$-ATPase with fluorescein isothiocyanate and subsequent tryptic cleavage yielded a peptide consisting of 11 amino acids. This peptide carrying the isothiocyanate label on $—NH_2$ group of lysine was at first considered to represent the pillar structure of the ATP binding site of $(Na^+ + K^+)$-ATPase (Farley et al. 1984; Otha et al. 1985). Nevertheless, this tryptic fragment contained neither the —SH group of cysteine which binds the adenine part of the ATP molecule (Patzelt-Wenczler and Schoner 1981; Schoner et al. 1982) nor the —COOH group of aspartic acid. The latter was demonstrated to form with ATP an acylphosphate bond during the reaction cycle of Na-pump (Jørgensen 1982). In addition, isothiocyanates were found to inhibit phosphohydrolase activity of the Na-pump via interaction with an essential —SH group localised in the ATP binding site of the enzyme (Ziegelhöffer et al. 1983). In view of the above contradictions it seems unrealistic that the peptide fragment carrying the isothiocyanate label on the $—NH_2$ group of lysine may represent the pillar structure of the ATP binding site of $(Na^+ + K^+)$-ATPase. However, it still might belong to structures in the nearest vicinity of the binding site. This image is absolutely probable because in slightly alkaline milieu (re-

Presented at the 14th IUB Congress, July 10—15, 1988, Prague, Czechoslovakia.

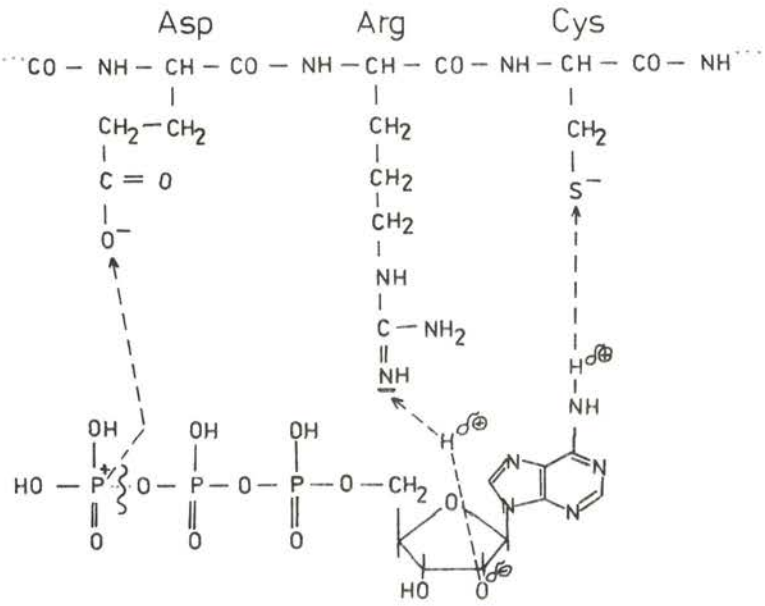
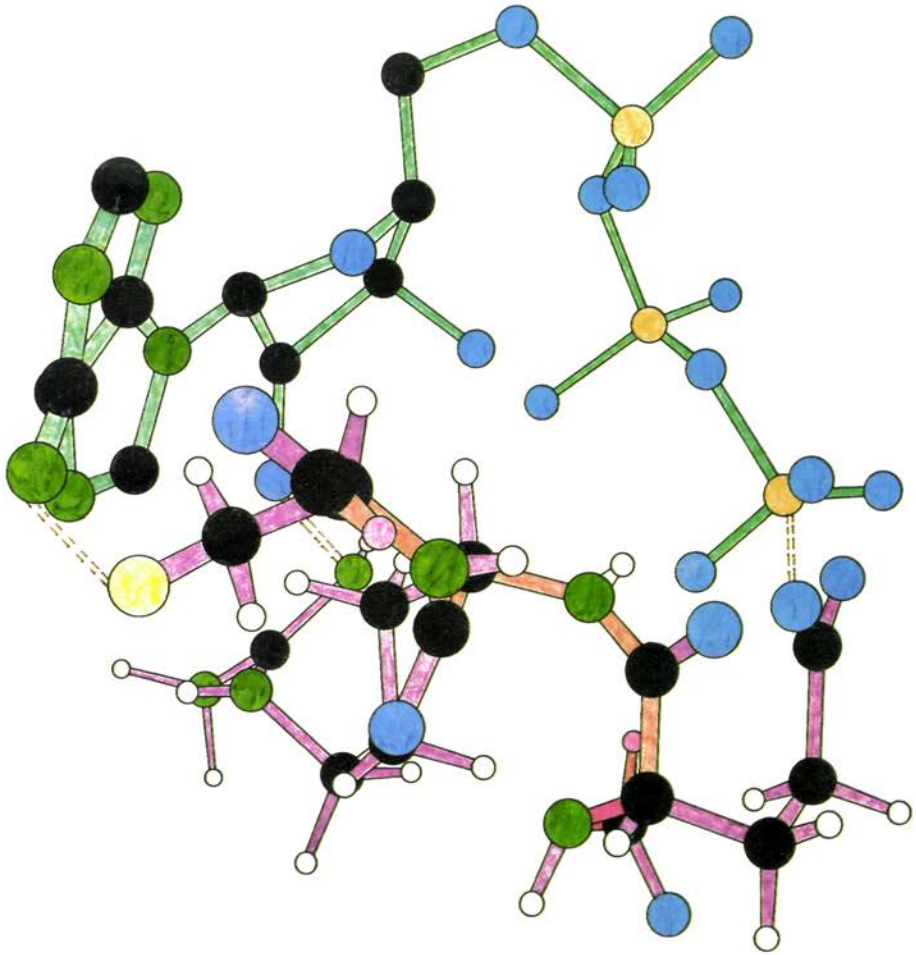


Fig. 1. Schematic representation of the proposed structure of the active site of $(Na^+ + K^+)$-ATPase; location of ATP is indicated.

quired for tryptic cleavage to occur) S-esthers of dithiocarbamic acid are very unstable. Thus, the isothiocyanate label would jump over easily from its original place on the —SH group to a near $—NH_2$ group offering at alkaline pH a more stable bond.

According to the primary structure of $(Na^+ + K^+)$-ATPase (Schull et al. 1985) the isothiocyanate-labelled peptide represents the sequence of amino acids 496—506 numbered from the N-terminal. Near to this place is localized a triplet of amino acids consisting of aspartic acid (509), arginine (510) and cysteine (511). This tripeptide could represent the actual ATP binding site of the enzyme: the —SH group of cysteine can react with the 6—NH_2 group of adenine by forming a hydrogen bond (Patzelt-Wenczler and Schoner 1981; Schoner et al.

Fig. 2. Three-dimensional simulation of the proposed structure of the active site of $(Na^+ + K^+)$-ATPase. Atoms: white — hydrogen; yellow — sulphur; black — carbon, blue — oxygen; orange — phosphorus; green — nitrogen. Bonds: green — chemical bonds in the ATP molecule; red — peptide bonds; violet — chemical bonds in the side chains of the tripeptide. Brown stripes indicate interaction between ATP and the tripeptide.



1982), and the —COOH group of aspartic acid with the γ-phosphate of ATP (acylphosphate bond, Jørgensen 1982). The essential arginine residue in the ATP binding site of $(Na^+ + K^+)$-ATPase (De Pont et al. 1984; Schneider-Bobos and Schoner 1985) might interact with the ribose moiety of ATP molecule by forming a hydrogen bond with the 2—OH group of the ribose (Monošíková et al. 1987). This composition of the ATP binding site (Ziegelhöffer et al. 1987) would be capable of differentiating between adenine and other nucleotides as well as between ATP and deoxy-ATP, ADP or AMP.

In resolving the three-dimensional structure of tripeptide 509—511 proposed to form the true active site of $(Na^+ + K^+)$-ATPase we utilised the method of molecular graphics. Standard bond lengths and standard values of valence angles (Hopfinger 1973) were applied in geometric simulation of the model tripeptide. Data concerning the geometry of the ATP molecule were adapted from results of X-ray structural analysis (Kennerd et al. 1971; Sugarawa and Iwasaki 1984). During geometrical simulation the following parameters were varied: i) the dihedral angles of the tripeptide both in the peptide backbone and in the side chains; ii) the dihedral angles in the triphosphate moiety of ATP and the dihedral angle between the ribose ring and the purine moiety of ATP molecule. As fitting was considered the geometrical arrangement in which the interacting atoms were reaching mutual distances of $\langle 0.16\,\text{nm}—0.28\,\text{nm}\rangle$ and mutual distances between chemically nonbonded atoms always exceeded the total of their van der Waals radii.

The resulting model represents one of several possible fitting arrangements documenting at the same time the sterical accessibility of the proposed biding site for ATP and excluding any interference of other parts of the peptide chain with the open active site. The probability of the proposed structure has been supported also by the results of a recent NMR study performed independently (Stewart et al. 1988).

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Final version accepted September 1, 1988