Journal of Thermal Analysis and Calorimetry, Vol. 77 (2004) 471–481

NUCLEOTIDE/PROTEIN INTERACTION
Energetic and structural features of Na,K-ATPase*

*E. Grell***, *E. Lewitzki, A. Schacht and M. Stolz*

Max-Planck-Institut für Biophysik, D-60596 Frankfurt, Germany

Abstract

Microcalorimetric titrations allow to recognize and investigate high-affinity ligand binding to Na,K-ATPase. Titrations with the cardiac glycoside ouabain, which acts as a specific inhibitor of the enzyme, have provided not only the thermodynamic parameters of high-affinity binding with a stoichiometric coefficient of about 0.6 but also evidence for low-affinity binding to the lipid. The marked enthalpic contribution of –95 kJ mol^{-1} at 298.2 K is partially compensated by a large negative entropy change, attributed to an increased interaction between water and the protein. The calorimetric ADP and ATP titrations at 298.2 K are indicative of high-affinity nucleotide binding either in 3 mM NaCl, 3 mM $MgCl_2$ or at high ionic strength such as 120 mM choline chloride. However, no binding is detected in the buffer solution alone at low ionic strength. The affinities for ADP and ATP are similar, around 10^6 M^{-1} and the stoichiometric coefficients are close to that of ouabain binding. The exothermic binding of ADP is characterized by a ΔH and ΔS value of –65 kJ mol^{-1} and –100 J mol^{-1} K^{-1}, respectively. The ΔH value for ATP binding is larger than for ADP and is compensated by a larger, unfavorable ΔS value. This leads to an enthalpy/entropy compensation, which could express that H-bond formation represents the major type of interaction. As for ouabain, the negative ΔS values that are also characteristic of nucleotide binding can indicate an increase of solvate interaction with the protein due to a conformational transition occurring upon to the binding process. The resulting binding constants are discussed with regard to the results of other studies employing different techniques. A molecular interaction model for nucleotide binding is suggested.

Keywords: ATP binding, cardiac glycoside, microcalorimetry, Na,K-ATPase, nucleotide binding, nucleotide protein interaction, ouabain, titration calorimetry

Introduction

The interaction between nucleotides and proteins is of great importance for many biological recognitions and functions. The involved specificities are not only related to the chemical nature of the nucleotide's base and sugar part but also to the mode and degree of phosphorylation. The resulting affinities are often in the range between 10^6 to 10^7 M^{-1}. In general, the involvement of coordinated metal ions such as Mg^{2+} or

* From contributons to the 6th Internat. Conference on Pharmacy and Thermal Analysis, Ascona and the 10th Internat. Conference on Na,K-ATPase and Related Cation Pumps, Elsinore

** Author for correspondence: E-mail: ernst.grell@mpibp-frankfurt.mpg.de

1388–6150/2004/ $ 20.00

Akadémiai Kiadó, Budapest
Kluwer Academic Publishers, Dordrecht

Ca^{2+} is thought to represent an important aspect of nucleotide/protein interaction. The sequence motifs of ATP binding proteins such as P-type ATPases have been analyzed, also with regard to evolutionary aspects [1, 2]. The side group of a Lys residue has been assumed to interact with the β- or γ-phosphate of the triphosphate residue, that of Thr and of Asp or Glu with the coordinated Mg^{2+}.

Detailed binding domains have been characterized by X-ray crystallography for the elongation factor Tu (EF-Tu) [3] with a resolution of 1.45 Å, for F_1-ATPase [4, 5] and for Ca^{2+}-ATPase [6] with lower resolution. As a representative example, the non-hydrolyzable β,γ-N-GTP derivative, bound to EF-Tu, exhibits as much as 31 direct interaction contacts in its bound state: Oxygens of the β- and γ-phosphate coordinate Mg^{2+} and interact with a Lys side chain. The ether oxygen of the sugar moiety forms a H-bond with the amino group of a second Lys. Nine additional contacts are due to hydrogen bonding with –OH and $–CONH_2$ containing amino acid side chains. In addition, eight interactions of this substrate analogue are due to hydrogen bond formation with main chain amide NH groups and in addition eight hydrogen bonds are formed with water molecules [3]. In the case of the α-subunit of F_1-ATPase according to [5], β- and γ-phosphate oxygens are also in contact with Mg^{2+} and in addition with a Lys residue. Here, the adenine part is located in a much more hydrophobic pocket than the guanosine residue in the EF-Tu complex structure mentioned before. For the trinitrophenyl-AMP (TNP-AMP) complex of Ca-ATPase [6], the adenine residue is again located in a fairly hydrophobic region. The α-phosphate group is in contact with a Lys side chain and in addition an Arg residue is located in its neighbourhood. The Asp side chain, which is phosphorylated during the enzymatic reaction, and the Mg^{2+} binding site relevant for catalysis too, are not located close to the nucleotide binding site, as generally assumed prior to the publication of the crystal structure. This Asp residue is separated from the ATP binding site by a distance of about 25 Å according to [6].

Based on these selected examples, it can be concluded that nucleotides are bound in form of a multidentate interaction with amino acid side chains of the proteins involving also specifically bound water molecules. The resulting strength of the interaction is usually discussed on a free energy level, based on equilibrium constants determined employing radioactively labeled nucleotides, where the values sometimes even differ markedly, as in the case of Na,K-ATPase [7, 8]. However, it is often not considered that the strength of direct binding interactions, which preferentially contribute to the enthalpic term (ΔH), can only be correlated with the equilibrium constant (K_{th}, here defined as thermodynamic association constant) in a direct manner if the entropic contribution (ΔS, cf. Eq. (1)) is either zero or its value is identical for all examples of the system under consideration.

$$-\ln K_{th} = \left(\Delta H - T\Delta S\right) / RT \tag{1}$$

The equilibrium constant K_{th} for a one-step binding process such as between a nucleotide N and an enzyme E, leading to the formation of the complex EN, is a dimensionless quantity and is given by

$$K_{\mathrm{th}} = \frac{c_{\mathrm{EN}}\, c_{\mathrm{S}}}{c_{\mathrm{N}}\, c_{\mathrm{E}}} K_{\gamma}, \qquad \text{with} \quad K_{\gamma} = \frac{\gamma_{\mathrm{EN}}}{\gamma_{\mathrm{N}}\, \gamma_{\mathrm{E}}} \tag{2}$$

where c_{EN}, c_N, c_E represent equilibrium concentrations, c_S is the standard concentration, expressed in molar units (c_S = 1 M) and γ_i are the related activity coefficients [9]. K_γ is a constant under conditions of low concentrations (K_γ = 1 because γ_i = 1) or of constant ionic strength. If K_γ is constant, the experimentally determined equilibrium constant K corresponds to

$$K = \frac{K_{\mathrm{th}}}{K_{\gamma}} = \frac{c_{\mathrm{EN}}\, c_{\mathrm{S}}}{c_{\mathrm{N}}\, c_{\mathrm{E}}}, \tag{3}$$

The term c_S is often not written explicitely when it is 1 M. The expression for K is then

$$K = \frac{c_{\mathrm{EN}}}{c_{\mathrm{N}}\, c_{\mathrm{E}}} \tag{4}$$

According to Eq. (4), K is expressed with the dimension M^{-1}, as usually given in the literature. The numerical value of K according to Eq. (4) is, however, identical to that of Eq. (3), provided c_S is equal 1 M.

For a meaningful interpretation of equilibrium constants in structural terms we have to differentiate between enthalpic and entropic contributions. This can be achieved by employing titration calorimetry, for example, where it is possible to determine the equilibrium constant together with the stoichiometric coefficient and ΔH, provided it is not equal to zero. The entropy change can be calculated according to Eq. (1). In addition, calorimetry offers the advantage of a direct determination, independent of assumptions related to other applied techniques.

As an illustrative example, a calorimetric study of the interaction of adenine nucleotides with Na,K-ATPase, is reported here. Na,K-ATPase, the receptor molecule for car-

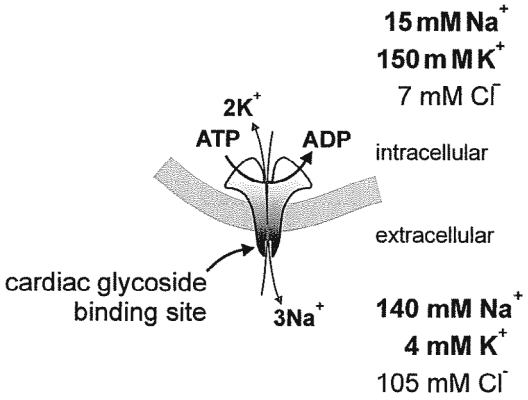

Fig. 1 Schematic illustration of Na,K-ATPase in a plasma membrane

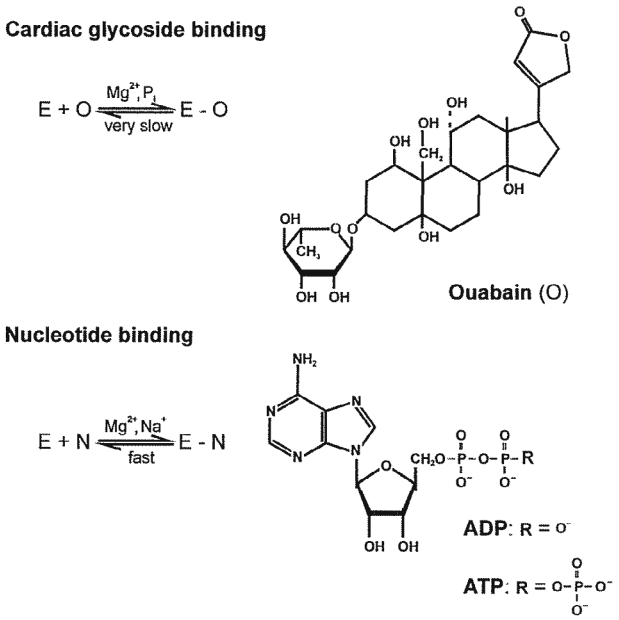

Fig. 2 Structure of the cardiac glycoside ouabain (O) and of the adenine nucleotides (N) ADP and ATP and illustration of the corresponding one-step binding equilibria

diac glycosides, consists of an α, β and γ subunit per protomer. It is an integral membrane protein, which exists in the plasma membranes of all higher organisms. This enzyme is ATP-driven and actively transports Na^+ out of and K^+ into the cells to maintain the well known alkali ion concentration gradients across cellular membranes. A schematic illustration is given in Fig. 1. The resulting concentration gradients contribute significantly to the membrane potential and can act as driving force for other important physiological processes. Purified membrane-bound Na,K-ATPase is isolated in form of nanoparticulate discs (mean diameter around 300 nm), consisting of densely packed and oriented protein molecules. The results of our thermodynamic study concerning the interaction between the nucleotides ADP and ATP and the binding site of Na,K-ATPase, located at the former intracellular membrane side, are correlated with the binding data obtained for Ouabain (Fig. 1). This cardiac glycoside acts as a specific inhibitor at the former extracellular side of Na,K-ATPase. Certain medium requirements have to be fulfilled to obtain high-affinity binding. For Ouabain binding, the presence of Mg^{2+} and P_i is required for example, whereas Mg^{2+} and Na^+ is generally assumed to be necessary for high-affinity ADP and ATP binding (cf. Fig. 2).

Experimental

Materials and methods

Chemicals

Chemicals were of analytical grade, supplied by Merck and Fluka. Imidazole (MicroSelect, Fluka) as buffer compound has been adjusted with HCl (Ultrapur, Merck). Trans-1,2-diamino-cyclohexane-N,N,N',N'-tetraacetic acid (CDTA) of quality puriss. p.a. originated from Fluka. NaCl (Suprapur) was from Merck and choline chloride (ChCl) of quality Microselect from Fluka. To prepare solutions nominally free of K^+, the contact of all solutions with the combined pH electrode for the pH adjustment was omitted. ATP has been used as Tris (tris(hydroxymethyl)aminomethane) salt (Sigma); ADP (acid form, Fluka) and phosphoric acid (MicroSelect, Fluka) have been neutralized with Tris (MicroSelect, Fluka). The nucleotide concentrations have been determined spectrophotometrically (ε = 15'400 at 260 nm).

Enzyme

Purified membrane-bound Na,K-ATPase has been isolated from dogfish (*Squalus acanthias*) rectal gland and pig kidney according to [10, 11]. Protein concentrations were determined according to Lowry and Popov [11–13]; enzymatic activities under standard conditions (120 mM NaCl, 20 mM KCl, 3 mM $MgCl_2$, 3 mM ATP in 30 mM histidine/HCl pH 7.5) were around 37 μmol mg^{-1} min^{-1} at 310.2 K. Glycerol has been added to reduce strong particle aggregation. The calculation of the protein concentrations are based on a protomer molecular mass of 155 kDa.

Microcalorimetry

Calorimetric studies have been carried out with the MCS ITC titration calorimeter of MicroCal at 298.2 K. All solutions have been vacuum degassed. Evaluation was performed with the manufacturer's program (single site model according to [14]). The association constants K for inhibitor and nucleotide binding correspond to the expression given in Eq. (4).

Results

Inhibitor binding

In order to obtain information about the state or properties of a protein such as the number of available binding sites per protomer, microcalorimetric titrations can be carried out with a specific inhibitor exhibiting high-affinity binding. In the case of Na,K-ATPase, ouabain represents the best choice provided the titration is performed in a suitable medium, for example consisting of Mg^{2+} and Tris/phosphoric acid (Tris/P_i) as a type of minimal requirement (cf. Fig. 2 top). In Fig. 3, a typical calorimetric saturation titration of dogfish Na,K-ATPase with ouabain is shown. The bind-

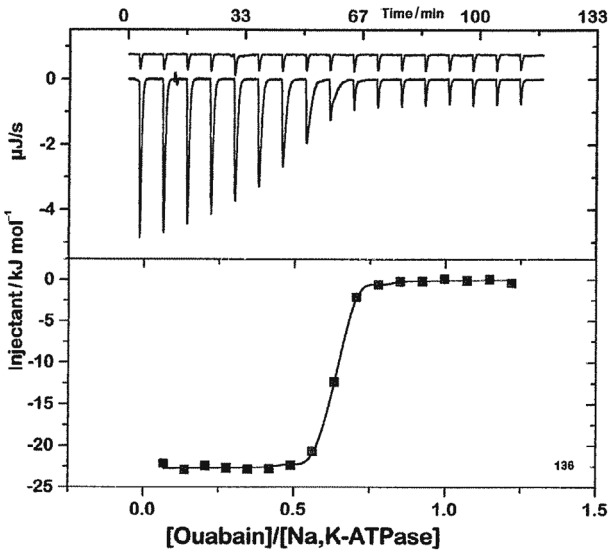

Fig. 3 Determination of the stoichiometric coefficient and the enthalpy change of ouabain binding to purified Na,K-ATPase: Calorimetric titration of 20 µM dogfish enzyme in 10 mM imidazole/HCl, 3 mM $MgCl_2$, 3 mM Tris/P_i pH 7.0 containing 25% glycerol (*w*/*w*) with 17×7 µL 0.25 mM ouabain in the same buffer medium at 298.2 K together with the corresponding reference titration (shifted by an offset of 0.75 µJ s^{-1}) in the absence of the enzyme (top). Plot of heat changes attributed to ligand binding as a function of the concentration ratio between ouabain and Na,K-ATPase. The solid line corresponds to the theoretical curve related to the resulting parameters given in the text (bottom)

ing of this inhibitor is characterized by an exothermic process. Based on the protein determination of Popov *et al*. [13], a stoichiometric coefficient of 0.6 per protomer results from this experiment. For different titrations with the pig kidney and dogfish enzyme an average stoichiometric coefficient of about 0.55 is obtained. Furthermore, the titration shown in Fig. 3 allows to determine the ΔH value (about –95 kJ mol^{-1}) after subtracting the mean heat of dilution of the last 5 signals of the titration experiment (Fig. 3 bottom). A realistic determination of the association constant K is not possible under conditions of such a high protein concentration as used for the experiment described in Fig. 3. Other studies have revealed a log*K* value of 7.8 for the dogfish enzyme [11]. After saturation of the specific high-affinity site, small additional heat changes are observed in the titration curve. These heat changes are significantly larger than the heat changes of ouabain dilution, resulting from every addition, as obtained in the corresponding reference titration (Fig. 3 top). The additional heat changes are assigned to low-affinity binding of ouabain to the lipid part of the membrane fragment. The determined thermodynamic parameters for high-affinity binding have not been sensitive to the ionisation enthalpies of the chosen buffer compounds.

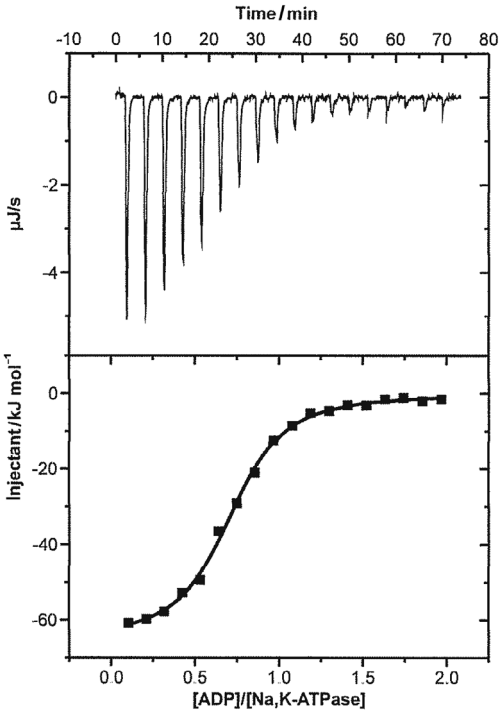

Fig. 4 Determination of thermodynamic parameters of ADP binding to purified Na,K-ATPase: Calorimetric titration of 17.5 μM dogfish enzyme in 10 mM imidazole/HCl, 0.25 mM CDTA and 3 mM NaCl pH 7.5 containing 25% glycerol (*w*/*w*) with 18×5 μl 0.5 mM TrisADP in the same buffer medium at 298.2 K (top). Plot of heat changes attributed to ligand binding as a function of the concentration ratio between ADP and Na,K-ATPase. The solid line corresponds to the theoretical curve related to the resulting parameters given in the text (bottom)

Nucleotide binding

For the investigation of ATP binding to Na,K-ATPase by titration calorimetry it is necessary to choose media where the added ATP is not appreciably hydrolysed during the time required to carry out the measurement. This problem can be circumvented by investigating more stable substrate analogues for binding studies such as ADP or β,γ-N-ATP. Therefore, binding experiments have been mainly performed with ADP in media of different compositions. Such titrations should allow not only to determine the corresponding stoichiometric coefficient and the related thermodynamic parameters but it should also provide information about the minimal medium requirements to achieve high-affinity nucleotide binding.

In the presence of 10 mM imidazole/HCl, 0.25 mM CDTA pH 7.5 containing 25% glycerol (*w*/*w*) and without of any additionally added salt (low ionic strength conditions), almost no heat signal due to nucleotide binding has been detected, which

represents a surprising observation. This implies that either the binding enthalpy is close to zero or that no high-affinity binding occurs in this medium.

In contrast to this result, Fig. 4 shows the result of an ADP titration of the dogfish enzyme in the medium mentioned before but containing now 3 mM NaCl in addition. A clear titration profile, characteristic of exothermic binding, has been observed, which indicates high-affinity nucleotide binding. After achieving saturation of the high-affinity nucleotide binding site (Fig. 4 top), the resulting areas of the heat signals remain nearly constant. The corresponding peak areas are, however, slightly larger than those obtained only from nucleotide dilution, as observed in the corresponding reference titrations (not shown in Fig. 4). For the purpose of quantitative evaluation, the mean value of the last four signals of the titration is subtracted from the heat signals of the complete titration (Fig. 4 bottom). The evaluation provides values for log$K$ of about 6.2, ΔH of –65 kJ mol^{-1} and a stoichiometric coefficient around 0.65. This stoichiometric coefficient is similar to the one found for ouabain binding. The calculated ΔS value is about –100 J mol^{-1} K^{-1}. High-affinity ADP binding has not only been observed in the presence of 3 mM or higher concentrations of NaCl. In the absence of NaCl, it has also been detected upon titrations in the presence of 3 mM $MgCl_2$ or alternatively with 120 mM ChCl, corresponding to high ionic strength conditions. Although it is generally assumed that the presence of Mg^{2+} leads to an increase of the nucleotide binding affinity, as reported recently [7], our calorimetric studies indicate the opposite behavior. We find a decrease of the affinity by a factor of about four, compared to the medium containing 3 mM NaCl. Since the presence of KCl does antagonize nucleotide binding, it is not surprising that no heat changes due to high-affinity nucleotide binding have been detected during a corresponding control titration in the presence of 120 mM KCl. The results with the pig kidney enzyme are closely related. The determined thermodynamic parameters do not depend on the ΔH value of the applied buffer compound. Thus no marked heat change resulting from protolytic reactions appears to contribute to the determined ΔH value.

Very similar calorimetric results have also been observed for ATP binding in the same media. However, in a medium containing the combination of 3 mM $MgCl_2$ and 120 mM NaCl, fast hydolysis of ATP after every addition occurs in the titration experiment. For the dogfish enzyme in the presence of 3 mM NaCl, essentially the same equilibrium constant as for ADP is obtained. However, the magnitudes of the negative ΔH and the ΔS value are markedly higher than for ADP. Also here, addition of Mg^{2+} decreases ATP affinity. No marked differences have been found between the membrane-bound pig kidney and dogfish enzyme. No high-affinity binding has been detected for AMP binding by titration calorimetry.

Discussion and conclusions

The interaction stoichiometry in terms of number of active sites n per protomer of Na,K-ATPase for nucleotide and ouabain binding as well as for phosphorylation has often been investigated and debated [15–20]. Up to now, the majority of binding studies

have been carried out with radioactive ligands at low temperature. Although analytical protein determinations differ between different labs and thus can affect the results, *n* was usually markedly lower than 1. Our calorimetric results, which depend on the liberation or consumption of heat as a very fundamental property, provide an average value for inhibitor and substrate binding under suitable conditions around 0.6 for the membrane-bound pig kidney and dogfish enzyme. This implies that nearly half of the protein molecules are in a non-binding state with regard to the investigated ligands in the chosen concentration ranges. As possible reasons we have to take into consideration that part of the enzyme may have been isolated in denatured form although our enzymatic activities reach the maximum values hitherto obtained, or may be of reduced activity due to a regulatory modification. Binding to a diprotomer state exhibiting negative cooperativity could represent an additional explanation but appears rather unlikely because this would have to concern both type of ligands, ouabain and nucleotides. These ligands do not only differ with regard to their chemical nature, they bind also to completely different sites that are located on opposite sides of the membrane. The regulatory aspect appears to represent an attractive possibility because a value of *n* close to 1 has been reported recently for a preparation, isolated accordingly but obtained from the nasal salt gland of specially feeded ducks [21], where the regulatory barrier could have been released to achieve higher enzymatic activity.

The exothermic binding of ouabain to the former extracellular side of the protein is characterized by a large enthalpy change of about –95 kJ mol^{-1} at 298.2 K. This is characteristic of a very strong interaction, assumed to be preferentially caused by hydrogen bond formation. This large enthalpic contribution to the free energy of ouabain binding is markedly compensated by a large negative entropy change of about –150 J mol^{-1} K^{-1} [22]. This unfavorable entropic contribution is assumed to be mainly due to an increased interaction between water molecules and the protein as a consequence of a conformational transition occurring upon the binding of this cardiac glycoside.

Under our low ionic strength conditions such as 10 mM imidazole/HCl pH 7.5, there is no evidence for high-affinity nucleotide binding because no marked heat change has been observed during the titrations with ADP and ATP. Although prevented nucleotide binding does not seem probable to occur, we can not rule out that binding takes place, however, without of a resulting enthalpy change. In principle, also strong membrane particle aggregation could perhaps prevent ligand binding. However, this is ruled out because high-affinity K^+ binding can easily be observed under our conditions [23]. Upon addition of 3 mM NaCl, which leads to the formation of the Na^+ complex of Na,K-ATPase [24], high-affinity ADP and ATP binding is possible and association constants around 10^6 M^{-1} are obtained. This value is consistent with those of early investigations [15–19], but it is markedly lower than in the case of recent fast separation studies with radioactive nucleotides performed at low temperature [7, 8]. The discrepancy can not be explained only on the basis of the different temperatures applied but may be due to not fulfilled assumptions introduced for the determination of the water content in the concentrated sample of the radioactive protein/nucleotide complex [7]. As for Ca-ATPase [25], ADP and ATP nucleotides can

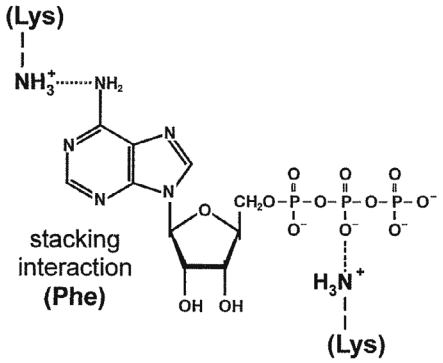

Fig. 5 Proposed molecular model for the interactions of deprotonated ATP in the nucleotide binding pocket of Na,K-ATPase. Adenine part: stacking interaction of the heterocyclic ring system with an aromatic residue such as Phe and hydrogen bonding of the amino group with a protonated basic residue such as Lys; triphosphate part: salt bridge of the ß-phosphate group with a protonated basic residue such as Lys

also bind to Na,K-ATPase in the absence of added Mg^{2+}. Furthermore, according to calorimetry the presence of Mg^{2+} does not lead to an increased affinity for nucleotide binding as generally assumed and reported recently [7]. We even observe a reduction of the affinity in the presence of 3 mM $MgCl_2$. High-affinity ADP and ATP binding can, however, also be detected in the absence of Na^+ or Mg^{2+} provided high ionic strength conditions such as 120 mM ChCl are present. This result suggests that the conformational properties and thus the binding properties of Na,K-ATPase depend in a sensitive manner on the ionic strength of the medium. The relevance of general cationic interactions such as with ChCl on the conformation of Na,K-ATPase has already been emphasized earlier [26].

In a recent investigation [7], the affinities have been interpreted in terms of molecular interactions. This is only meaningful if either ΔS for different nucleotides is equal to zero or adopts a constant value. Although the association constants for ADP and ATP binding are similar according to our calorimetric results, the magnitude of the enthalpy change for ATP binding is larger than that for ADP binding. This is indicative of an increased number of protein interactions for the tri- than for the diphosphate. Similar to ouabain, the entropy change of ADP and ATP binding is large and negative. As for ouabain binding, this can imply that the protein offers more access to water molecules in the bound state, for example as a consequence of a structural change upon ADP and ATP binding. This unfavorable entropy change is larger for ATP than for ADP, leading to an enthalpy/entropy compensation. Such a compensation is characteristic of the same type of protein/nucleotide interaction such as H-bond formation. As AMP is not strongly bound to Na,K-ATPase, our results thus question the molecular interpretations given in [7], where the interaction between the negatively charged α-phosphate group of the nucleotides and the protein is considered to provide the most relevant contribution for the binding affinity. We

rather postulate that the negative charge of the β-phosphate group is of special importance for this interaction with at least one positively charged group of the Na,K-ATPase, for example a lysine side chain as indicated schematically in Fig. 5. The adenine part of ADP and ATP is likely to be bound in a hydrophobic pocket, stabilized by a stacking interaction (cf. Fig. 5). Concerning general structural features, all our results can be easily understood on the basis of a structure model similar to that reported for Ca-ATPase [6].

References

1 J. E. Walker, M. Saraste, M. J. Runswick and N. J. Gay, EMBO J., 1 (1982) 945.
2 M. Galperin and E. V. Koonin, TIBS, 23 (1998) 127.
3 H. Berchtold, L. Reshetnikova, Ch. O. A. Reiser, N. K. Schirmer, M. Sprinzl and R. Hilgenfeld, Nature, 365 (1993) 126.
4 J. P. Abrahams, A. G. W. Leslie, R. Lutter and J. E. Walker, Nature, 370 (1994) 621.
5 M. A. Bianchet, J. Hullihen, P. L. Pedersen and L. M. Amzel, Proc. Natl. Acad. Sci. USA, 95 (1998) 11065.
6 C. Toyoshima, M. Nakasako, H. Nomura and H. Ogawa, Nature, 405 (2000) 647.
7 N. Feodosova, P. Champeil and M. Esmann, Biochemistry, 41 (2002) 1267.
8 P. L. Jorgensen, J. R. Jorgensen and P. A. Pedersen, J. Bioenerg. Biomembr., 33 (2001) 367.
9 M. K. Gilson, J. A. Given, B. L. Bush and J. A. McCammon, Biophys. J., 72 (1997) 1047.
10 P. L. Jorgensen, Biochim. Biophys. Acta, 356 (1974) 36.
11 E. Grell, E. Schick and E. Lewitzki, Thermochim. Acta, 380 (2001) 245.
12 O. H. Lowry, N. J. Rosenbrough, A. L. Farr and R. J. Randall, J. Biol. Chem., 193 (1951) 265.
13 N. Popov, M. Schmitt, S. Schulzeck and H. Matthies, Acta Biol. Med. Germ., 34 (1975) 1441.
14 T. Wiseman, S. Williston, J. F. Brandts and L.-N. Lin, Anal. Chem., 17 (1989) 131.
15 C. Hegyvary and R. L. Post, J. Biol. Chem., 246 (1971) 523.
16 J. G. Norby and J. Jensen, Biochim. Biophys. Acta, 233 (1971) 104.
17 F. M. A. H. Schuurmans Stekhoven, H. G. Swarts, J. J. De Pont and S. L. Bonting, Biochim. Biophys. Acta, 649 (1981) 533.
18 J. G. Norby, I. Klodos and N. O. Christiansen, J. Gen. Physiol., 82 (1983) 725.
19 W. Schoner, H. Pauls and R. Patzelt-Wenczler, In Myocardial Failure (G. Riecker, A. Weber and J. Goodwin, Eds), Springer, Heidelberg 1977, p. 104.
20 M. Esmann and N. U. Fedosova, In Na/K-ATPase and Related ATPases (K. Taniguchi and S. Kaya, Eds), Elsevier Science B. V., Amsterdam 2000, p. 311.
21 D. W. Martin and J. R. Sachs, Biochemistry, 38 (1999) 7485.
22 M. Stolz, E. Lewitzki, E. Schick, M. Mutz and E. Grell, In Na,K-ATPase and Related Cation Pumps; Structure, Function, and Regulatory Mechanisms (P. L. Jorgensen, S. J. D. Karlish and A. B. Maunsbach, Eds), Ann. N.Y. Acad. Sci., 986 (2003) 245.
23 E. Grell and E. Lewitzki, unpublished results.
24 H. Ruf, E. Lewitzki and E. Grell, In The Sodium Pump (E. Bamberg and W. Schoner, Eds), Steinkopff, Darmstadt 1994, p. 569.
25 J.-J. Lacapère, N. Bennett, Y. Dupont and F. Guillain, J. Biol. Chem., 265 (1990) 348.
26 E. Grell, R. Warmuth, E. Lewitzki and H. Ruf, Acta Physiol. Scand., 146 (1992) 213 with Erratum in Acta Physiol. Scand., 147 (1993) 343.