Critical role of $\gamma$-phosphate in structural transition of Na,K-ATPase upon ATP binding

Irina Yu. Petrushanko1*, Vladimir A. Mitkevich1*, Anastasia A. Anashkina1, Elizaveta A. Klimanova2, Elena A. Dergousova2, Olga D. Lapina2 & Alexander A. Makarov1

1Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Vavilov Str. 32, 119991 Moscow, Russia, 2Faculty of Biology, M.V. Lomonosov Moscow State University, 119234 Moscow, Russia.

Active transport of sodium and potassium ions by Na,K-ATPase is accompanied by the enzyme conformational transition between E1 and E2 states. ATP and ADP bind to Na,K-ATPase in the E1 conformation with similar affinity but the properties of enzyme in complexes with these nucleotides are different. We have studied thermodynamics of Na,K-ATPase binding with adenine nucleotides at different temperatures using isothermal titration calorimetry. Our data indicate that $\beta$-phosphate is involved in complex formation by increasing the affinity of adenine nucleotides to Na,K-ATPase by an order of magnitude, while $\gamma$-phosphate does not affect it. ATP binding to Na,K-ATPase in contrast to ADP binding generates a structural transition in the enzyme, which is consistent with the movement of a significant portion of the surface area to a solvent-protected state. We propose that ATP binding leads to convergence of the nucleotide-binding and phosphorylation domains transferring the enzyme from the “E1-open” to “E1-closed” conformation ready for phosphorylation.

In all animal cells, active transport of sodium and potassium ions across the plasma membrane is facilitated by Na,K-ATPase (sodium pump, EC 3.6.3.9). The transport is followed by conformational transition between the two main states of the enzyme, E1 and E2, that bind three Na$^+$ and two K$^+$ ions, respectively$^1$. Na,K-ATPase in the E1 conformation has a high affinity to ATP and Na$^+$, while the enzyme in the E2 conformation has a low affinity to ATP and high affinity to K$^+$ ions$^1$. Functional monomer of the Na,K-ATPase consists of the catalytic $\alpha$-subunit and $\beta$-subunit, the latter is required for routing of the $\alpha$-subunit to the plasma membrane and for the K$^+$ ions binding$^2$. The $\alpha$-subunit contains sites for the binding of nucleotide, Na$^+$ and K$^+$, and is responsible for the cation translocation as well as for the ATP hydrolysis. A major part of $\alpha$-subunit is localised in the cytosol and consists of the large cytosolic loop forming the nucleotide binding (N) and phosphorylation (P) domains, and the small cytosolic loop, which together with the N-terminus of the molecule forms the actuator (A) domain. Transition from the E1 to E2 conformation is accompanied by formation of the phosphorylated intermediate: at first $\gamma$-phosphate of ATP bonded to the N domain is transferred to the carboxyl group of Asp369 located in the P domain, and then the acyl-phosphate bond is hydrolyzed$^1$. Recently a crystal structure of the protein in intermediate [Na$_3$E1P-ADP state (pig renal $\alpha_1$$\beta_1$ enzyme) at 4.3 Å and 2.8 Å resolution has been solved. The structure of Na,K-ATPase in the E1 free state is absent and it remains unclear which conformational transitions the enzyme undergoes when passing from the free E1 state to the nucleotide-binding state.

ATP and ADP bind to Na,K-ATPase in the E1 conformation with similar affinity$^{6,7}$, however ATP and ADP affect Na,K-ATPase in a different way. ATP was shown to protect the enzyme from inactivation by hydroxyl radicals and ADP does not produce such effect$^8$. Structural organization of the free Na,K-ATPase and as complexes with ATP or ADP may be different. This assumption is supported by the fact that crystal structures of the Na,K-ATPase homolog – sarco (endo) plasmic reticulum Ca$^{2+}$-ATPase (SERCA) in the Ca$^{2+}$-bound E1 state$^{9,10}$, and in complex with a nonhydrolyzable ATP analog - adenosine (β - γ methylene)-triphosphate$^{11}$, differ from each other by the arrangement of N and A domains with respect to the P domain.

In this work, we have used isothermal titration calorimetry (ITC) to estimate the equilibrium constants along with the enthalpic and entropic components for the A-nucleotides (AMP, ADP and ATP) binding to Na,K-ATPase in the E1 state at different temperatures. Previously the Na,K-ATPase interactions with adenine nucleotides have been studied by ITC, at a single temperature value and the thermodynamic parameters of the enzyme binding with ATP were not reported$^{12}$. Thus no conclusions could be made regarding conformational changes in

Received 20 December 2013
Accepted 14 May 2014
Published 4 June 2014

Correspondence and requests for materials should be addressed to A.A.M. (aamakarov@eimb.ru)

* These authors contributed equally to this work.
the protein upon binding of nucleotides. In this study we have found that β-phosphate is involved in complex formation by increasing the affinity of nucleotides to Na,K-ATPase by an order of magnitude, while γ-phosphate does not affect it. Furthermore, we have demonstrated that ATP binding to Na,K-ATPase in contrast to the ADP binding generates a structural transition in the enzyme, which is compatible with the movement of molecular surface area (ranging from 4021 to 5891 Å²) from a solvent-exposed to solvent-protected state. Comparison of the ITC data with the structure modeling results for Na,K-ATPase in the E1 free state and in the ATP-bound state allowed us to conclude that this structural change is caused by the locking of the N domain of Na,K-ATPase to the P domain induced by the γ-phosphate of ATP, thereby reducing the exposed surface area of the protein.

**Results**

**Affinities of Na,K-ATPase to adenine nucleotides.** We used ITC to estimate the affinities (association ($K_a$) and dissociation ($K_d$) equilibrium constants) of AMP, ADP and ATP to duck ATPase at different temperatures (Table 1). It should be noted that the experimental conditions exclude ATP hydrolysis and formation of phosphorylated intermediate (see Methods). A typical set of ITC data for AMP, ADP and ATP binding to ATPase at 37°C is shown in Figure 1, including the “raw” calorimetric data for the ligand-into-protein titration (upper parts) and the binding curves (lower parts). The latter were fitted to a model with one adenine nucleotide binding site per ATPase molecule.

In the presence of 3 mM NaCl we observed a 13-fold and 15-fold higher affinity for the binding of ATPase to ADP compared to AMP at 25°C (Kd values equal to 0.07 and 0.9 µM) and at 37°C (Kd values equal to 0.04 and 0.6 µM), respectively (Table 1). Affinities of ATPase to ADP and ATP were similar (Table 1). The data show that β-phosphate significantly contributes to the affinity of nucleotides to ATPase, while γ-phosphate of ATP does not affect the affinity during complex formation. These data confirm previous suggestions regarding the key role of β-phosphate in the formation of Na,K-ATPase complexes with nucleotides. The obtained binding constants for ADP and ATP, are close to the values determined previously.

In the presence of 3 mM KCl instead of NaCl (E2 state), we failed to observe any measurable ATP or ADP binding to ATPase either at

| Table 1 | Thermodynamic parameters of the duck Na,K-ATPase binding to AMP, ADP and ATP determined by isothermal titration calorimetry |
| --- | --- | --- | --- | --- | --- | --- |
| Ligand | $T$, °C | $K_a$, M⁻¹ | $K_d$, µM | $\Delta H^\circ$, kcal/mole | $\Delta S^\circ$, kcal/mole | $\Delta G^\circ$, kcal/mole |
| AMP | 25 | $1.1 \times 10^6$ | 0.91 | −1.40 | 6.83 | −8.23 |
| AMP | 30 | $1.8 \times 10^6$ | 0.56 | −1.81 | 6.86 | −8.67 |
| AMP | 37 | $1.7 \times 10^6$ | 0.59 | −2.19 | 6.65 | −8.84 |
| ADP | 25 | $1.4 \times 10^7$ | 0.07 | −5.68 | 4.06 | −9.74 |
| ADP | 30 | $2.4 \times 10^7$ | 0.04 | −6.00 | 4.23 | −10.23 |
| ADP | 37 | $2.6 \times 10^7$ | 0.04 | −6.21 | 4.31 | −10.52 |
| ATP | 10 | $1.2 \times 10^7$ | 0.08 | −7.70 | 16.87 | −9.17 |
| ATP | 25 | $1.6 \times 10^7$ | 0.06 | −4.00 | 5.82 | −9.82 |
| ATP | 37 | $2.0 \times 10^7$ | 0.05 | −12.80 | −2.44 | −10.36 |

*All measurements were performed three to four times in imidazole buffer (25 mM imidazole, 1 mM DTT, 1 mM EDTA, 3 mM NaCl and 250 mM sucrose, pH 7.5).* 
*Kₐ – affinity constant; standard deviation did not exceed ±20%.* 
*Kₖ – dissociation constant; calculated as 1/Kₐ.* 
*$\Delta H^\circ$ – enthalpy variation; standard deviation did not exceed ±10%.* 
*$\Delta S^\circ$ – entropy variation; calculated from the equation $\Delta S^\circ = \Delta H^\circ / T$.* 
*$\Delta G^\circ$ – Gibbs energy; calculated from the equation $\Delta G^\circ = -RT \ln K_a$. 

*Figure 1 | Na,K-ATPase interaction with adenine nucleotides measured by ITC. Titration curves (upper panels) and binding isotherms (lower panels) for duck Na,K-ATPase interaction with AMP (A), ADP (B) and ATP (C) at 37°C and pH 7.5.*
had low D (Figure 2, Table 2). Na,K-ATPase binding both to AMP and ADP formation. The large decrease in solvent accessible surface area (SAA) during the process of complex formation was equal to 450 μM. The concentrations of Na,K-ATPase used in this study render it not possible to determine the Kd values by ITC.

Energetics of complex formation between Na,K-ATPase and adenine nucleotides. In addition to the binding constant estimates, obtained from the shape of the binding curves, we also estimated the change of enthalpy, ΔH, upon binding of an adenine nucleotide to Na,K-ATPase (Table 1). Plotting the enthalpy of the interaction versus temperature we obtained heat capacity change (ΔCp) values for the ATPase interactions with adenine nucleotides as d(ΔH)/dT (Figure 2, Table 2). Na,K-ATPase binding both to AMP and ADP had low ΔCp of −66 and −43 cal·mol⁻¹·K⁻¹, respectively, and the ATP binding to Na,K-ATPase was characterized by massive ΔCp of −760 cal·mol⁻¹·K⁻¹ (Table 2). On the assumption of additive contributions of the different phosphor groups to the ΔCp values, we found that the contribution of γ-phosphate is equal to −717 cal·mol⁻¹·K⁻¹.

Changes in heat capacity are believed to reflect the change in the solvent accessible surface area (SAA) during the process of complex formation. The large decrease in ΔCp upon complex formation between Na,K-ATPase and ATP suggests that ATP binding to Na,K-ATPase significantly decreases its SAA. To assess changes in SAA by ITC a number of empirical formulas are used, which connect the changes in ΔCp and the changes of water-accessible polar and nonpolar surface area in proteins. In the review it was shown that formulas representing individual contributions of polar and nonpolar groups can be replaced by the formula with a single coefficient: ΔCp = k × ΔA tot, where ΔA tot is the total change in SAA in Å². The value of k reported in different studies varies from 0.129 to 0.189 cal/(K·mol·Å²). Using these values we estimated the total decrease in protein SAA when enzyme complexes with nucleotides are formed (Table 2).

Molecular modeling of the Na,K-ATPase structure changes upon ATP binding. Models have been constructed of the Na,K-ATPase in the “open” E1 free state and “closed” ATP-bound state (Figure 3). In the E1 “open” state the N and P domains are separated in space. In the E1 “closed” state the N domain is positioned closer to the boundary between the P and A domains, with the ATP γ-phosphate in the N domain interacting with the side chain of residue Asp369 in the P domain. The SAA, estimated for an “open” E1 and “closed” E1 conformations were 39436 Å² and 34687 Å², respectively. Thus, the difference in SAA between the “open” and “closed” ATP-binding state conformations is 4749 Å². This is in good agreement with the data of ΔA tot obtained from ITC (Table 2).

**Table 2 | Heat capacity changes and change in the solvent-accessible surface area for the duck Na,K-ATPase binding to AMP, ADP and ATP.**

| Ligand | ΔCp, cal·mol⁻¹·K⁻¹ | ΔAtot_min, Å² | ΔAtot_max, Å² |
|--------|------------------|---------------|---------------|
| AMP    | −66              | 349           | 551           |
| ADP    | −43              | 228           | 333           |
| ATP    | −760             | 4021          | 5891          |

**Discussion**

The classical scheme of Na,K-ATPase function consists of the two major conformational transitions: between the phosphorylated forms of the enzyme E1P-E2P and between the non-phosphorylated forms E2-E1. From the crystal structure of Na,K-ATPase it is known that in the E2 conformation the N and P domains are positioned close to each other, while limited proteolysis results showed that in the E1 conformation these domains reside apart in three-dimensional space. Conformational transitions in the Na,K-ATPase molecule when binding nucleotides up to now has not been established.
changes in the γRF3 factors. Modeling of the Na,K-ATPase complex with the structures of elongation (EF-G) and translation termination catalytic subunit of Na,K-ATPase, SERCA, it was demonstrated mentally for ATP binding (Table 2). Thus, the structural changes of the Na,K-ATPase (Figure 3B), and shows the change of solvent accessible phosphate in the N domain and Asp369 in the P domain of Na,K-ATPase, produces results demonstrating convergence of the ATP binding and phosphorylation domains, resulting in the protein adopting the E1-closed conformation, ready for phosphorylation. Thus, γ-phosphate is a trigger for the structural change in Na,K-ATPase, converting it from the relaxed state to the ratcheted state.

Methods

Na,K-ATPase purification and activity measurements. Na,K-ATPase (α1β2 isoform) was purified from duck salt gland (for details see25–27) to the purity grade of 99% of total protein as confirmed by polyacrylamide gel electrophoresis in the presence of SDS. The enzyme preparations also contain a 13 kDa protein that is similar to proteins of the FXYD family28. Specific activity of the duck Na,K-ATPase reached ~1800–2000 μmol P i (mg protein × h)−1 at 37°C.

Hydrolytic activity of the duck Na,K-ATPase was estimated as ATP cleavage in the medium containing (in mM): 130 NaCl, 20 KCl, 3 MgCl2, 3 ATP, and 30 imidazole, pH 7.4 at 37°C26,29. Na,K-ATPase-induced ATP hydrolysis was measured as a difference in ATP cleavage rate in the presence or absence of the Na,K-ATPase specific inhibitor ouabain (1 mM)26. Reaction of the ATP cleavage was carried out in the volume 0.5 ml. It was initiated by the addition of the protein (2 μg) and stopped 2 min later by the addition of 0.5 ml of the 3 M cold acetate buffer. The method developed by Rathbun and Betlach26 was used to quantify the amount of inorganic phosphate (P i) spectrophotometrically. In the presence of ouabain, ATPase activity in the enzyme preparation was not detected, indicating the absence of other ATPases in the preparation.

Isothermal titration calorimetry (ITC). The thermodynamic parameters of adenosine nucleotides (ATP, ADP and AMP) binding to duck Na,K-ATPase were measured using a MicroCal iTC200 instrument (MicroCal, Northampton, MA), as described elsewhere26. Experiments were carried out at 10, 25, 30 and 37°C in imidazole buffer containing 5 mM imidazole, 1 mM EDTA, 250 mM sucrose, 0.1 mM DTT, and either 3 mM NaCl or 3 mM KCl, pH 7.5. Experiments were conducted in the absence of Mg2+, preventing the formation of phosphorylated intermediate25. Absence of ATP hydrolysis by the enzyme was monitored by detecting P i. Aliquots (2.6 μl) of ligands were injected into a 0.2-ml cell containing the protein solution to achieve a complete binding isotherm. Protein concentration in the cell ranged from 5 to 20 μM, and ligand concentration in the syringe ranged from 50 to 200 μM. The heat of dilution was measured by injection of the ligand into the buffer solution or by additional injections of ligand after saturation; the values obtained were subtracted from the heat of reaction to obtain the effective heat of binding. The resulting titration curves were fitted using the MicroCal Origin software, assuming one set of binding sites. Affinity constants (K D) and enthalpy variations (ΔH) were determined and the Gibbs energy (AG) and entropy variations (ΔS) were calculated from the equation: ΔG = −RTlnK D = ΔH − TΔS.

Modelling. Three-dimensional models of the Na,K-ATPase catalytic τ-subunit in the E1 “opened” and “closed” state were created on the basis of the previously published 2.8 A structure of a porcine τ-subunit in the [Na]+[E1P-ADP] state (PDB code 3wgu)30. For modeling of the complex of Na,K-ATPase in the ATP E1 “closed” state the ATP molecule was placed into the cavity of the ATP-binding site of the enzyme similarly to the ADP molecule19. Modeling the enzyme in the “opened” E1 free state was carried out by changing the geometry of the polypeptide chain of the boundary segments 375–379 and 586–590 of the N-domain, by analogy to the changes observed in SERCA upon nucleotide binding, specifically exemplified by the rigid body motion of N-domain relative to the A- and P-domains31. The resulting model structures of Na,K-ATPase were minimized in the force field MMFF94x using the MOE version 2013.08 modeling software (Chemical Computing Group Inc., Montreal, Quebec, Canada). The solvent accessible surface area of the τ-subunit of Na,K-ATPase was calculated with the server http://structure.pitt.edu/anchor.

1. Schainer-Bobis, G. The sodium pump. Its molecular properties and mechanics of ion transport. Eur. J. Biochem. 269, 2424–2433 (2002).
2. Apell, H. J. & Karlish, S. I. Functional properties of Na,K-ATPase, and their structural implications, as detected with biophysical techniques. J. Membr. Biol. 180, 1–9 (2001).
3. Geering, K. The functional role of beta subunits in oligomeric P-type ATPases. J. Bioenerg. Biomembr. 33, 425–431 (2001).
4. Nyblom, M. et al. Crystal structure of Na+, K(+)•ATPase in the Na(+)-bound state. Science 342, 123–127 (2011).
5. Kani, R., Ogawa, H., Vilsen, B., Cornelius, F. & Toyoshima, C. Crystal structure of Na+, K(+)•ATPase preceding the E1P state. Nature 502, 201–206 (2013).
6. Grell, E., Lewitzki, E., Schacht, A. & Stolz, M. Nucleotide/Protein Interactions: Energetic and Structural Features of Na,K-ATPase. J. Therm. Anal. Calorim. 77, 471–481 (2004).
7. Fedosova, N. U., Chapmell, P. & Esmann, M. Rapid titration analysis of nucleotide binding to Na,K-ATPase. Biochemistry 42, 3536–3543 (2003).
8. Xu, K. Y., Zweier, J. L. & Becker, L. C. Oxygen-free radicals directly attack the ATP binding site of the cardiac Na+, K(+)•ATPase. Ann. N.Y. Acad. Sci. 834, 680–683 (1997).
9. Toyoshima, C., Nakasako, M., Nomura, H. & Ogawa, H. Crystal structure of the calcium pump of sarcoplasmic reticulum at 2.6 Å resolution. Nature 405, 647–655 (2000).
10. Winther, A. M. et al. The sarcoplasmic-bound calcium pump stabilizes calcium sites exposed to the cytoplasm. Nature 495, 265–269 (2013).
11. Sorensen, T. L., Muller, J. V. & Nissen, P. Phosphorylation and calcium ion occlusion in the calcium pump. Science 304, 1672–1675 (2004).
12. Pilotelle-Bunner, A. et al. ATP binding equilibria of the Na(+), K(+)•ATPase. Biochemistry 47, 13103–13114 (2008).
13. Grycova, L. et al. ATP and magnesium drive conformational changes of the Na(+) / K(+)•ATPase cytoplasmic headpiece. Biochim. Biophys. Acta. 1081–1091 (2009).
14. Skou, J. C. The Na,K-pump. Methods Enzymol. 156, 1–25 (1988).
15. Ladbury, J. E. & Doyle, M. L. Biocatalysis 2. Applications Calorimetry In The Biological Sciences. (The Sussex John Wiley & Sons, Ltd, 2004).
16. Hedwig, G. R. & Hinz, H. J. Group additivity schemes for the calculation of the partial molar heat capacities and volumes of unfolded proteins in aqueous solution. Biochim. Phys. 100, 239–260 (2003).
17. Jelezovski, I. & Bosshard, H. R. Isothermal titration calorimetry and differential scanning calorimetry as complementary tools to investigate the energetics of biomolecular recognition. J. Mol. Recognit. 12, 3–18 (1999).
18. Kairane, C., Mahlapuu, R., Ehrlich, K., Zilmer, M. & Soomets, U. The effects of different antioxidants on the activity of cerebrocortical MnSOD and Na,K-ATPase from post mortem Alzheimer’s disease and age-matched normal brains. Curr. Alzheimer Res. 11, 79–85 (2014).
19. Hauriulk, V. et al. The pretranslocation ribosome is targeted by GTP-bound EF-G in partially activated form. Proc. Natl. Acad. Sci. U S A 105, 15678–15683 (2008).
20. Peruzo, R., Folkers, G. & Scapoza, I. Thermodynamics of protein-ligand interactions: history, presence, and future aspects. J. Recept Signal Transduct Res. 24, 1–2 (2004).
21. Nordt, A. D. & Murphy, K. P. Protein Structure and the Energetics of Protein Stability. Chem Rev. 97, 1251–1268 (1997).
22. Shinoda, T., Ogawa, H., Cornelius, F. & Toyoshima, C. Crystal structure of the sodium-potassium pump at 2.4 Å resolution. Nature 459, 446–450 (2009).
23. Segall, L., Javid, Z. Z., Carl, S. L., Lane, J. K. & Blostein, R. Structural basis for alpha1 versus alpha2 isoform-different behavior of the Na,K-ATPase. J. Biol. Chem. 278, 9027–9034 (2003).
24. Fuller, W., Parmar, V., Eaton, P., Bell, J. R. & Shattock, M. J. Cardiac ischemia causes inhibition of the Na,K ATPase by a labile cytosolic compound whose production is linked to oxidant stress. Cardiovasc Res. 57, 1044–1051 (2003).
25. Kononenko, A. V. et al. GTP-dependent structural rearrangement of the eRF1:eRF3 complex and eRF3 sequence motifs essential for PABP binding. *Nucleic Acids Res.*, 38, 548–558 (2010).

26. Boldyrev, A. A., Lopina, O. D., Kenney, M. & Johnson, P. Characterization of the subunit isoforms of duck salt gland Na/K adenosine triphosphatase. *Biochem Biophys Res Commun.*, 216, 1048–1053 (1995).

27. Petrushanko, I. Y. et al. S-glutathionylation of the Na,K-ATPase catalytic α subunit is a determinant of the enzyme redox sensitivity. *J. Biol. Chem.*, 287, 32195–32205 (2012).

28. Jørgensen, P. L. Purification of Na+, K+ -ATPase: enzyme sources, preparative problems, and preparation from mammalian kidney. *Methods Enzymol.*, 156, 29–43 (1988).

29. Smith, T. W. Purification of Na+, K+ -ATPase from the supraorbital salt gland of the duck. *Methods Enzymol.*, 156, 46–48 (1988).

30. Yakushev, S. S., Kumskova, E. M., Rubtsov, A. M. & Lopina, O. D. Effect of colchicine on sensitivity of duck salt gland Na,K-ATPase to Na+. *Biochemistry (Mosc)*, 73, 990–994 (2008).

31. Petrushanko, I. et al. Na-K-ATPase in rat cerebellar granule cells is redox sensitive. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, 290, R916–925 (2006).

32. Komniski, M. S., Yakushev, S., Bogdanov, N., Gassmann, M. & Bogdanova, A. Interventricular heterogeneity in rat heart responses to hypoxia: the tuning of glucose metabolism, ion gradients, and function. *Am. J. Physiol. Heart Circ. Physiol.*, 300, H1645–1652 (2011).

33. Rathbun, W. B. & Betlach, M. V. Estimation of enzymically produced orthophosphate in the presence of cysteine and adenosine triphosphate. *Anal. Biochem.*, 28, 436–445 (1969).

34. Mitkevich, V. A. et al. Termination of translation in eukaryotes is mediated by the quaternary eRF1*eRF3*GTP*Mg2+ complex. The biological roles of eRF3 and prokaryotic RF3 are profoundly distinct. *Nucleic Acids Res.*, 34, 3947–3954 (2006).

**Acknowledgments**

This study was funded by the Molecular and Cellular Biology Program of the Russian Academy of Sciences and by the Russian Foundation for Basic Research (grants 14-04-01737 and 12-04-00403).

**Author contributions**

I.Yu.P. and V.A.M. conceived the project, coordinated the study and drafted the paper with contributions from A.A.A., O.D.L. and A.A.M. I.Yu.P., V.A.M., E.A.K. and E.A.D. performed experiments. A.A.A. analyzed the structural data. A.A.M. coordinated the study and contributed materials and reagents.

**Additional information**

Competing financial interests: The authors declare no competing financial interests.

**How to cite this article**: Petrushanko, I.Y. et al. Critical role of γ-phosphate in structural transition of Na,K-ATPase upon ATP binding. *Sci. Rep.*, 4, 5165; DOI:10.1038/srep05165 (2014).

This work is licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported License. The images in this article are included in the article’s Creative Commons license, unless indicated otherwise in the image credit; if the image is not included under the Creative Commons license, users will need to obtain permission from the license holder in order to reproduce the image. To view a copy of this license, visit http://creativecommons.org/licenses/by-nc-sa/3.0/