Localization of a K\(^+\) binding Site Involved in Dephosphorylation of the Sarcoplasmic Reticulum Ca\(^{2+}\)-ATPase*  

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The atomic coordinates and structure factors (code 1T5T) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ 08903-0993 (http://www.rcsb.org/).}
Table 1

Summary of crystallographic parameters

| Form               | Ca$_2^+$-ADP:AlF$_4^-$ (RbCl) |
|--------------------|--------------------------------|
| Space Group        | C2                             |
| Cell dimensions    | a = 162.1, b = 75.1, c = 150.0, β = 109.3$^\circ$ |
| Wavelength (Å)     | 0.81                           |
| Resolution         | 40–3.3 (3.42–3.3)              |
| $R_{sym}$          | 0.099 (0.42)                   |
| $I/σ$              | 11.1 (2.1)                     |
| $R_{sym}$          | 9.8%                           |
| Completeness (%)   | 91.4 (61.0)                    |

$^a$ $R_{sym} = \sum I(h) - \langle I(h) \rangle / \sum I(h)$, where $I$ is the $i$th measurement.

$^b$ $R_{sym}$ is calculated like $R_{sym}$ upon scaling the data set to the Ca$_2^+$-ADP:AlF$_4^-$ (RbCl) form.

$^c$ Anomalous data.

Results and Discussion

Structural Analysis—Crystals of the Ca$_2^+$-ATPase in the Ca$_2^+$-ADP:AlF$_4^-$ form grown in the presence of either 80 mM KCl or 80 mM RbCl belong to the same crystal form. The structure of the Ca$_2^+$-ADP:AlF$_4^-$ form refined at 2.6-Å resolution was used as the starting model for structure determination and refinement of the Ca$_2^+$-ADP:AlF$_4^-$ (KCl) form at 2.9-Å resolution as described previously (11). Crystals grown in the presence of Rb$^+$ diffracted to 3.3-Å resolution, and data were collected using synchrotron radiation with a wavelength of 0.81 Å where a significant anomalous signal of rubidium is present ($I = 3.5\, e$) (Table I). CNS was used to generate an anomalous difference Fourier map (17) based on data from 40- to 3.3-Å resolution and phases previously derived from the refined model of the KCl form. A single positive peak at 7.1Å (background maximum of 4.5σ) was identified at a site displaying the structure of a typical cation-binding site as defined by interactions with several carbonyl and carboxylate oxygens (Fig. 1A). Similarly, a $F_o(RbCl) - F_o(KCl)$ difference Fourier map showed a positive peak at 7.2Å in the same site (Fig. 1A). The site was therefore assigned as a K$^+$ site in the native enzyme.

Description of the Identified Rb$^+$/K$^+$ Site—Fig. 1B shows the overall structure of the Ca$_2^+$-ATPase in the E1-ADP:AlF$_4^-$ conformation with the K$^+$ site located between the two C-terminal helices of the P-domain, over the M3 and M5 segments. This localization in the cytoplasmic domain is in agreement with biochemical data demonstrating that K$^+$ exerts its effect on the function of the Ca$_2^+$-ATPase when present on the cytoplasmic side (8).

In the electron density maps of the Ca$_2^+$-ATPase with bound AMPPCP or ADP:AlF$_4^-$ we had previously identified additional density at this position that could not originate from the atoms of the enzyme. Also, the density could not be accounted for by a water molecule since this led to unrealistic low B-factors compared with the B-factors of surrounding protein atoms and an impossible hydrogen bonding network. On the other hand, placing a potassium ion at the site provided a sensible chemical model which was substantiated by further refinement at 2.6-Å resolution of the Ca$_2^+$-ADP:AlF$_4^-$ form, in agreement with the finding with rubidium. This leads to the conclusion that K$^+$ is coordinated with carbonyl oxygens from residues 711, 712, and 714 together with one side chain oxygen of Glu$^{732}$, thereby providing a total of four of the six ligands required for an octahedral coordination of the potassium ion. Moreover, the observed site is surface-located, resulting in the potassium ion being exposed (Fig. 1B), thereby allowing solvent molecules to fulfill the coordination.

A Na$^+$ to (Rb$^+$ or K$^+$) ratio of 2.5:1 was present at the given experimental conditions and a partial occupancy by Na$^+$ in the crystals cannot be excluded, since Na$^+$ also stimulates dephosphorylation, although with a somewhat lower affinity than K$^+$ (2, 3). Indeed, Toyoshima and co-workers (10, 18) place a Na$^+$ ion at the same site in their refined structures of the Ca$_2^+$-E1 and Ca$_2^+$-AMPPCP forms obtained in the absence of K$^+$. However, at typical physiological conditions with a very high K$^+$ to Na$^+$ ratio the site is most likely occupied by K$^+$.

We have also identified density corresponding to this K$^+$ site in preliminary maps of the Ca$_2^+$-ATPase in the E2-thapsig-
Evidence has accumulated that dephosphorylation rates of Ca\(^{2+}\)-ATPase activity, relative to wild type, in the absence of K\(^+\) are diminished throughout the enzyme cycle. From these data it is clear that Glu\(^{732}\) is important for the function of the thyroparathyroid hormone receptor, and that the mutation to alanine relieves an inhibition on the Ca\(^{2+}\) sensitivity. 

The Glu\(^{732}\) hydrolysis of the ADP-insensitive phosphoenzyme intermediate, E\(^2P\), is one of the partial reaction steps contributing to rate limitation of the enzyme cycle under the experimental conditions used for measurement of ATPase activity described above. 

### Mutagenesis Analysis of the Importance of Glu\(^{732}\) in K\(^+\) Modulation of Function

To test the functional importance of the K\(^+\) site described above, Glu\(^{732}\) was replaced by either alanine or glutamate, and the mutant and wild-type enzymes, expressed in COS-1 cell endoplasmic reticulum membranes, were compared with respect to K\(^+\) modulation of function. Table II, left part, shows the steady-state turnover rates for ATP hydrolysis determined at 37 °C in the presence of optimal concentrations of Ca\(^{2+}\) and ATP, with and without K\(^+\) added. It is seen that the addition of 100 mM K\(^+\) induced a ~2-fold increase of the turnover rate for wild-type enzyme, whereas mutant Glu\(^{732}\) → Ala showed close to maximum activity already in the absence of K\(^+\) with little further activation occurring upon the addition of K\(^+\). Mutant Glu\(^{732}\) → Gln, on the other hand, behaved much like wild type, with K\(^+\) inducing a ~2-fold activation.

The hydrolysis of the ADP-insensitive phosphoenzyme intermediate, E\(^2P\), is one of the partial reaction steps contributing to rate limitation of the enzyme cycle under the experimental conditions used for measurement of ATPase activity described above, particularly in the absence of K\(^+\). This step was examined separately by determining the dephosphorylation rate following phosphorylation of the enzyme with \(^{32}\)P, (Fig. 2 and Table II, right part). The addition of 100 mM K\(^+\) increased the dephosphorylation rate by a factor of 14 in wild type and by a factor of 11 in mutant Glu\(^{732}\) → Ala. The dephosphorylation rate was activated only 2.6-fold, thus indicating a very significant reduction of the sensitivity to K\(^+\). In the absence of K\(^+\), the dephosphorylation rate of Glu\(^{732}\) → Ala was 1.5-fold higher than that corresponding to wild type, in line with the finding of higher ATPase activity, relative to wild type, in the absence of K\(^+\). From these data it is clear that Glu\(^{732}\) is important for the modulation of E\(^2P\) dephosphorylation by K\(^+\) and that this function is disrupted by removal of both side chain oxygen atoms of Glu\(^{732}\) (in Glu\(^{732}\) → Ala) but not by the selective removal of only one of them (in Glu\(^{732}\) → Gln), thus indicating that Glu\(^{732}\) donates one of its side chain oxygen atoms, but not necessarily both, to K\(^+\) binding. This notion correlates well with the crystal structure, as shown in Fig. 1A. It is also clear that the mutation to alanine relieves an inhibition existing when the side chain oxygen is not coordinated by K\(^+\).

The Glu\(^{732}\) → Ala mutation will destabilize the K\(^+\)–binding site described above, Glu\(^{732}\) was replaced by either alanine or glutamine leading to a 5 Å distance of the K\(^+\)–binding site identified here, and the models for the structure of the vanadate-bound

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E2 form suggested on the basis of analysis of two-dimensional membrane crystals (25, 26) also indicate that these sites come close together. Hence they might interact directly in the E2P state (23), and the K⁺ ion could, thus, play a role as a “cross-linker” that stabilizes the interaction between the lower part of domain P and the A-M3 linker. Indeed, it should be possible for the K⁺ ion with free, solvent-exposed ligand-binding sites to coordinate incoming oxygen atoms from residues of the A-M3 linker.

Which advantages would the K⁺ ion offer as such an inter-domain cross-linker, compared with, for example, a salt bridge? First of all, the interaction must conform to the coordination of the K⁺ ion, and it will therefore be sensitive to conformational changes. This may serve as a regulatory switch in the E2P to E2 transition, where a close yet transient interaction between the P-domain and the A-M3 linker may stimulate the dephosphorylation reaction. In support of this view, Na⁺ and Rb⁺ display coordination chemistries similar to K⁺ and are also observed to have a stimulatory effect on the dephosphorylation rate, whereas the chemically far more distinct Li⁺ does not.

Is the K⁺ Site Conserved in the P-type ATPase Family?—The alignment in Fig. 1c shows that the Glu⁷³ residue is highly conserved as either Glu or Asp. The residues making up the 710–715 loop are only partly conserved, but since only the backbone carbonyls are contributing to the binding site this is not surprising. More importantly Pro⁷⁰⁹ is highly conserved and is likely to be essential for the formation of the loop by discontinuing the preceding helix. Taken together, this suggests that the site may be a general feature of P-type ATPases. A stimulatory effect of K⁺ has also been found in the plant H⁺-ATPase (27), indicating the possible functional role of the site in this pump, as well. For Na⁺, K⁺ and H⁺, K⁺-ATPases, K⁺ is a direct substrate for intramembrane cation-binding sites, which then either activate or inhibit the pump (when binding from the extracellular or cytoplasmic side, respectively). An additional, modulatory effect of the cytoplasmic K⁺ site described here may therefore be obscured in most functional studies of Na⁺, K⁺- and H⁺, K⁺-ATPases, and the importance of a possible, modulatory effect of K⁺ on these pumps may need to be addressed by renewed interpretation and acquisition of experimental data similar to those described here for the Ca²⁺-ATPase.

REFERENCES

1. Møller, J. V., Juul, B., and le Maire, M. (1996) Biochim. Biophys. Acta 1286, 1–51
2. Shigekawa, M., and Pearl, L. J. (1976) J. Biol. Chem. 251, 6947–6952
3. Shigekawa, M., and Dougherty, J. P. (1978) J. Biol. Chem. 253, 1451–1457
4. Andersen, J. P., Lassen, K., and Møller, J. V. (1985) J. Biol. Chem. 260, 371–380
5. De Meis, L., and Hasselbach, W. (1971) J. Biol. Chem. 246, 4759–4763
6. Champeil, P., Henao, F., and de Foresta, B. (1997) Biochemistry 36, 12383–12393
7. Lee, A. G., Baker, K., Khan, Y. M., and East, J. M. (1985) Biochem. J. 215, 225–231
8. Shigekawa, M., and Wakabayashi, S. (1985) J. Biol. Chem. 260, 11679–11687
9. Buoninsegni, F. T., Bartolommei, G., Moncelli, M. R., Inesi, G., and Guidelli, R. (2004) Biophys. J. 86, 3671–3686
10. Toyoshima, C., and Inesi, G. (2004) Annu. Rev. Biochem. 73, 269–292
11. Sørensen, T. L., Møller, J. V., and Nissen, P. (2004) Science 304, 1672–1675
12. Doyle, D. A., Morais Cabral, J., Pfuetzner, R. A., Kuo, A., Gulbis, J. M., Cohen, S. L., Chait, B. T., and MacKinnon, R. (1998) Science 280, 69–77
13. Nissen, P., Hansen, J., Ban, N., Moore, P. B., and Steitz, T. A. (2000) Science 289, 920–930
14. Otwinowski, Z., and Minor, W. (1997) Methods Enzymol. 276, 307–326
15. Sørensen, T. L., and Andersen, J. P. (2000) J. Biol. Chem. 275, 28854–28861
16. Clausen, J. D., and Andersen, J. P. (2003) Biochemistry 42, 2585–2594
17. Brunger, A. T., Adams, P. D., Clore, G. M., DeLano, W. L., Gross, P., Grosse-Kunstleve, R. W., Jiang, J. S., Kuszniewski, J., Nilges, M., Pannu, N. S., Read, R. J., Rice, L. M., Simonson, T., and Warren, G. L. (1998) Acta Crystallogr Sect. D Biol. Crystallogr. 54, 905–921
18. Toyoshima, C., and Mizutani, M. (2004) Natuure 430, 529–535
19. Champeil, P., le Maire, M., Andersen, J. P., Guilain, F., Gingold, M., Lund, S., and Møller, J. V. (1996) J. Biol. Chem. 261, 16372–16384
20. Clausen, J. D., Vilsen, B., McIntosh, D. B., Einholm, A. P., and Andersen, J. P. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 2776–2781
21. Montigny, C., Jaxel, C., Shainskaya, A., Vinh, J., Labas, V., Møller, J. V., Karlsh, S. J., and Le Maire, M. (2004) J. Biol. Chem. 279, 43971–43981
22. Lenoir, G., Picard, M., Gauron, C., Montigny, C., Le Marechal, P., Falson, P., Le Maire, M., Møller, J. V., and Champeil, P. (2004) J. Biol. Chem. 279, 9156–9166
23. Møller, J. V., Lenoir, G., Marchand, C., Montigny, C., le Maire, M., Toyoshima, C., Juul, B. S., and Champeil, P. (2002) J. Biol. Chem. 277, 38647–38659
24. Toyoshima, C., and Nomura, H. (2002) J. Biol. Chem. 277, 38647–38659
25. Toyoshima, C., Nakasako, M., Nomura, H., and Ogawa, H. (2000) Nature 405, 647–655
26. Xu, C., Rice, W. J., He, W., and Stokes, D. L. (2002) J. Mol. Biol. 316, 201–211
27. Varà, F., and Serrano, R. (1982) J. Biol. Chem. 257, 12826–12830
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