Regulation of the Na-pump by the Catalytic Subunit of Protein Kinase*

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The (Na^+,K^+)-ATPase (Na^+-pump) is responsible for the bulk transport of Na^+ and K^+ in most cells.[1] β-Adrenergic stimulation leads to an increase of intracellular c' AMP and a concomittant increase of monovalent cation transport.[2] A primary effect of c'AMP in the cell is activation of a protein kinase by a dissociation of its regulatory and catalytic subunits.[3] Therefore, in this study direct effects of a highly purified preparation of the catalytic subunit of protein kinase on (Na^+,K^+)-ATPase are investigated in order to gain information about possible hormonal regulation of cellular ion transport.

Experimental. The Tris-salt of ATP was prepared as described previously.[4] [γ-^{32}P] ATP was a product of New England Nuclear. (Na^+,K^+)-ATPase was prepared from the outer medulla of pig kidneys.[5] Catalytic subunit of protein kinase was prepared as described previously.[5,6] (Na^+,K^+)-ATPase activity was assayed at 30 °C and at 1 mM ATP, 2 mM $MgCl_2$, 100 mM NaCl and 10 mM KCl in 30 mM Tris-HCl buffer, pH 7.4. The activity was generally about 7 μmol (mg protein)$^{-1}$ min^{-1}. *p*-Nitrophenylphosphatase activity of the (Na^+,K^+)-ATPase preparation was assayed at 30 °C and at 3 mM *p*-nitrophenylphosphate (*p*-NPP), 3 mM $MgCl_2$ and 10 mM KCl in 20 mM imidazole-HAc buffer, pH 7.4. The phosphoenzyme intermediate of (Na^+,K^+)-ATPase was obtained at 100 μM ATP, 2 mM $MgCl_2$ and 100 mM NaCl in 30 mM Tris-HCl buffer, pH 7.4. In other experiments about 5 μg of the catalytic subunit of protein kinase (C) was included in the incubation medium and Na^+ was omitted. (Na^+,K^+)-ATPase was incorporated into liposomes and ^{22}Na-transport into these liposomes was measured as described.[7]

Result and discussion. Only a low extent of phosphorylation of (Na^+,K^+)-ATPase was obtained with Mg^{2+} only in the incubation medium (Table 1). A further addition of Na^+ resulted in the formation of the phosphoenzyme

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Table 1. Phosphorylation of (Na^+,K^+)-ATPase.

Addition	nmol ^{32}P/mg
ATPase	0.06
ATPase + Na	1.48
ATPase + C	2.36
C	0.11

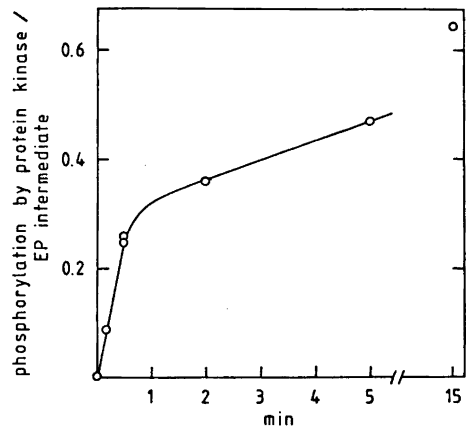

Fig. 1. Phosphorylation of (Na^+,K^+)-ATPase by the catalytic subunit of protein kinase.

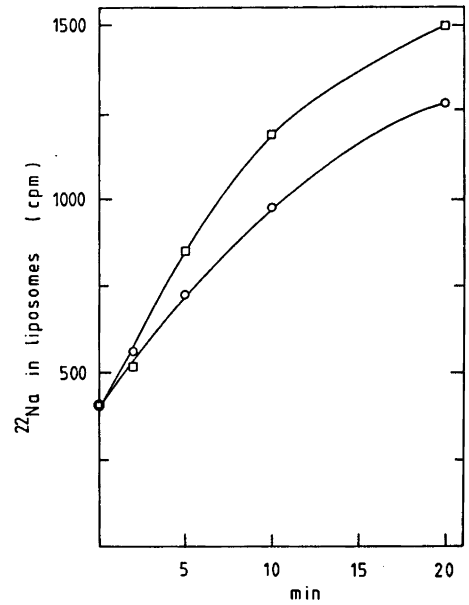

Fig. 2. ^{22}Na-Transport into liposomes with unphosphorylated (Na^+,K^+)-ATPase (○), and (Na^+,K^+)-ATPase phosphorylated by the catalytic subunit for 5 min before incorporation into liposomes (□).

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intermediate with a high extent of phosphorylation. Replacing Na^+ by the catalytic subunit of protein kinase resulted in a high extent of phosphorylation, too. The intermediary phosphorylation is extremely rapid,[4] while the protein kinase dependent phosphorylation was slow (Fig. 1). The rate of incorporation of phosphate into (Na^+,K^+)-ATPase by protein kinase appeared to be diphasic indicating that more than one site is phosphorylated. There was no significant change of either the Na^+ plus K^+-stimulated hydrolysis of ATP or the *p*-nitrophenylphosphatase activity after phosphorylation of (Na^+,K^+)-ATPase by the protein kinase catalytic subunit. In five separate experiments the ^{22}Na-transport, however, always was increased (15 – 50 %) in liposomes containing enzyme which had been phosphorylated by the catalytic subunit. Fig. 2 shows a time dependent study of ^{22}Na-transport in a reconstituted system. These results indicate that the increased rate of Na^+-transport, which has been reported to occur in intact cells upon stimulation by β-adrenergic hormones, at least partly might be due to an activation of (Na^+, K^+)-ATPase by a regulatory phosphorylation catalyzed by an intracellular c'AMP-stimulated protein kinase.

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1. Glynn, I. M. and Karlish, S. J. D. *Annu. Rev. Physiol. 37* (1975) 13.
2. Gardner, J. D., Klaevemen, H. L., Bilezikian, J. R. and Aurbach, G. D. *J. Biol. Chem. 248* (1973) 5590.
3. Soderling, T. R. and Park, C. R. *Adv. Cyclic Nucleotide Res. 4* (1974) 283.
4. Mårdh, S. and Post, R. L. *J. Biol. Chem. 252* (1977) 633.
5. Mårdh, S. Skou, J. C. and Nørby, J. G., Eds., *Na,K-ATPase, Structure and Kinetics*, Academic, London 1979, p. 359
6. Beavo, J. A., Bechtel, P. J. and Krebs, E. G. *Methods Enzymol. 38* (1974) 299.
7. Anner, B. M., Lane, L. K., Schwartz, A. and Pitts, B. J. R. *Biochim. Biophys. Acta 467* (1977) 340.

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Adenylate Kinase Activity Associated with Coupling Factor ATPase in *Rhodospirillum rubrum**

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The measurement of photophosphorylation and ATPase activity in chromatophores by continuous monitoring of the ATP concentration with a purified firefly reagent has been described.[1-3] Under the conditions used in the assay of photophosphorylation it was noted that there is some ATP formation from added ADP even without illumination of the chromatophores. This ATP formation does not require phosphate and is tentatively defined as an adenylate kinase activity. It could not be removed by extensive washings or by the specific adenylate kinase inhibitor diadenosine pentaphosphate, indicating that the adenylate kinase activity in question is not of the ordinary type. A transphosphorylation reaction of bound ADP to bound ATP and AMP has previously been reported to occur on the chloroplast coupling factor CF_1.[4] The present study was performed in order to find out if the adenylate kinase activity was associated with the coupling factor ATPase in *Rhodospirillum rubrum* chromatophores.

Experimental. The solubilization and reconstitution of coupling factor ATPase from chromatophores of *R. rubrum* have been described.[5] The coupling factor was solubilized by sonication of chromatophores in the presence of 1 mM EDTA and coupling factor depleted chromatophores were collected by centrifugation. Photophosphorylation and ATPase activities were reconstituted to the depleted chromatophores by incubation with solubilized coupling factor in the presence of 10 mM Mg^{2+}-ions.

Photophosphorylation, ATPase and adenylate kinase activities were measured in the original chromatophore preparation, the coupling factor depleted chromatophores, the solubilized coupling factor and the reconstituted chromatophores. In the assays the ATP concentration was monitored by continuously measuring the light emitted in the firefly luciferase reaction [1-3] using the ATP Monitoring Reagent according to the manufacturer (LKB-Wallac, Turku, Finland). The ATPase and adenylate kinase activities were measured in an LKB-Wallac Luminometer 1250 and photo-

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Table 1. Solubilization and reconstitution of coupling factor associated activities.

Fraction	Photophosphorylation [a] ATP/BChl, min	ATPase [b] ATP/BChl, min	Adenylate kinase [c] ATP/BChl, min
Chromatophores (original suspension)	6.7	0.056	0.64
Chromotophores (partially depleted)	1.0	0.022	0.09
Coupling factor [d] (crude prep.)	0.2	0.001 [e]	0.28
Chromotophores (partially reconst.)	3.6	0.046	0.40

[a] Measured with 20 μM ADP and 1 mM inorganic phosphate. [b] Measured with 0.5 μM ATP. [c] Measured with 20 μM ADP. [d] The activity in this chlorophyll-free fraction was measured in a concentration corresponding to the original chlorophyll containing suspension. [e] This low activity is due to the fact that solubilized coupling factor is Ca^{2+}-activated and Mg^{2+}-inhibited.[6]

phosphorylation in a previously described instrument.[3]

Results and discussion. Table 1 shows that in the chromatophores partially depleted of coupling factor all three activities, *i.e.* ATPase, adenylate kinase and photophosphorylation, were reduced to 39, 14 and 15 %, respectively, of the initial activity. Removed adenylate kinase activity was found in the solubilized coupling factor ATPase preparation. Table 1 also shows that all three activities could be restored to the partially depleted chromatophores by incubation with solubilized coupling factor.

The present study strongly indicates that the adenylate kinase activity in some way is associated with the coupling factor ATPase. Studies are under way in an attempt to find out if the adenylate kinase activity can be attributed to any of the subunits of purified coupling factor ATPase and if a corresponding adenylate kinase activity is associated also with chloroplast and mitochondrial coupling factor ATPase.

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1. Lundin, A., Thore, A. and Baltscheffsky, M. *FEBS Lett. 79* (1977) 73.
2. Lundin, A. and Baltscheffsky, M. In DeLuca, M., Ed., *Methods in Enzymology*, Academic, New York 1978, Vol. 57, p. 50.
3. Lundin, A. and Baltscheffsky, M. In Schram, E. and Stanley, P., Eds., *Proc. Int. Symp. on Analytical Applications of Bioluminescence and Chemiluminescence*, Brussels 1978. *In press.*
4. Roy, H. and Moudrianakis, E. N. *Proc. Natl. Acad. Sci. U.S.A. 68* (1971) 2720.
5. Johansson, B. C. *FEBS Lett. 20* (1972) 339.
6. Johansson, B. C., Baltscheffsky, M., Baltscheffsky, H., Buccarini-Melandri, A. and Melandri, B. *Eur. J. Biochem. 40* (1973) 109.

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