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CONVERSION OF THE Ca^{2+}-ATPase FROM RHODOSPIRILLUM RUBRUM INTO A Mg^{2+}-DEPENDENT ENZYME BY 1,N^6-ETHENO ATP

Hans-Jochen Schäfer, Hans Werner Müller°, and Klaus Dose

Institut für Biochemie der Johannes Gutenberg-Universität, Johann Joachim Becher-Weg 30, D-6500 Mainz, W.Germany

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SUMMARY: Nucleoside triphosphate hydrolysis of R. rubrum ATPase complexes can be changed from Ca^{2+}-dependence to Mg^{2+}-dependence by replacing ATP with 1,N^6-etheno ATP. Four ATPase complexes which have been prepared by different procedures hydrolyze ATP and 1,N^6-etheno ATP at different rates in dependence on the added metal ions. These differences allow an easy distinction of the various enzyme forms.

INTRODUCTION

The F_1 factor of the adenosine triphosphatase (ATPase) complex (EC 3.6.1.3) of the photobacterium Rhodospirillum rubrum (R. rubrum) is tightly bound to the chromatophore membrane probably via the δ subunit (1). This factor can be removed by different preparation methods (2-5). The various enzyme forms thus obtained differ in their molecular weight, electrophoretic mobility, and subunit composition (5). The oligomycin-sensitive ATPase complex can be released from chromatophore membranes by treatment with the nonionic detergent Triton X-100 (6,7). Preparations of this complex have also been analyzed for the subunit composition (8,9).

We have compared the hydrolysis of ATP and 1,N^6-etheno ATP (ε ATP) by four different ATPase preparations from R. rubrum in the presence of equimolar concentrations of magnesium or calcium ions. We demonstrate a change in the cation requirement

°Present address: Friedrich-Miescher-Laboratorium der Max-Planck-Gesellschaft, Spemannstr. 39, D-7400 Tübingen, W.Germany

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when replacing the substrate ATP by its analog εATP and suggest the use of this effect for a distinction of the various ATPase forms.

MATERIALS AND METHODS

Chloroacetaldehyde was obtained from Fluka AG. ATP disodium salt and all other chemicals were obtained from E. Merck. 1,N6-Etheno adenosine 5'-triphosphate (εATP) was synthesized by the reaction of ATP with chloroacetaldehyde according to Secrist et al. (10).

Chromatophores (membrane-bound ATPase) from R. rubrum, strain FR1 (DSM-No. 1068), were prepared by ultrasonication as described by Horio et al. (11). The oligomycin-sensitive ATPase complex was extracted from sodium cholate washed chromatophore membranes by 0.2 % Triton X-100 according to a published procedure (8). The purification of a chloroform-released F_1 ATPase complex was carried out as described earlier by our group (5). The ATPase complex solubilized by EDTA-ultrasonic treatment was prepared according to Johansson (4).

ATPase activity was determined continuously in 50 mM Tris-HCl buffer (pH 8.0) by measuring the liberated inorganic phosphate at 37 °C (12). The nucleotide hydrolysis was determined at the following Me^{2+}/nucleotide concentrations [mM]: 0.05, 0.1, 0.2, 0.5, 1.0, and 1.5.

RESULTS AND DISCUSSION

The membrane-bound ATPase activity of R. rubrum chromatophores is stimulated by either magnesium or calcium ions (3). As shown in Tab. 1, line a, Mg^{2+} ATP is more efficiently hydrolyzed than Ca^{2+} ATP by this enzyme form if the metal ions are present in amounts equimolar to the nucleoside triphosphate. The highest activity of the membrane-bound ATPase, however, is observed with Mg^{2+} ε ATP as substrate.

The Ca^{2+} ATPase activity of the isolated oligomycin-sensitive ATPase complex (Tab. 1, line b) is about three times that of the membrane-bound complex whereas its Mg^{2+} ε ATPase activity is almost unchanged. Mg^{2+} ATP is less efficiently hydrolyzed than Ca^{2+} ε ATP. This ATPase preparation contains the five subunits of the F_1 ATPase (α- ε) as well as at least three additional polypeptides (8).

Tab. 1 Relative velocity of nucleoside triphosphate hydrolysis ([Me^{2+}], [nucleotide] = 1.5 mM). Specific activity in brackets [µmoles Pi/min·mg protein].

		Ca·ATP	Mg·ATP	Ca·ε ATP	Mg·ε ATP
a	membrane-bound ATPase complex	100 (0.10)	158 (0.16)	10 (0.02)	434 (0.48)
b	oligomycin-sensitive ATPase complex [α, β, γ, δ, ε, and at least 3 additional polypeptides]	100 (0.30)	23 (0.07)	37 (0.11)	163 (0.50)
c	chloroform-released ATPase complex [α, β, γ, δ, ε]	100 (21.0)	7 (1.5)	6 (1.3)	120 (25.2)
d	EDTA-ultrasonic-released ATPase complex [α, β, γ, (δ)]	100 (12.0)	0 (0.0)	5 (0.6)	22 (2.4)

Tab. 1, line c, and Fig. 1 show the hydrolysis of ATP and ε ATP by the chloroform-released ATPase complex. This enzyme preparation hydrolyzes Ca^{2+} ATP and Mg^{2+} ε ATP at similarly high rates. But low rates are found for both Mg^{2+} ATP and Ca^{2+} ε ATP. The chloroform-released enzyme complex is composed of the five subunit polypeptides α- ε (5) known to be present in the F_1 moiety of coupling factor ATPases (13).

For the EDTA-ultrasonic-released ATPase complex Ca^{2+} ATP is the best of the four tested substrates (Tab. 1, line d). The rate of Mg^{2+} ε ATP hydrolysis by this enzyme preparation is only about 20 % that of Ca^{2+} ATP hydrolysis. Ca^{2+} ε ATP is hydrolyzed at much lower rate; Mg^{2+} ATP is no substrate for this enzyme complex. EDTA-ultrasonic treatment of chromatophore membranes from R. rubrum leads to the release of an enzymatically active complex which mainly consists of the α, β, and γ subunits of F_1 (1).

The relative rate of Mg^{2+} ε ATP hydrolysis is decreased stepwise from the membrane-bound ATPase via the isolated oligo-

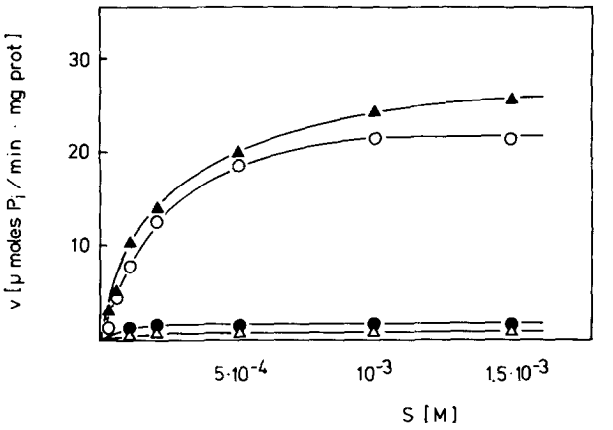

Fig. 1 Chloroform-released ATPase complex: hydrolysis of Ca·ATP (○), Mg·ATP (●), Ca·εATP (△), and Mg·εATP (▲); $[Me^{2+}]$:[nucleotide] = 1:1.

mycin-sensitive ATPase and the chloroform-released F_1 factor to the EDTA-ultrasonic-released enzyme complex. The corresponding Mg^{2+} ε ATPase/Ca^{2+} ATPase activity ratios are 4.8, 1.6, 1.2, and 0.2, respectively. These differences in the activity ratios of the ATPase preparations tested allow their easy distinction. As the stepwise decrease in the relative rate of Mg^{2+} ε ATP hydrolysis (Tab. 1) parallels a decrease in the number of the different subunits present in the isolated enzyme forms, Mg^{2+} ε ATP may also be a valuable tool to pursue the reconstitution of ATPase complexes.

The different cation requirement for the hydrolysis of ATP and εATP by one and the same enzyme form, e.g., by the chloroform-released ATPase complex (Fig. 1) can be interpreted by the different structural properties of the nucleotides or their metal complexes, respectively (14-17). Masking of the free N-1 and 6-NH_2 group by the etheno bridge seems to prevent the formation of a hydrolyzable Ca^{2+} ε ATP complex. On the other hand, coordination of Mg^{2+} ions with εATP leads to a hydrolyzable complex. Mg^{2+} ε ATP probably gains a structure different from

that of Ca^{2+} ε ATP, likely due to the different ion radii of Ca^{2+} and Mg^{2+} ions (0.099 and 0.065 nm, respectively).

On the other hand, the interaction of one of the Me^{2+} nucleotide complexes with the various ATPase modifications is not uniform. This observation can be interpreted by different abilities of the various enzyme forms to yield active nucleotide-metal-protein complexes. Recently, Soe et al. have reported a conversion of Ca^{2+} ATPase from an acetone powder of R. rubrum chromatophores into Mg^{2+} ATPase activity by pH indicators (18) and unsaturated fatty acids (19).

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REFERENCES

1 Müller, H.W., Schmitt, M., Schneider, E., and Dose, K. (1979) Biochim. Biophys. Acta 545, 77-85.
2 Philosoph, S., Binder, A., and Gromet-Elhanan, Z. (1977) J. Biol. Chem. 252, 8747-8752.
3 Johansson, B.C., Baltscheffsky, M., Baltscheffsky, H., Baccarini-Melandri, A., and Melandri, B.A. (1973) Eur. J. Biochem. 40, 109-117.
4 Johansson, B.C. (1972) FEBS Lett. 20, 339-340.
5 Müller, H.W., Schwuléra, U., Salzer, M., and Dose, K. (1979) Z. Naturforsch. 34c, 38-45.
6 Oren, R., and Gromet-Elhanan, Z. (1977) FEBS Lett. 79, 147-150.
7 Schneider, E., Müller, H.W., Rittinghaus, K., Thiele, V., Schwuléra, U., and Dose, K. (1979) Eur. J. Biochem. 97, 511-517.
8 Müller, H.W., and Baltscheffsky, M. (1979) Z. Naturforsch. 34c, 229-232.
9 Bengis-Garber, C., and Gromet-Elhanan, Z. (1979) Biochemistry 18, 3577-3581.
10 Secrist, J.A.III, Barrio, J.R., Leonard, N.J., and Weber, G. (1972) Biochemistry 11, 3499-3506.
11 Horio, T., Nishikawa, K., and Horiuti, Y. (1971) in Methods in Enzymology (San Pietro, A., ed.) Vol. 23, pp. 650-654, Academic Press, New York.
12 Arnold, A., Wolf, H.U., Ackermann, B.P., and Bader, H. (1976) Anal. Biochem. 71, 209-213.
13 Pedersen, P.L. (1975) J. Bioenerg. 6, 243-275.
14 Mildvan, A.S. (1970) in The Enzymes (Boyer, P.D., ed.) Vol. 2, pp.445-536, Academic Press, New York.
15 Diebler, H., Eigen, M., and Hammes, G.G. (1960) Z. Naturforsch. 15b, 554-560.

16 McCubbin, W.D., Willick, G.E., and Kay, C.M. (1973) Biochem. Biophys. Res. Commun. 50, 926-933.
17 Willick, G.E., Oikawa, K., McCubbin, W.D., and Kay, C.M. (1973) Biochem. Biophys. Res. Commun. 53, 923-928.
18 Soe, G., Nishi, N., Kakuno, T., and Yamashita, J. (1978) J. Biochem. 84, 805-814.
19 Soe, G., Nishi, N., Kakuno, T., Yamashita, J., and Horio, T. (1980) J. Biochem. 87, 473-481.