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Conversion of coupling factor 1 of *Rhodospirillum rubrum* from a Ca^{2+}-ATPase into a Mg^{2+}-ATPase

Birgitta Norling, Åke Strid and Pål Nyrén

Department of Biochemistry, Arrhenius Laboratory, University of Stockholm, Stockholm (Sweden)

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Isolation of F_1-ATPase from *Rhodospirillum rubrum* by chloroform extraction of chromatophores, followed by purification on a glycerol gradient, results in a very pure enzyme preparation containing five subunits with high Ca^{2+}-ATPase activity (15 μmol per min per mg protein). Furthermore, conditions are reported under which the purified F_1 exhibits Mg^{2+}-dependent ATPase activity of about 35 μmol per min per mg protein. $NaHCO_3$ stimulates the Mg^{2+}-activity from 1.5 μmol per min per mg protein to 5 μmol per min per mg protein giving a maximal activity at a concentration of about 60 mM $NaHCO_3$. Lauryl dimethylamine oxide (LDAO), octyl glucoside and nonanoyl *N*-methylglucamide enhance the Mg^{2+}-ATPase activity from 1.5 to 14, 22 and 35 μmol per min per mg protein, respectively, in the absence of $NaHCO_3$, and from 5 to 34, 30 and 37 μmol per min per mg protein, respectively, in the presence of 50 mM $NaHCO_3$. The V_{max} is increased, but the K_m for ATP remains the same, about 0.22 mM, both in the absence of activators and in the presence of $NaHCO_3$, LDAO or $NaHCO_3$ plus LDAO. Ca^{2+}-dependent ATPase activity is slightly stimulated by $NaHCO_3$ but strongly inhibited by octyl glucoside.

Introduction

The ATPase activity of *Rhodospirillum rubrum* chromatophores is dependent on the presence of various divalent cations, including Ca^{2+} and Mg^{2+} [1,2]. In contrast, it has been shown that purified coupling factor F_1 exhibits only Ca^{2+}-ATPase activity which is competitively inhibited by Mg^{2+} [3]. A similar change in divalent cation requirement also takes place upon resolution of the chloroplast ATPase. It has been described as allotopic, which implies that the substrate specificity is modified by membrane binding [4].

However, the latent Mg^{2+}-ATPase activity of F_1 isolated from *Rhodospirillum rubrum* chromatophores and from spinach chloroplasts can be stimulated. Certain detergents have been reported to stimulate Mg^{2+}-ATP hydrolysis of the two F_1-ATPases [5–7]. Isolated F_1 from *R. rubrum* was shown to exhibit a Mg^{2+}-ATPase activity of, maximally, 9 μmol per min per mg protein in the presence of dodecylsulfonic acid [5]. F_1 isolated from spinach chloroplasts showed a very high Mg^{2+}-ATPase activity, 120 μmol per min per mg protein, in the presence of octyl glucoside [7]. Various anions are also known to increase the rate of Mg^{2+}-dependent ATPase activity of the two types of isolated F_1 [8,9]. Sulphite has been reported to stimulate Mg^{2+}-dependent ATPase of F_1

Abbreviations: RrF_1, F_1-ATPase isolated from *Rhodospirillum rubrum* chromatophores; OG, *n*-octyl glucoside; LDAO, lauryl dimethylamine oxide; MEGA-9, nonanoyl *N*-methylglucomide; cmc, critical micellar concentration.

Correspondence: B. Norling, Department of Biochemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden.

isolated from *R. rubrum*, giving a maximal activity of 6 μmol per min per mg protein [8].

The present study shows that certain detergents, in combination with the anion HCO^-, reversibly enhance the Mg^{2+}-ATPase activity of isolated F_1 from *R. rubrum*. On the other hand, the Ca^{2+}-ATPase activity of the isolated enzyme is inhibited by octyl glucoside and only slightly stimulated by $NaHCO_3$.

Materials and Methods

R. rubrum cells were grown and harvested and chromatophores were prepared according to Ref. 10.

F_1-ATPase was isolated by chloroform extraction of chromatophores, according to a modified method described by Fisher et al. [11] for the mitochondrial enzyme. 2 ml of the chromatophore preparation, suspended in 0.2 M glycylglycine (pH 7.4) to about 2 mM bacteriochlorophyll, was incubated for 5 min at 37°C in 8 ml 25% ethylene glycol containing 0.2 mM EDTA. 50 μl chloroform was added to each batch of 2.5 ml, and the mixture was shaken for 20 s in a Vortex mixer. After centrifugation for 4 min in a bench centrifuge, the supernatant was centrifuged for 60 min at 4°C at 133500 × *g*. The supernatant was concentrated 8-fold by ultrafiltration through an Amicon XM-100 membrane. 0.4 ml was layered on top of a linear glycerol gradient, 20–50% (v/v) containing 50 mM Tris-Cl (pH 7.5) and 1 mM $MgCl_2$. The gradient was centrifuged for 18 h at 4°C at 35000 rpm in a Beckman SW 40 rotor. The active fractions were stored on ice. The purified enzyme was stable for at least 1 week. It showed five bands on silver stained Tricine-DISC-polyacrylamide electrophoresis [12]. Protein sequencing of the N-terminals of these five polypeptides showed that they were the products of the previously sequenced genes of the *atp* operon from *R. rubrum* (Walker, J.E., Falk, G. and Strid, Å., unpublished results and Ref. 13).

Mg^{2+}-ATPase activity was measured at 30°C by coupling the reaction to the pyruvate kinase and lactate dehydrogenase reactions and following the oxidation of NADH at 340 nm [14]. The reaction mixture contained 5 units of pyruvate kinase, 5 units of lactate dehydrogenase, 3 mM ATP, 3 mM $Mg(Ac)_2$, 30 mM KAc and 25 mM Tris-Ac (pH 8.0) unless otherwise stated.

The Ca^{2+}-ATPase activity was assayed at 30°C according to a modification [15] of the Rathbun method [16] previously described, except that 25 mM Tris-Ac (pH 8.0), 30 mM KAc, 5 mM $CaCl_2$ and 2.5 mM ATP were used in the assay medium.

The cmc for octyl glucoside and MEGA-9 was determined according to Ref. 17.

The Bio-Rad protein assay was used for determination of protein concentration.

Octyl glucoside was purchased from Sigma and MEGA-9 was from OXYL, Bobingen, F.R.G. LDAO, purified with mixed-bed ion-exchange chromatography, was a kind gift from Dr. Timo Pentillä.

Results

The specific Mg^{2+}-ATPase activity of F_1, isolated from chromatophores of *R. rubrum* by the chloroform extraction method described in the Materials and Methods, is only 1.4 μmol per min per mg protein. This can be compared with the Ca^{2+}-dependent activity, which is 12 μmol per min per mg protein. However, the Mg^{2+}-dependent ATPase activity can be stimulated 4-fold by $NaHCO_3$, which results in a maximal activity of almost 5 μmol per min per mg protein (Fig. 1) at a concentration of about 60 mM $NaHCO_3$. Ca^{2+}-

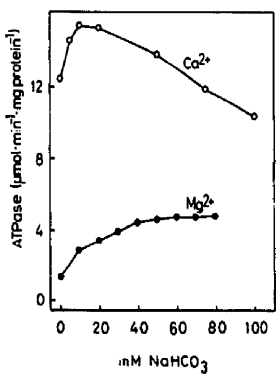

Fig. 1. Effect of $NaHCO_3$ on the ATPase activity of isolated RrF_1. Mg^{2+}-dependent ATPase (●———●); Ca^{2+}-dependent ATPase, (○———○). Enzyme activities were measured as described in Materials and Methods.

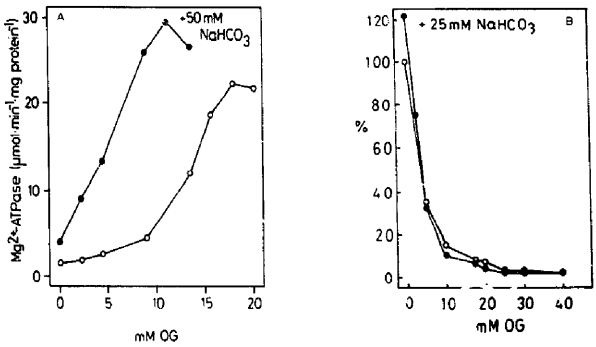

Fig. 2. Effect of octyl glucoside in the presence or absence of $NaHCO_3$ on the ATPase activity of isolated RrF_1 (A) Mg^{2+}-dependent ATPase; (B) Ca^{2+}-dependent ATPase. 100% activity corresponded to 12 µmol per min per mg protein. Enzyme activities were measured as described in Materials and Methods.

dependent ATPase activity is only slightly stimulated by bicarbonate, which increases the rate from 12 to 15 µmol per min per mg protein; maximal activity is obtained at 25 mM $NaHCO_3$ (Fig. 1).

As seen in Fig. 2A, octyl glucoside can stimulate the Mg^{2+}-dependent ATPase activity of isolated RrF_1 further, almost 8-fold, by increasing the activity from 4 µmol per min per mg protein to 30 µmol per min per mg protein; maximal activity is obtained at 11 mM octyl glucoside. Higher concentrations of the detergent are inhibitory. In the absence of bicarbonate, Mg^{2+}-ATPase activity is also stimulated by octyl glucoside. In this case, however, there is a sigmoidal dependence on the octyl glucoside concentration. Very little stimulation is accomplished below 10 mM of the detergent. On the other hand, as seen in Fig. 2B, Ca^{2+}-dependent ATPase activity is inhibited by octyl glucoside, both in the absence and presence of bicarbonate. The titers are identical, resulting in 50% inhibition at about 5 mM octyl glucoside.

From Fig. 3 it can be seen that the detergent MEGA-9 behaves very similarly to octyl glucoside. In the presence of 50 mM $NaHCO_3$, there is an 8-fold stimulation of the Mg^{2+}-ATPase activity, which is linearly dependent on the concentration of the detergent, whereas in the absence of bicarbonate the activity is sigmoidally dependent on the detergent concentration. The effect of MEGA-9 on the Ca^{2+}-ATPase activity of RrF_1 could not be studied, as MEGA-9 inhibited the color development of the phosphate assay.

The amphipathic detergent LDAO also stimulates the Mg^{2+}-dependent ATPase activity of isolated RrF_1 (see Fig. 4). In the presence of 50 mM $NaHCO_3$, again there is an 8-fold stimulation. The activity increases linearly from 4 to 33 µmol per min per mg protein and maximal activity is obtained at 1 mM LDAO. A higher concentration of LDAO causes a decrease in activity. In the absence of bicarbonate, there is a 10-fold activation. However, the specific activity is lower, ranging

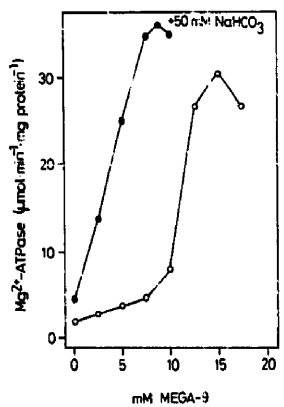

Fig. 3. Effect of MEGA-9 in the presence or absence of $NaHCO_3$ on the Mg^{2+}-ATPase activity of isolated RrF_1. Enzyme activity was measured as described in Materials and Methods.

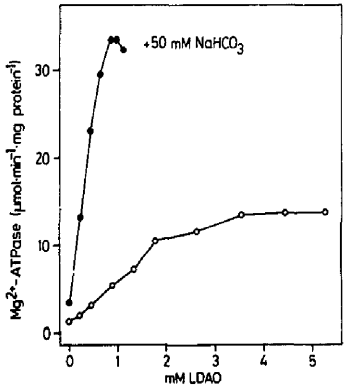

Fig. 4. Effect of LDAO in the presence or absence of $NaHCO_3$ on the Mg^{2+}-ATPase activity of isolated RrF_1. Enzyme activity was measured as described in Materials and Methods.

from 1.2 μmol per mg per mg protein in the absence of LDAO to 13 μmol per min per mg protein in the presence of 4 mM LDAO. The activity is not inhibited by higher concentrations of LDAO. The effect of LDAO on Ca^{2+}-ATPase activity could not be studied, since LDAO precipitated in the molybdate solution used in the colorimetrical determination of phosphate.

The kinetic analysis of Mg^{2+}-ATPase activity shows that the V_{max} is increased by the activators, resulting in a 45-fold activation in the presence of LDAO plus $NaHCO_3$ (Table I). However, the K_m for ATP is similar, about 0.22 mM, in both the absence and presence of activators. Free Mg^{2+} is known to inhibit Mg^{2+}-ATPase activity of isolated RrF_1 [3]. The present study shows that this is true for the non-activated as well as for the activated enzyme (Table I).

Discussion

F_1-ATPase, isolated from bacteria, mitochondria and chloroplasts, have basically similar structure and function. The enzymes are not identical molecules, however, and exhibit different catalytical and immunological properties. F_1-ATPases can exist in forms with low and high ATP hydrolytic activity, and several different treatments have been reported which can convert a latent ATPase to an active enzyme. Furthermore, some ATPases change their cationic specificity upon isolation.

As shown in this work, RrF_1, isolated by chloroform extraction of chromatophores followed by purification on a glycerol gradient, has a high Ca^{2+}-ATPase activity, 12 μmol per min per mg protein. Similar to earlier preparations obtained from acetone powder of *R. rubrum* chromatophores, the present preparation has low, but significant, Mg^{2+}-ATPase activity. It is also demonstrated that this low Mg^{2+}-ATPase activity, in the presence of the anion HCO^{3-} and certain detergents, can be stimulated to about 35 μmol per min per mg protein. To our knowledge, this is the highest Mg^{2+}-ATPase activity reported for F_1 isolated from *R. rubrum*.

Anions present in millimolar concentrations are known to stimulate various F_1-ATPases [9,19,20]. For stimulation of the *R. rubrum* Mg^{2+}-ATPase activity, sulphite has been reported to be the most efficient activator [8]. In accordance with that

TABLE I

K_m, V_{max} AND SPECIFIC ACTIVITIES OF ISOLATED RrF_1 IN THE PRESENCE OR ABSENCE OF ACTIVATORS

The K_m and V_{max} were determined in the presence of 10 mM $Mg(Ac)_2$ and with ATP concentrations varying between 0.3 mM and 3 mM. Spec. act. was determined at 3 mM ATP and with 3 mM or 10 mM $Mg(Ac)_2$ as indicated.

Activator	K_m (mM)	V_{max} (μmol·min^{-1}·mg^{-1})	Spec. act. (μmol·min^{-1}·mg^{-1})	
			3 mM $Mg(Ac)_2$	10 mM $Mg(Ac)_2$
–	0.220	0.5	1.2	0.5
40 mM $NaHCO_3$	0.210	2.1	4.9	2.0
4.4 mM LDAO	0.220	6.9	13.8	5.8
0.88 mM LDAO + 40 mM $NaHCO_3$	0.230	22.9	34.0	21.2

report, Mg^{2+}-ATPase activity of the preparation used in the present study is stimulated maximally 8-fold by Na_2SO_3 (not shown) and 4-fold by $NaHCO_3$. There are also several reports on the effects of detergents on various F_1-ATPases. F_1s from *Escherichia coli* [21], spinach chloroplasts [6,7] and the thermophilic bacterium PS3 [22,23] are stimulated by certain detergents, whereas F_1 from bovine heart [23], yeast [23] and *Micrococcus luteus* [24] are inhibited by various detergents. Mg^{2+}-ATPase activity of F_1 solubilized from acetone powder of *R. rubrum* chromatophores has been shown to be stimulated by detergents. The most effective stimulator was dodecyl sulfonic acid, which, at concentrations significantly below the cmc, gave a maximal stimulation resulting in an activity of 9 μmol per min per protein [5]. Various unsaturated fatty acids also exhibited stimulation, palmitoleic acid being the most effective with a Mg^{2+}-ATPase activity of 16 μmol per min per mg protein [25].

The present work shows that octyl glucoside and MEGA-9 are able to stimulate the Mg^{2+}-ATPase activity of isolated RrF_1. With both detergents, stimulation was accomplished at monomer concentrations and very little effect was obtained above the cmc. The cmc was determined to be 24 mM for octyl glucoside and 15 mM for MEGA-9. The cmc values were not altered in the presence of 50 mM $NaHCO_3$. In the presence of $NaHCO_3$ plus octyl glucoside or MEGA-9, even lower concentrations of the detergents stimulated the Mg^{2+}-ATPase activity, which results in very high activities. This effect of detergent is similar to that found for F_1 isolated from spinach chloroplasts. Heat-activated chloroplast F_1 according to Lien and Racker [26], was also stimulated by octyl glucoside in concentrations below the cmc, whereas the non-activated enzyme was suggested to be stimulated by micelles of octyl glucoside [7]. It is also shown in the present work, that octyl glucoside inhibits the Ca^{2+}-dependent ATPase-activity of RrF_1, in accordance with the chloroplast enzyme [6]. The Ca^{2+}-ATPase activity of chloroplast F_1 can be irreversibly activated by preincubation of the enzyme with octyl glucoside, followed by dilution of the detergent [6]. However, this cannot be achieved with F_1 from *R. rubrum* (not shown). The different effects of octyl glucoside upon the Mg^{2+}- and Ca^{2+}-ATPase of isolated RrF_1 were paralleled by the action of the hydrophobic compound diethylstilbestrol. This artificial steroid also showed a strong inhibition of the Ca^{2+}-ATPase and a stimulation of the Mg^{2+}-ATPase activity [18] of RrF_1 prepared in the same manner as that described in this report. The same inhibition/stimulation pattern was also obtained with diethylstilbestrol for isolated, liposome-reconstituted F_0F_1-ATPase and for the native ATPase of *Rhodospirillum rubrum* chromatophores [28].

Another amphipathic detergent, LDAO, also stimulated the Mg^{2+}-ATPase activity of RrF_1, both in the absence and presence of bicarbonate. However, the stimulation occurs above the cmc (0.125 mM [29]) in both cases, although higher concentrations are needed in the absence of bicarbonate. Furthermore, a combination of $NaHCO_3$ and LDAO is much more effective than LDAO alone. The concentration range (0.2–4 mM) of LDAO which causes, maximally, a 10-fold stimulation in the absence of bicarbonate is identical to that causing a 5-fold stimulation of the *E. coli* enzyme [21]. The effect of LDAO on F_1 from *E. coli* was suggested to be due to the release of the inhibitory action of subunit ε on the catalytic subunit. This was demonstrated by a lower extent of cross-linking between ε and β in the presence of LDAO, upon treatment of F_1 with a cross-linking agent [21].

The ATPase activity of F_1 isolated from the thermophilic bacterium PS3, is stimulated at 30°C by several detergents, including octyl glucoside [22,23] and LDAO (Norling, B., unpublished results), to activities found at the optimal temperature, 75°C. The effect of the detergents was concluded to be exerted at the level of subunit interactions, which resulted in a sufficient degree of flexibility of the subunits for optimal catalysis to be obtained. Some of the activation of chloroplast F_1-ATPase achieved by octyl glucoside, seems to be due to the release of the ε-subunit, which is thought to act as an inhibitor [28]. However, another mode of activation seems to be of importance, since ε-deficient F_1 from chloroplast can also be activated by the detergent [28]. F_1 isolated from chloroplasts has a high temperature optimum, both 65°C [29] and 52°C [27] have been reported. Therefore, it is possible that, similar to

the case of the thermophilic enzyme, strong hydrophobic interactions between the subunits limit the enzyme activity and that these interactions can be relieved by amphiphatic detergents.

Hydrophobic entities, such as hydrocarbon tails of detergents and compounds like diethylstilbestrol probably act at the hydrophobic interfaces between subunits in the F_1. This action induces conformational changes in the enzyme molecule. One possibility is that the active sites of F_1 are altered. The residues responsible for the binding of Ca^{2+}-ATP may come closer to each other, which would make binding of Ca-ATP to the site impossible, but at the same time would stabilize the binding of Mg^{2+}-ATP, since Mg^{2+} is smaller than Ca^{2+} (0.65 and 0.99 Å, respectively). This would result in an inhibition of the Ca^{2+}-ATPase and a stimulation of the Mg^{2+}-ATPase.

Yet another conformation which can facilitate the binding of both Mg^{2+}-ATP and Ca^{2+}-ATP would be ruling in the enzyme in the native, membrane-bound state, since chromatophores of *R. rubrum* catalyze hydrolysis of Ca^{2+}-ATP and Mg^{2+}-ATP at similar rates [28]. Also in the membrane-bound state, diethylstilbestrol induces a conformation in the enzyme preferential to Mg^{2+}-ATP [18].

To sum up, plentiful information from studies on different ATPases seems to indicate that detergents can exert their stimulatory effect through at least two different mechanisms. For some ATPases, the ε-subunit seems to regulate the ATPase activity and certain detergents seem to be able to abolish the inhibitory effect of the ε-subunit. In addition, some ATPases, especially those with a high temperature optimum, seem to be activated by the weakening of the hydrophobic interactions between the subunits. An increased flexibility between the subunits is induced, which is necessary for optimal activity. Further studies of the effect of detergents on RrF_1 are required in order to establish whether one or both of these mechanisms, or any other, could be responsible for the stimulation of the Mg^{2+}-ATPase activity.

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