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COUPLING FACTOR ATPase COMPLEX OF *RHODOSPIRILLUM RUBRUM*

PURIFICATION AND CHARACTERIZATION OF AN OLIGOMYCIN AND *N,N'*-DICYCLOHEXYLCARBODIIMIDE-SENSITIVE (Ca^{2+} + Mg^{2+})-ATPase

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Summary

An ATPase complex sensitive to the energy transfer inhibitors oligomycin, dicyclohexylcarbodiimide and venturicidin has been solubilized from *Rhodospirillum rubrum* chromatophores with Triton X-100 and further purified by centrifugation on a glycerol gradient. The partially purified $RrF_0 \cdot F_1$ contains 13 distinct polypeptide subunits, as revealed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, including the subunits of the oligomycin-insensitive, water-soluble RrF_1 ATPase.

The ATPase activity of $RrF_0 \cdot F_1$ as that of the membrane-bound enzyme complex depends on Ca^{2+} or Mg^{2+} and from detailed kinetic studies it is concluded that the divalent cation-ATP complex is the substrate for both ATPase complexes. Free ATP and free Mg^{2+} act as competitive inhibitors, with K_i values of 1 mM and 7 μM, respectively.

The subunit composition of the purified $RrF_0 \cdot F_1$ and its similarity to the membrane-bound ATPase with respect to cation dependence and sensitivity to energy transfer inhibitors suggests that it contains all the subunits of the *R. rubrum* coupling factor-ATPase complex.

Abbreviations: DCCD, *N,N'*-dicyclohexylcarbodiimide; $CF_0 \cdot F_1$, $F_0 \cdot F_1$ and $RrF_0 \cdot F_1$, detergent-solubilized ATPase complexes isolated, respectively, from chloroplasts, mitochondria and *Rhodospirillum rubrum* chromatophores; CF_1 and F_1 and RrF_1, water-soluble DCCD-insensitive ATPase moieties of $CF_0 \cdot F_1$, $F_0 \cdot F_1$ and $RrF_0 \cdot F_1$, respectively; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; SDS, sodium dodecyl sulfate; Tricine, *N*-tris(hydroxymethyl)methylglycine.

Introduction

A coupling factor-ATPase complex has been found to play a dominant role in the terminal steps of ATP synthesis in oxidative phosphorylation [1] as well as in photophosphorylation [2]. The activity of the various membrane-bound ATPase complexes is inhibited by energy-transfer inhibitors, such as oligomycin, rutamycin and DCCD. These inhibitors do not affect the water-soluble F_1- or CF_1-ATPases, but do inhibit detergent-extracted $F_0 \cdot F_1$-ATPases. A detergent-solubilized rutamycin-sensitive ATPase has first been isolated from beef heart mitochondria [3]. Subsequently such oligomycin or DCCD-sensitive ATPase complexes have been highly purified from beef heart mitochondria [4], yeast mitochondria [5] and from a thermophilic bacterium [6]. Only recently have such ATPase complexes been isolated from photosynthetic membranes. Winget et al. [7] have isolated by cholate extraction a DCCD-sensitive ATPase complex from spinach chloroplasts ($CF_0 \cdot F_1$) and Oren and Gromet-Elhanan [8,9] have solubilized by Triton X-100 extraction an oligomycin-sensitive ATPase ($RrF_0 \cdot F_1$) from chromatophore membranes of the photosynthetic bacterium *Rhodospirillum rubrum*.

This paper describes the purification and characterization of $RrF_0 \cdot F_1$ from chromatophores. The ATPase activity of $RrF_0 \cdot F_1$, as that of the membrane-bound enzyme, can be activated by either Ca^{2+} or Mg^{2+} and is inhibited by the energy-transfer inhibitors oligomycin, DCCD and venturicidin.

Experimental

R. rubrum strain S1 cells were grown photosynthetically in the medium of Ormerod et al. [10]. Chromatophores were isolated from harvested cells as previously described [11,12]. Bacteriochlorophyll was measured using the in vivo extinction coefficient given by Clayton [13]. Protein was assayed according to Lowry et al. [14] using the modification of Chandrarajan and Klein [15] for samples containing Triton X-100. The assay of membrane-bound protein included incubation in 0.5 N NaOH at 60°C for 1 h as described by Drews et al. [16]. The bacteriochlorophyll/protein ratio for eight different preparations of chromatophores was 40 μg bacteriochlorophyll/mg protein.

ATPase activity was assayed by following the hydrolysis of [γ-^{32}P]ATP, which was prepared according to the method of Avron [17]. The reaction mixture contained, in a final volume of 1 ml: 10 mM Hepes-NaOH, pH 8.0; chromatophores (containing 10 μg bacteriochlorophyll) or $RrF_0 \cdot F_1$ (20 μg protein) and either 4 mM $CaCl_2$ or 2 mM $MgCl_2$ unless otherwise stated. The reaction was started by addition of 4 mM ATP containing $2 \cdot 10^5$ cpm [γ-^{32}P]-ATP and stopped by the addition of cold trichloroacetic acid to a final concentration of 5%. The released $NaH_2{}^{32}PO_4$ was separated by the isobutanol/benzene procedure [18]. In some experiments ATPase activity was followed by the change in pH [19]. The reaction was started by addition of either chromatophores or $RrF_0 \cdot F_1$. A linear rate of acid production was recorded for at least 5 min. The amount of hydrolyzed ATP was calculated from the decrease in pH.

Polyacrylamide gel electrophoresis was carried out on a composite agarose-

polyacrylamide gel containing 3% polyacrylamide, 0.5% agarose and 0.1% Triton X-100. Samples were run in 15 mM Tricine-NaOH, pH 8.0, for 1.5 h under 200 V and stained by Coomassie brilliant blue. For SDS-polyacrylamide gel analysis samples were mixed with electrophoresis sample buffer [20] to a final concentration of 5% glycerol, 0.05% β-mercaptoethanol, 2% SDS, 62.5 mM Tris-HCl (pH 6.8), 0.01% bromophenol blue, and boiled for 2 min. Aliquotes of 10—40 μl were subjected to electrophoresis for 2.5 h at 150 V and 24°C through a slab gel containing a 10%—20% polyacrylamide gradient [21].

All chemicals used were of analytical grade. Oligomycin was purchased from Sigma; DCCD, from Fluka and venturicidin from Cambrian Chemicals. Aurovertin was a gift from Drs. H. Lardy and T.A. Out.

Results

Purification of $RrF_0 \cdot F_1$

Solubilization and purification of the ATPase was carried out by a modification of the method of Tzagaloff and Meagher [47]. Chromatophores were suspended at 5 mg protein/ml in a solution containing 0.2% Triton X-100 (v/v) and 1 mM Hepes-NaOH, pH 8.0, and allowed to stand for 30 min at room temperature. The suspension was centrifuged at 140 000 × *g* for 90 min at 4°C and all subsequent operations were carried out at 0—4°C. The supernatant contained about 1 mg protein/ml and was concentrated to 8 mg protein/ml by ultrafiltration through a Diaflo XM-300 membrane (Amicon). The concentrated material was layered on top of a linear glycerol gradient (5—15%, v/v) containing 0.1% Triton X-100 and 5 mM Hepes-NaOH, pH 8.0. The gradient was centrifuged for 15 h at 26 000 rev./min in the SW-27 Beckman rotor.

As illustrated in Fig. 1 ATPase activities dependent on both Ca^{2+} and Mg^{2+} appeared in fractions 3—5, well separated from the bulk protein. Samples from various fractions of the gradient were analyzed on an agarose-polyacrylamide gel and the most active fraction revealed only one band (Fig. 2). The polypeptide composition of this fraction has been determined, using the SDS-polyacrylamide gel electrophoresis method of Laemmli [20], that gives high resolution of low molecular weight components. As is shown in Fig. 3 this method visualized in $RrF_0 \cdot F_1$ 13 bands of which five correspond to the subunits of the RrF_1, the water-soluble ATPase isolated from the same bacterium [22]. The molecular weights calculated from the mobilities in the gel are: 64 000; 57 000 (α); 53 000 (β), 41 000; 34 000 (γ); 31 000; 26 000; 21 000; 16 000 (δ); 14 500; 13 000; 12 000 and 11 000. With beef heart $F_0 \cdot F_1$ the gel electrophoresis system of Laemmli [20] has been reported by Glaser et al. [23] to resolve 17 bands as compared to 8—12 bands visualized by the method of Weber and Osborn [24]. Using the Weber and Osborn method Sone et al. [6] found eight polypeptide subunits in their $F_0 \cdot F_1$ preparation from a thermophilic bacterium. But they have recently reduced this number to seven since one of the three subunits originally attributed to F_0 was found to be a contaminant [25]. The minimal number of polypeptide subunits in $F_0 \cdot F_1$ as well as in $RrF_0 \cdot F_1$ is thus still uncertain. However, the above-described $RrF_0 \cdot F_1$ preparation does contain all the subunits of the *R. rubrum* coupling factor-ATPase complex, since it has been found to catalyze ATP formation when

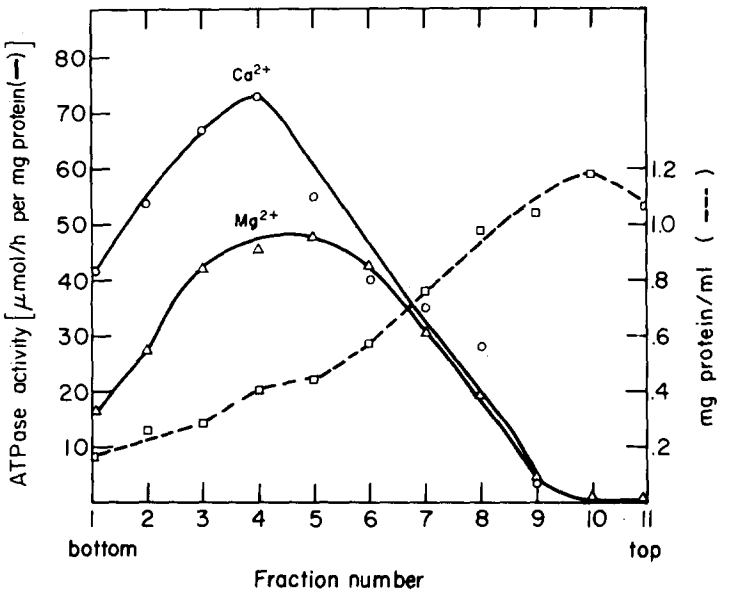

Fig. 1. Distribution profile of $RrF_0 \cdot F_1$ on a glycerol gradient. 4 ml of the Triton extract were applied to 32 ml of a 5—15% glycerol gradient as described in the text. ○———○, Ca^{2+}-dependent ATPase activity; △———△, Mg^{2+}-dependent ATPase activity; □- - - - -□, protein concentraton.

reconstitution into liposomes together with bacteriorhodopsin [26].

The recovery of protein and ATPase activity of $RrF_0 \cdot F_1$ obtained in a typical experiment is summarized in Table I. Both Ca^{2+}- and Mg^{2+}-dependent ATPase activities are purified, but to a different extent, so that the ratio between these ATPase activities changes during solubilization and purification. In the chromatophores the Mg^{2+}-dependent ATPase was always 2—3 fold more active than the Ca^{2+}-dependent one. Solubilization by Triton X-100 decreased the ratio between the Ca^{2+}- and Mg^{2+}-dependent ATPase activities to 1 : 1.5—2.0 and in the active glycerol gradient fractions this ratio was further reduced to 1 : 0.5—1.0 (see Fig. 1). This pattern was observed in a large number of purification experiments starting with different chromatophore preparations, in which the specific ATPase activity varied up to 3-fold. In all these experiments the extent of pufirication and overall yield of the $RrF_0 \cdot F_1$-ATPase activities varied by less than 25%.

The trend of decrease in the Mg^{2+}-dependent ATPase activity as compared to the Ca^{2+}-dependent one is even more pronounced in preparations of RrF_1, which have been reported to contain a Ca^{2+}-dependent ATPase [27,28] that is competitively inhibited by Mg^{2+} [27]. Indeed the Triton-extracted $RrF_0 \cdot F_1$ is the first solubilized *R. rubrum* ATPase preparation that can be activated by both cations. This enzyme complex is unstable at room temperature even in the presence of ATP. The glycerol gradient fractions can, however, be kept for at least 6 months in liquid air without any loss of activity.

Effect of inhibitors on the ATPase activity of $RrF_0 \cdot F_1$

Oxidative phosphorylation in mitochondria as well as their membrane-

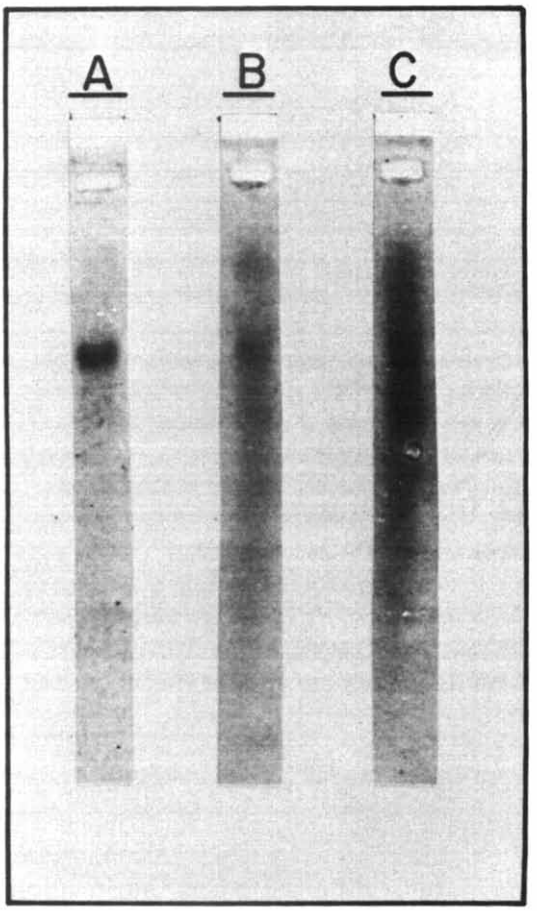

Fig. 2. Polyacrylamide gel electrophoresis of $RrF_0 \cdot F_1$. (A) 5 μg protein from fraction 4 of the glycerol gradient (see Fig. 1); (B) 12 μg protein from fraction 10 of the glycerol gradient (see Fig. 1); (C) 30 μg protein from the sample applied to the glycerol gradient.

bound and detergent-solubilized $F_0 \cdot F_1$-ATPase activities are blocked by energy transfer inhibitors, such as oligomycin, rutamycin and DCCD, which do not inhibit the water-soluble F_1-ATPase [29,30]. DCCD inhibits ATPase activity also in chloroplasts and bacterial vesicles, whereas oligomycin is inactive in these systems [30]. In *R. rubrum* chromatophores, as in mitochondria, oligomycin has been shown to act as an energy transfer inhibitor blocking both ATP formation and hydrolysis [31—33]. Fig. 4 illustrates that the ATPase activity of $RrF_0 \cdot F_1$ is also sensitive to oligomycin. For 50% inhibition of the membrane-bound and $RrF_0 \cdot F_1$ Mg^{2+}-ATPase activities 1 μM and 5 μM oligomycin are required, respectively. According to Johansson et al. [34] the Ca^{2+}-dependent ATPase activity of *R. rubrum* chromatophores is also inhibited by oligomycin, although it is somewhat less sensitive than the Mg^{2+}-dependent activity. A similar situation has been found in the $RrF_0 \cdot F_1$ Ca^{2+}-ATPase [8].

The ATPase activity of *R. rubrum* is sensitive not only to oligomycin but to a number of other energy transfer inhibitors. Thus, DCCD inhibits both the

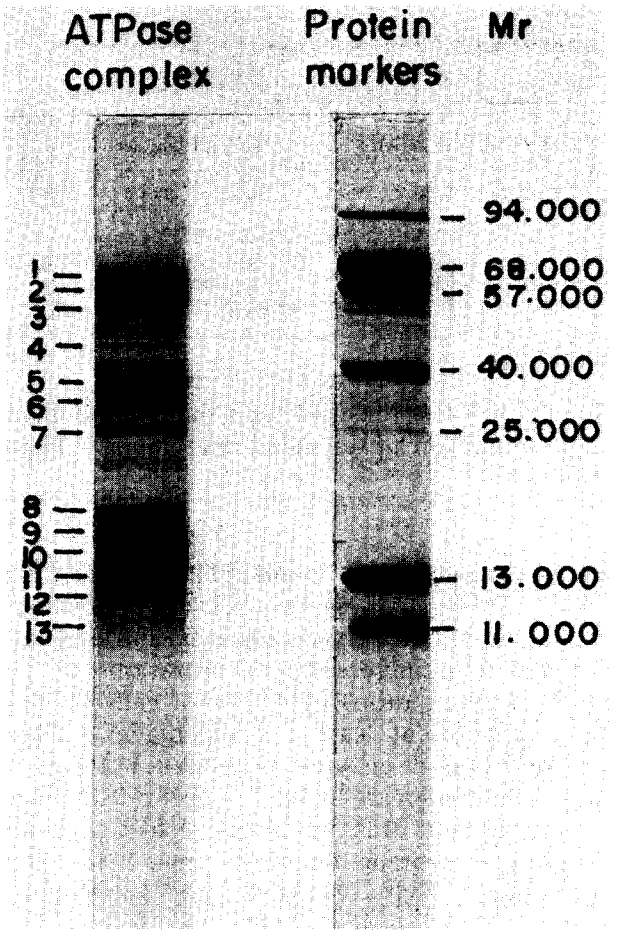

Fig. 3. SDS-polyacrylamide gel electrophoresis of the purified $RrF_0 \cdot F_1$. A 20 µl solution, containing the amounts of protein indicated below, was applied on a slab SDS gel as described under Experimental. (A) 12 µg protein from fraction 4 of the glycerol gradient (see Fig. 1). (B) Protein markers: 2 µg of phosphorylase *A* (M_r = 94 000; 3 µg of bovine serum albumin (M_r = 68 000): 3 µg of pyruvic kinase (M_r = 57 000); 2 µg aldolase (M_r = 39 000); 5 µg of α-chymotrypsin (M_r = 25 000, for the whole enzyme and 13 000 and 11 000 for its subunits).

membrane-bound and $RrF_0 \cdot F_1$ (Ca^{2+} + Mg^{2+})-ATPases (Fig. 5). Here the Mg^{2+}-dependent activity of $RrF_0 \cdot F_1$ is even somewhat more sensitive than that of the membrane-bound enzyme. Venturicidin, which like oligomycin and DCCD, has been shown to inhibit ATPase activity in submitochondrial particles but not in the water-soluble F_1-ATPase [35] is also active in *R. rubrum*. It inhibits to a similar extent the membrane-bound as well as $RrF_0 \cdot F_1$ Mg^{2+}-ATPase (Fig. 6), but in both preparations the inhibition levelled off at around 50% of control.

Another type of inhibitor is aurovertin [36]. This compound, unlike oligomycin, DCCD and venturicidin, has been shown to inhibit the F_1-ATPase of mitochondria [30]. In *R. rubrum* it has been reported to block both the membrane-bound [33] and RrF_1-ATPase activity [37]. As illustrated in Fig. 7 the effect of aurovertin is indeed different from that of other inhibitors, since

TABLE I

SUMMARY OF PURIFICATION OF $RrF_0 \cdot F_1$

Specific activities are expressed in μmol ATP hydrolyzed per h mg protein.

Step	Total protein (mg)	Mg^{2+}-ATPase activity			Ca^{2+}-ATPase activity		
		Specific	Total (units)	Yield (%)	Specific	Total (units)	Yield (%)
Chromatophores	48.0	5.2	250	100	2.4	115	100
Triton extract	10.0	16.0	160	64	9.0	90	78
Glycerol gradient (fractions 3—5)	1.1	48.0	53	21	73.0	80	70

the ATPase activity of $RrF_0 \cdot F_1$ is much less sensitive than that of the membrane-bound enzyme. Here too the inhibition of the membrane-bound ATPase levels off at around 50% of control.

Catalytic properties

Although the $RrF_0 \cdot F_1$ as well as the membrane-bound ATPase activity can be induced by either Mg^{2+} or Ca^{2+} (Table I), these two ATPase systems are differently affected by the cations (Fig. 8). At a fixed concentration of 4 mM ATP both ATPase activities increase with increasing concentrations of the divalent cations reaching maximal values at ratios of 1–2 mol cation/4 mol ATP. At these ratios Mg^{2+} always induces at least a 2-fold higher ATPase activity than Ca^{2+} in the membrane-bound enzyme (Fig. 8A), but not in $RrF_0 \cdot F_1$ (Fig. 8B). Moreover, any further increase in Mg^{2+} concentration leads to inhibition of the ATPase activity and this inhibitory effect is more pronounced in $RrF_0 \cdot F_1$ than in the membrane-bound enzyme. Thus, at a molar ratio of 1 Mg^{2+}/1 ATP the ATPase activity decreases by 85% in the first (Fig. 8B) as compared to less than 20% in the second (Fig. 8A). Excess Ca^{2+}

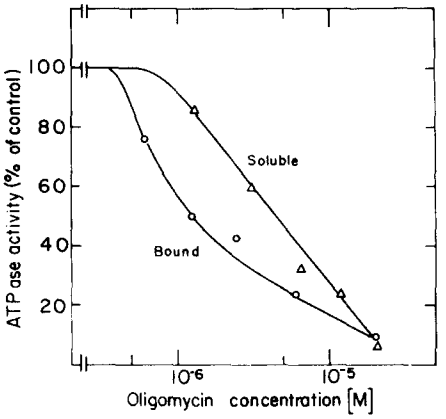

Fig. 4. Effect of oligomycin on the Mg^{2+}-ATPase activity of *R. rubrum* membranes and of $RrF_0 \cdot F_1$. Conditions as described under Experimental. ○, membrane-bound ATPase. The reactions contained 14 μg bacteriochlorophyll (=350 μg protein) and the control activity was 180 μmol ATP hydrolyzed/h per mg bacteriochlorophyll. △, $RrF_0 \cdot F_1$-ATPase activity. The reactions contained 75 μg protein and the control activity was 38 μmol ATP hydrolyzed/h per mg protein.

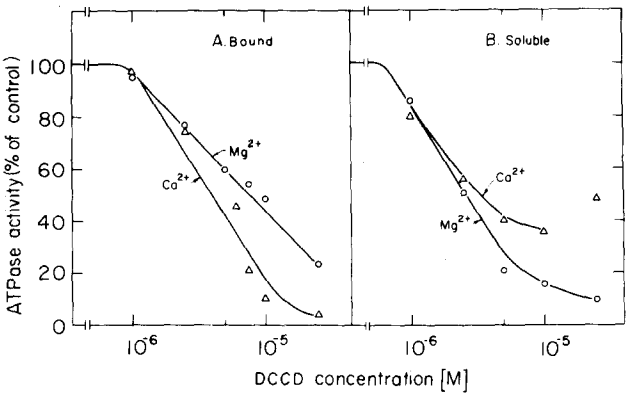

Fig. 5. Effect of DCCD on the (Ca^{2+} + Mg^{2+})-ATPase activity of *R. rubrum* membranes and of $RrF_0 \cdot F_1$. (A) Membrane-bound ATPase. The reactions contained 8 μg bacteriochlorophyll (=200 μg protein) and control activities expressed in μmol ATP hydrolyzed/h per mg bacteriochlorophyll were, for Ca^{2+}-ATPase 57 (△) and for Mg^{2+}-ATPase 180. (○). (B) $RrF_0 \cdot F_1$ activity. The reactions contained 65 μg protein and control activities expressed in μmol ATP hydrolyzed/h per mg protein were, for Ca^{2+}-ATPase 53 (△) and for Mg^{2+}-ATPase 25 (○).

is much less inhibitory in both enzyme systems and even at a molar ratio of 3 Ca^{2+}/1 ATP no more than 25% decrease in ATPase activity is recorded.

The effect of increasing concentrations of ATP at two constant concentrations of Mg^{2+} on $RrF_0 \cdot F_1$ Mg^{2+}-ATPase activity is demonstrated in Fig. 9. Maximal activity is observed here as in Fig. 8B at a molar ratio of 1 Mg^{2+}/2—4 ATP. A larger excess of ATP results in marked inhibition of the ATPase activity. Thus, 50% inhibition is already obtained at a molar ratio of 1 Mg^{2+}/8—10 ATP (Fig. 9). Similar results were obtained also for the Ca^{2+}-ATPase in both $RrF_0 \cdot F_1$ and in the membrane-bound system (not shown).

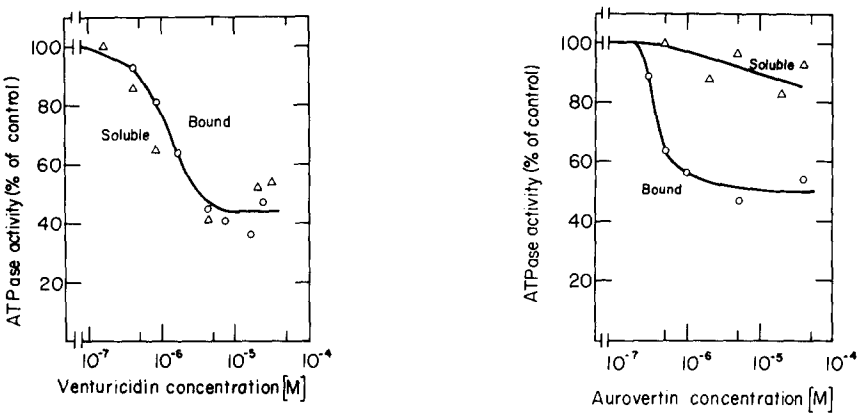

Fig. 6. Effect of venturicidin on the Mg^{2+}-ATPase activity of *R. rubrum* membranes and of $RrF_0 \cdot F_1$, conditions as described in Fig. 4. ○, membrane-bound ATPase (control activity 180 μmol ATP hydrolyzed/h per mg bacteriochlorophyll); △, $RrF_0 \cdot F_1$-ATPase activity (control activity 38 μmol ATP hydrolyzed/h per mg protein).

Fig. 7. Effect of aurovertin on the Mg^{2+}-ATPase activity of *R. rubrum* membranes and of $RrF_0 \cdot F_1$. Conditions as described in Fig. 4. ○, membrane-bound ATPase (control activity 180 μmol ATP hydrolyzed/h per mg bacteriochlorophyll); △, $RrF_0 \cdot F_1$-ATPase activity (control activity 38 μmol ATP hydrolyzed/h per mg protein).

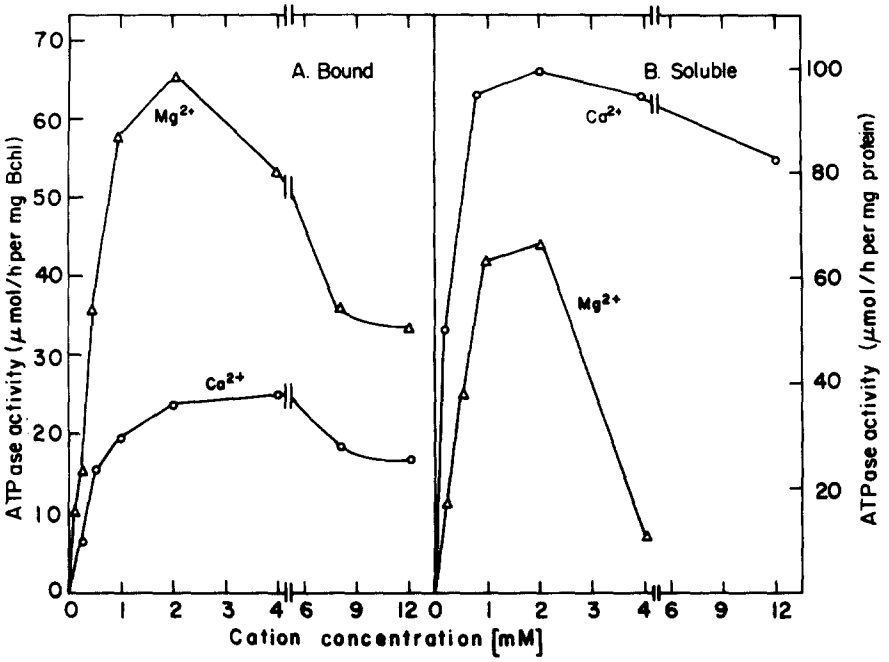

Fig. 8. Dependence of membrane-bound and $RrF_0 \cdot F_1$-ATPase activity on the concentration of divalent cations. The reaction mixture was as described under Experimental, except that the cation concentration was varied as indicated. (A) Membrane-bound ATPase, and (B) $RrF_0 \cdot F_1$-ATPase. Bchl, bacteriochlorophyll.

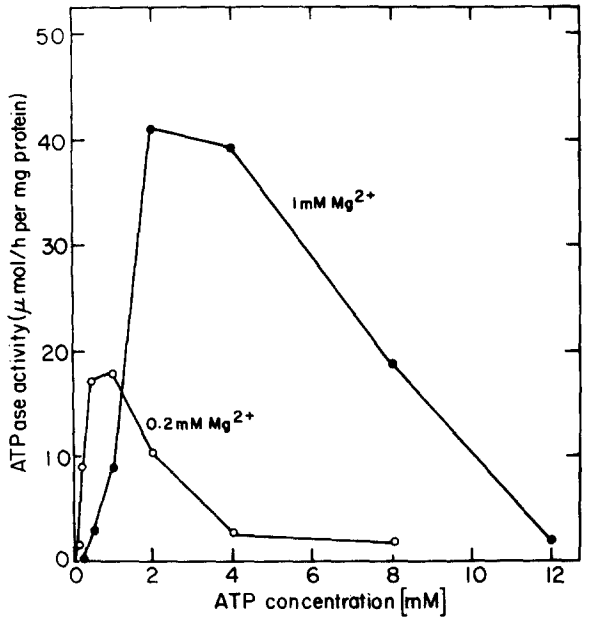

Fig. 9. Dependence of the Mg^{2+}-ATPase activity of $RrF_0 \cdot F_1$ on the concentration of ATP. The reaction mixture was as described under Experimental with two fixed Mg^{2+} concentrations of 0.2 mM and 1.0 mM. The ATP concentration was varied as indicated.

TABLE II

KINETIC PARAMETERS OF THE MEMBRANE-BOUND AND $RrF_0 \cdot F_1$-ATPase ACTIVITY

ATPase activity was assayed as described under Experimental. K_m and K_i were determined as described under Results.

Type of ATPase	Substrate	Component in excess (mM)	K_m (mM)	K_i (mM)	V (μmol/h per mg protein)
Bound	$MgATP^{2-}$	Mg^{2+} (0.1; 0.5)	0.3	0.15	56 *
Soluble	$MgATP^{2-}$	Mg^{2+} (0.05; 0.15)	0.025	0.007	44
Soluble	$MgATP^{2-}$	ATP (1.5; 3)	0.018	0.7	50
Soluble	$CaATP^{2-}$	ATP (1; 3)	0.5	1.0	100

* V values of the bound ATPase are given in μmol per h per mg bacteriochlorophyll.

The inhibition of mitochondrial F_1-ATPase activity by free Mg^{2+} and by free ATP led Selwyn [38] to suggest that the $MgATP^{2-}$ complex is the substrate for the enzyme. Recently, a cation-ATP complex was suggested to be the substrate for the water-soluble ATPase isolated from chloroplasts [39] and from *Chromatium* chromatophores [40]. The possibility that a cation-ATP complex is the substrate of *R. rubrum* membrane-bound as well as $RrF_0 \cdot F_1$-ATPase complexes was tested by setting up a system in which the concentration of the cation-ATP complex was varied keeping the free cation or free ATP at a fixed excess. The concentrations of the cation-ATP complex and its free components were calculated according to the stability constants determined by Walaas [41]. The activity of the membrane-bound and $RrF_0 \cdot F_1$ enzyme system was tested as a function of the $MgATP^{2-}$ complex concentration at two constant free Mg^{2+} concentrations. Values of V and K_m for the $MgATP^{2-}$ complex and K_i for the excess free Mg^{2+} calculated from Lineweaver-Burk plots of the data are summarized in Table II. This table contains also data from similar kinetic analysis done for the ATPase activity of $RrF_0 \cdot F_1$ using either $MgATP^{2-}$ complex or $CaATP^{2-}$ complex as substrates and an excess of free ATP. The results indicate that both the membrane-bound and $RrF_0 \cdot F_1$-ATPase systems are competitively inhibited by free Mg^{2+}, but $RrF_0 \cdot F_1$ is about 20-fold more sensitive, with a K_i of 7 μM for the free Mg^{2+}. Free ATP is also a competitive inhibitor, although much less effective than free Mg^{2+}. It inhibits the ATPase activity of $RrF_0 \cdot F_1$ to the same extent when either $CaATP^{2-}$ or $MgATP^{2-}$ are used as substrate, with a K_i of around 1 mM. On the other hand, free Ca^{2+} are very ineffective and did not inhibit even at an excess of 20 mM.

Discussion

The photosynthetic membranes of *R. rubrum* exhibit an ATPase activity which is dependent on either Ca^{2+} or Mg^{2+} [34] and is sensitive to the energy transfer inhibitor oligomycin [30—32]. This oligomycin-sensitive (Ca^{2+} + Mg^{2+})-dependent ATPase has been isolated from *R. rubrum* membranes by extraction with Triton X-100 (Table I and Fig. 4). The Triton-solubilized $RrF_0 \cdot F_1$-ATPase complex reported here is of high specific activity and purity (Figs. 1 and 2). It contains 13 different polypeptide subunits separable by SDS-

polyacrylamide gel electrophoresis (Fig. 3). Five of these are components of the water-soluble RrF_1 which has been isolated from *R. rubrum* chromatophores [22,42]. RrF_1 shows, however, only Ca^{2+}-dependent ATPase activity and is insensitive to oligomycin [27]. The sensitivity of the ATPase activity of $RrF_0 \cdot F_1$ to this inhibitor must, therefore, be due to its additional detergent-soluble subunits, but it cannot be specified at present to which of them. Studies on the specific role of the various subunits seen by SDS-polyacrylamide gel electrophoresis are now in progress. It has recently been shown that the catalytic activity of the membrane-bound [22] as well as $RrF_0 \cdot F_1$-ATPase [9] complexes is strictly dependent on the β-subunit which can be specifically removed from *R. rubrum* membranes by treatment with 2 M LiCl [22].

Although the purified $RrF_0 \cdot F_1$-ATPase and the membrane-bound one can be activated by Ca^{2+} or by Mg^{2+}, they differ in the degree of their activation by these cations. In the membrane-bound ATPase Mg^{2+} is 2—3-fold more active than Ca^{2+}, whereas in the purified $RrF_0 \cdot F_1$ Ca^{2+} is more effective than Mg^{2+} (Table I and Fig. 1). These results could be interpreted as an indication for the presence of two different species of ATPase in the membrane, each of them purified to a different extent. This possibility is, however, ruled out by the observations that the Ca^{2+}-dependent RrF_1-ATPase restores Mg^{2+}-dependent ATPase activity to depleted *R. rubrum* membranes [27,28]. Moreover, the purified β-subunit, which by itself has no ATPase activity [22], restores both Ca^{2+}- and Mg^{2+}-dependent ATPase activities to LiCl-depleted *R. rubrum* membranes [43].

It is, therefore, concluded that the Ca^{2+}- and Mg^{2+}-dependent ATP hydrolysis are different activities of the same enzyme, but the Mg^{2+}-dependent activity is more sensitive to various treatments. Thus, its activity decreases upon solubilization (Table I) and might even disappear altogether [27], but this is a reversible inactivation, since it is restored when the ATPase is reattached to the membrane. Moreover, a conversion of Ca^{2+}-ATPase activity into Mg^{2+}-activated ATPase has been found in the water-soluble CF_1 of chloroplasts [44] and recently Webster et al. [45] have reported a similar conversion in the RrF_1-ATPase isolated from *R. rubrum*. This conversion required the addition of anions, such as sulphite, and they have proposed that the anion blocks changes in properties of the enzyme which normally accompany its dislocation from the membrane and lead to a decrease in the Mg^{2+}-dependent ATPase activity.

Both $RrF_0 \cdot F_1$ and the membrane-bound ATPase have been found to be sensitive not only to oligomycin but also to DCCD and venturicidin (Figs. 5 and 6). This sensitivity to a wide range of energy transfer inhibitors is in accord with the sensitivity pattern reported for the mitochondrial detergent-solubilized ATPase [29,30]. In other systems, such as bacterial vesicles [30] or chloroplasts [7] only DCCD-sensitive ATPase complexes have been isolated. Mitochondrial $F_0 \cdot F_1$ and the *R. rubrum* $RrF_0 \cdot F_1$ differ, however, in their sensitivity to aurovertin. The ATPase activity of $F_0 \cdot F_1$ is as sensitive to this inhibitor as that of submitochondrial particles [46], whereas the ATPase activity of $RrF_0 \cdot F_1$ is much less sensitive to aurovertin than that of the *R. rubrum* chromatophores (Fig. 7). A difference in the effect of aurovertin on these systems has already been indicated in the reported absence of increase in the fluorescence of aurovertin upon addition of *R. rubrum* chromatophores [33].

Kinetics studies carried out on the water-soluble ATPase complexes, isolated from mitochondria [38], chloroplasts [39] and *Chromatium* strain D chromatophores [40] have been interpreted as indicating that the substrate for these enzymes is a cation-ATP complex. Such studies have not been reported up to now on any detergent-solubilized ATPase. Our observations that both the membrane-bound and $RrF_0 \cdot F_1$-ATPase systems of *R. rubrum* require a specific ratio of cation to ATP for optimal activity and are inhibited by an excess of cations (Fig. 8) or an excess of ATP (Fig. 9) indicate that a cation-ATP complex is the substrate also in *R. rubrum*. This indication is supported by the findings that free ATP and free Mg^{2+} are competitive inhibitors of both membrane-bound and $RrF_0 \cdot F_1$-ATPase complexes (Table II). $RrF_0 \cdot F_1$ has a K_m value of 0.02 mM for $MgATP^{2-}$ and 0.5 mM for $CaATP^{2-}$. The K_i for free ATP is about 1 mM with both divalent cation-ATP complexes indicating that these complexes are bound to the enzyme tighter than the free ATP. Free Mg^{2+} is a much more effective inhibitor with a K_i of 7 μM. The K_i values for both ATP and Mg^{2+} show a striking similarity to the respective K_i values reported for CF_1 in chloroplast [39] and for the water-soluble ATPase isolated from *Chromatium* chromatophores [40].

The findings that the ATPase activity of $RrF_0 \cdot F_1$ is very similar to the membrane-bound activity in respect to its kinetic parameters, cation dependency and sensitivity to energy transfer inhibitors suggest that $RrF_0 \cdot F_1$ contains all the subunits of the *R. rubrum* coupling factor. If this suggestion is correct, the isolated enzyme should be capable of catalyzing energy-linked reactions. This $RrF_0 \cdot F_1$ has recently been found to catalyze ATP synthesis when reconstituted into liposomes containing bacteriorhodopsin [26].

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