Reconstitution of the H+-ATPase Complex of *Rhodospirillum rubrum* by the β Subunit of the Chloroplast Coupling Factor 1*

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A method is described for isolating the β subunit from spinach chloroplast F₁ (CF₁). The isolated β subunit reconstituted an active F₁ hybrid with the F₁ of *Rhodospirillum rubrum* chromatophores from which the β subunit had been removed. The CF₁ β subunit was similar to the isolated β subunit of *Escherichia coli* F₁ (Gromet-Elhanan, Z., Khananshivili, D., Weiss, S., Kanazawa, H., and Futai, M. (1985) J. Biol. Chem. 260, 12635–12640) in that it restored a substantial rate of ATP hydrolysis and low, but significant light-dependent ATP synthesis to the β-less chromatophores. The low rate of photophosphorylation observed with the hybrid enzyme probably resulted from a looser coupling of the CF₁ β subunit to proton translocation in the *R. rubrum* F₀-F₁ complex.

The hybrid enzyme exhibited a high specificity for Mg⁺⁺-ATP as substrate for ATP hydrolysis and with the antibiotic tentoxin. In contrast, chromatophores reconstituted with the native *R. rubrum* β subunit actively hydrolyzed both Mg⁺⁺-ATP and Ca⁺⁺-ATP and were insensitive to tentoxin.

These results indicate a close functional homology between the β subunits of the prokaryotic and eukaryotic H⁺-ATPases and suggest a role for the β subunit in conferring the different metal ion specificities and inhibitor sensitivities upon the enzymes. They also demonstrate the feasibility of isolating the β subunit from CF₁ in a reconstitutively active form.

ATP synthesis by bacterial, chloroplast, and mitochondrial membranes is catalyzed by a protein complex that links ATP synthesis and hydrolysis to transmembrane proton fluxes. The protein complex is referred to as the H⁺-ATPase, ATP synthase, or F₁-F₀. The enzymes from different sources have a number of common features. All are comprised of two functionally distinct entities, a membrane-spanning, proton translocating segment, F₀, and a peripheral segment, F₁, which couples proton movement to ATP synthesis/hydrolysis.

The total molecular mass of the complex from different sources ranges between 500 and 600 kDa. The F₀ segment contains multiple copies of a small (approximately 8 kDa), very hydrophobic protein and at least two other proteins with molecular masses ranging between 12 and 30 kDa. F₁ is comprised of five protein subunits labeled α to ε in order of decreasing molecular mass (88–8 kDa) and with a probable subunit stoichiometry of 3α, 3β, γ, δ, ε.

The amino acid sequences of β subunits from various prokaryotes and eukaryotes (1) are strikingly homologous. A lesser, but pronounced, sequence homology exists between α subunits whereas, the amino acid sequences of the three smaller subunits from the different enzymes show less homology. A high degree of homology between β subunits was predicted on the basis of immunological evidence (2, 3). Evolutionary conservation of the β subunit is consistent with its proposed critical function in nucleotide binding and catalysis (4–6).

All of the F₁ subunits from a thermophilic bacterium (7), from *Salmonella typhimurium* (8), and from *Escherichia coli* (EcF₁) (9) have been purified and shown to be reconstitutively active. Purified β and γ subunits of *Rhodospirillum rubrum* (8) (RrF₁) reconstitute both ATPase activity and photophosphorylation to chromatophores deficient in these proteins (10–12). The F₁ from organellar membranes has proven to be more resistant to isolation of subunits in active form. Exceptions are the δ (13–15) and ε (16) subunits of chloroplast F₁ (CF₁) which restore the capacity for ATP synthesis to the enzyme lacking these subunits. The ε subunit is a potent inhibitor of the ATPase activity of CF₁, devoid of ε (16).

The α, β, and γ subunits of EcF₁, *S. typhimurium*, and thermophilic bacterium form F₁ hybrids active in ATP hydrolysis (8, 17). More recently, the β subunit from EcF₁ was shown to restore rapid rates of ATP hydrolysis and some ATP synthesis to *R. rubrum* chromatophores containing β-less F₁ (18). In addition to demonstrating extensive functional homology between F₁ subunits from different bacteria, these studies provide systems for testing functional homology between the bacterial F₁ and the eukaryotic organelle membrane F₁.

In this paper we describe a method for isolating the β subunit from spinach chloroplast F₁ in a substantially purified form. The CF₁ β reconstituted active ATP hydrolysis and a low rate of photophosphorylation to β-less chromatophores of *R. rubrum*.

**MATERIALS AND METHODS**

**Preparations**—CF₁ was purified from市场 spinach (19). Control and *R. rubrum* chromatophores deficient in the β subunit of *R. rubrum* F₁ (β-less chromatophores) and RrF₁ β subunit were prepared as described (11, 12). The β subunit of *E. coli* F₁ was a generous gift of Dr. M. Futai of Osaka University, Japan. The β subunit of CF₁ was prepared from CF₁ lacking the δ and ε subunits. Removal of the two...
smaller CF$_1$ subunits was accomplished by a modification of previous (16, 20) procedures. CF$_1$ (15–20 mg) desalted by gel filtration in 25 mM Tris-HCl (pH 8.0), 1 mM EDTA, 2 mM ATP, was applied to 1.6 × 5-cm column of DEAE-cellulose equilibrated with the same buffer at room temperature. The column was washed with 20 ml of the Tris/EDTA/ATP buffer and the $\delta$ and $\epsilon$ subunits removed by washing with 50–70 ml of the Tris/EDTA/ATP solution supplemented with 30% (v/v) glyceral, 20% (v/v) ethanol, 5 mM dithiothreitol, and 50 mM sodium phosphate (pH 7). The final pH was approximately 7.7. The column was then washed with 20–30 ml of the Tris/EDTA/ATP buffer and the CF$_1$ lacking the $\delta$ and $\epsilon$ subunits was eluted with the same buffer containing 0.4 M NaCl, (NH$_4$)$_2$SO$_4$ was added to 50% of saturation and the precipitated protein collected by centrifugation. The pellet was dissolved in a small volume of 5 mM Tris-HCl (pH 8), 0.2 mM EDTA. Residual (NH$_4$)$_2$SO$_4$ was removed by gel filtration. To the CF$_1$, (-$\delta$-$\epsilon$) (1–2 mg/ml, 8–12 mg total) were added, in final concentrations, 0.5 mM dithiothreitol, 0.5 mM ATP ($\leq$ 0.15 mM), 400 mM NaC10$_4$ (pH about 6), and 50 mM Tris succinate (pH 5.9). The solution was frozen in a dry ice/ethanol bath and stored at $-70^\circ$C overnight. The solution was thawed at room temperature and applied to a 1.6 × 5-cm column of hydroxyapatite (Bio-Rad HTP) at 4 $^\circ$C. The column was equilibrated with 25 mM Tricine/NaOH (pH 8.0), 0.2 mM EDTA, 0.1 mM ATP, 0.1 mM dithiothreitol. The column was prewashed with 2–3 column volumes of equilibration buffer to remove the ClO$_4$- . The $\beta$ subunit was eluted with the same buffer supplemented with 50 mM sodium phosphate (pH 8). ATP and glyceral were added to the pooled $\beta$-containing fractions, to final concentrations of 1 mM and 10% (v/v), respectively. The protein was concentrated by ultrafiltration at 4 $^\circ$C (Amicon PM 30 membrane), and in some cases dialyzed against the glycerol/Tris/EDTA/ATP solution to remove phosphate, and stored at $-20^\circ$C.

*Assays*—ATPase activities were determined at 35 $^\circ$C by two methods. When Mg$_2^+$ was present, ATP hydrolysis was coupled to NADH oxidation using pyruvate kinase and lactate dehydrogenase and the reaction was followed by the change in absorbance at 340 nm. The standard reaction mixture (0.5 ml) contained 50 mM Tricine/NaOH (pH 8.0), 10 mM KCl, 5 mM phosphoenolpyruvate, 4 mM ATP, 2 mM MgCl$_2$ (except where indicated), 0.2–0.4 mM NADH, 20 units/ml of pyruvate kinase, 50 units/ml of lactate dehydrogenase, and control or reconstituted chromatophores equivalent to 2.5 $\mu$g of bacteriochlorophyll. The light intensity was 2.5 ergs/cm$^2$/s of incandescent light. In a few cases, 0.2 mM diaminodurene was used as the mediator of cyclic electron flow instead of N-methylphenazonium methosulfate. To distinguish between $P_i$ incorporation into ATP (glucose 6-phosphate since glucose and hexokinase were present) and ADP, the organic phosphate content of the reaction mixtures was determined before and after treatment with 1 N HCl for 10 min.

Reconstitution was carried out at 35 $^\circ$C for 1–2 h as described using, in most cases, chromatophores equivalent to 3–4 $\mu$g of bacteriochlorophyll in 0.15 ml (12, 18). In most experiments, the pH during reconstitution was 8.0. Samples were assayed directly for ATP synthetase activity prior to assay of ATPase activity; mixtures were centrifuged at 90,000 $\times$ g in a Beckman airfuge. The supernatant fluids were discarded and the pellets resuspended in 30–50 $\mu$l of 50 mM Tricine/NaOH (pH 8).

Quinacrine (atebrin) fluorescence quenching was determined essentially as described (16, 17) at room temperature in an incubation mixture (2 ml) that contained 50 mM Tricine/NaOH (pH 8.0), 50 mM KCl, 0.75 mM MgCl$_2$, 1 $\mu$m valinomycin, 0.2 mM dianolaminodurene, 1 $\mu$m quinacrine, and chromatophores equivalent to approximately 8 $\mu$g of bacteriochlorophyll. ATP was added to 1.5 mM to the stirred cuvette.

Protein was determined by either the procedure of Lowry et al. (25) or by the Bradford method (26) using bovine serum albumin as the standard.

**Reagents**—Nucleotides, tetraxon, and oligomycin were from Sigma and $^{32}$P, from ICN. Pyruvate kinase and lactate dehydrogenase were purchased from Boehringer-Mannheim and valinomycin from Behring Diagnostics.

**RESULTS**

Preparation of the $\beta$ Subunit of CF$_1$—Reconstitutively active $\beta$ subunit in an overall yield of more than 50% was routinely obtained (Fig. 1). A trace contamination by the $\alpha$ subunit was usually evident on gels containing heavy loads of the purified $\beta$ subunit. Preparations were, however, routinely greater than 95% pure as judged by densitometric scanning of stained gels. Solutions of the $\beta$ subunit at 1–2 mg/ml showed no signs of turbidity or aggregation even after repeated freezing and thawing and remained fully active for at least several weeks when stored at $-20^\circ$C. As is the case for R. rubrum $\beta$ (10, 11, 27), ATP is essential for preservation of the reconstitutive activity of CF$_1$ $\beta$ during all stages of its extraction, purification, storage, and binding to $\beta$-less chromatophores. Increasing the ATP concentration to 0.5 mM or greater during the chromatography step led to an increased contamination of the $\beta$ subunit with the $\alpha$ subunit.

Reconstitution of ATPase Activity—Tested at equal concentrations (10 $\mu$g/$\mu$g of bacteriochlorophyll) the RrF$_1$ $\beta$ subunit (ATPase rate 381 pmol/h/mg of bacteriochlorophyll) was more effective than that of EcF$_1$ (ATPase rate 218) which was only slightly more effective than the chloroplast $\beta$ subunit (ATPase rate 172) under the conditions described. EcF$_1$ $\beta$ subunit is more effective when the reconstitution is performed at pH 6.2 (18). CF$_1$ $\beta$, however, like RrF$_1$ $\beta$, (28) had the same reconstitutive activity at pH 6.2 and 5 (not shown). Optimal conditions for reconstitution were remarkably similar for the RrF$_1$, $\beta$ subunit preparations this activity varied between 80 and 180 $\mu$mol of ATP hydrolyzed per h/mg of bacteriochlorophyll. The activity of $\beta$-less chromatophores without added $\beta$ ranged between 10 and 20, whereas saturating amounts of RrF$_1$ $\beta$ subunit yielded rates between 350 and 450. CF$_1$ $\beta$ had no

**FIG. 1.** Sodium dodecyl sulfate polyacrylamide-gel electrophoresis of the isolated chloroplast $\beta$ subunit. Electrophoresis was performed on 12% acrylamide gels. Lane 1, 35 $\mu$g of CF$_1$; Lane 2, 15 $\mu$g of isolated CF$_1$ $\beta$ subunit.
effect on the ATPase activity of control chromatophores. Approximately 3–5 µg of CF₁ β were required to reconstitute half-maximal activity to chromatophores equivalent to 1 µg of bacteriochlorophyll, which is about twice the required amount of native R. rubrum β subunit (11) (Fig. 2). Chromatophores reconstituted with either the native or chloroplast β subunit also showed a similar MgCl₂ dependence. Optimal rates of ATPase activity were obtained with a ratio of ATP to MgCl₂ of 2:1 (29) in both cases (not shown).

**Stimulation of Mg-dependent ATPase Activity by Sulfite**—Sulfite and similar oxyanions stimulate the Mg-dependent ATPase activities of F₁ enzymes from a variety of sources including mitochondria (30), R. rubrum (31), and chloroplasts (32, 33). This effect was attributed to relief of the inhibition of ATPase activity by free Mg²⁺ ions. The effects of Na₂SO₃ on chromatophores reconstituted with either RrF₁ or CF₁ β subunit were compared. Both systems showed a similar concentration dependence for sulfite stimulation (half-maximum stimulation at 2–3 mM), but the extent of stimulation was greater for chromatophores reconstituted with the CF₁ β. The latter show a 5–7-fold stimulation as compared to only a 2–3-fold stimulation of chromatophores containing the RrF₁ β.

The stimulation of ATPase activity by sulfite was not a consequence of uncoupling since Na₂SO₃ had no effect on light-induced quenching of quinacrine fluorescence in either control or reconstituted chromatophores (not shown). Moreover, sulfite-stimulated ATPase activity of control chromatophores was further enhanced by the uncoupler, gramicidin. As with control chromatophores, ATPase activity of chromatophores reconstituted with the RrF₁ β subunit was strongly stimulated (approximately 60%) by gramicidin, whereas the hybrid chromatophores were only slightly stimulated (approximately 16%) (Table I). The maximum rates obtained with the hybrid were consistently 50–60% of those obtained with chromatophores reconstituted with the native β subunit at optimal concentrations of sulfite and gramicidin. Similar results were obtained for ATP-induced quinacrine fluorescence quenching in reconstituted chromatophores. Chromatophores reconstituted with RrF₁ β subunit show a strong ATP-induced quenching of quinacrine fluorescence (18). Addition of 20 mM Na₂SO₃ to the assay mixture caused a 30% increase in ATP-induced quenching. On the other hand, chromatophores reconstituted with chloroplast β showed a barely detectable response to added ATP, and 20 mM Na₂SO₃ only slightly enhanced (about 19%) the quenching.

The two enzymes also exhibited differential behavior toward Ca²⁺-dependent ATP hydrolysis (Table II), the hybrid showing a high specificity for Mg²⁺ which is typical of membrane-bound CF₁ (34). On the other hand, membrane-bound RrF₁ catalyzes ATP hydrolysis in the presence of either Mg²⁺ or Ca²⁺ ions (28, 29; Table II).

**Reconstitution of ATP Synthesis**—The β subunit of CF₁ also restored a low, but significant rate of ATP synthesis to the β-less chromatophores. Photophosphorylation rates with saturating amounts of CF₁ β ranged between 13 and 55 µmol/h/mg of bacteriochlorophyll. In the absence of added β, the rate was approximately 5, and between 350 and 450 when chromatophores were reconstituted with saturating amounts of the native R. rubrum β subunit. Thus, although ATPase activity was restored to 50–60% of that obtained with RrF₁ β subunit, at best CF₁ β restored only 10–12% of the ATP synthesis observed with the native β subunit. The identity of the product of light-dependent ATP synthesis by chromatophores reconstituted with either RrF₁ β or CF₁ β as ATP, was confirmed by the demonstration that the ATP, was incorporated into an acid-stable form (glucose 6-phosphate). Low concentrations of oligomycin (20–50 nM) did not stimulate ATP synthesis in hybrid chromatophores, suggesting that uncoupling is not a cause of the low rates of ATP synthesis (35).

Half-maximal restoration of ATP synthesis occurred at a CF₁ β concentration similar to that required for half-maximum restoration of ATPase activity (see Fig. 2). Moreover, CF₁ β strongly inhibited the restoration of ATP synthesis by RrF₁ β (Fig. 3). Approximately 40 µg of chloroplast β were required to block 50% of the stimulation given by 23 µg of RrF₁ β. Thus, the low activity in ATP synthesis cannot be

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**Table I**

<table>
<thead>
<tr>
<th>β Subunit*</th>
<th>Gramicidin* (1 µM)</th>
<th>Mg²⁺-ATPase µmol h⁻¹ mg bacteriochlorophyll⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>R. rubrum</td>
<td>–</td>
<td>957</td>
</tr>
<tr>
<td>R. rubrum</td>
<td>+</td>
<td>1520</td>
</tr>
<tr>
<td>Chloroplast</td>
<td>–</td>
<td>635</td>
</tr>
<tr>
<td>Chloroplast</td>
<td>+</td>
<td>739</td>
</tr>
</tbody>
</table>

*β-less chromatophores were reconstituted with saturating amounts of the respective β subunits. Assay mixtures contained 40 mM Na₂SO₃ and ATPase measured by Pi released after 4 min incubation at 35 °C. Background rates obtained with β-less chromatophores in the absence of added β subunits were subtracted to give the rates shown.

Ggramicidin was present in the assay mixture where indicated.

**Table II**

<table>
<thead>
<tr>
<th>Chromatophores*</th>
<th>β Subunit</th>
<th>Me⁺⁺ ATPase µmol h⁻¹ mg bacteriochlorophyll⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control None</td>
<td>Mg²⁺</td>
<td>453</td>
</tr>
<tr>
<td>Control None</td>
<td>Ca²⁺</td>
<td>99</td>
</tr>
<tr>
<td>β-Less R. rubrum</td>
<td>Mg²⁺</td>
<td>222</td>
</tr>
<tr>
<td>β-Less R. rubrum</td>
<td>Ca²⁺</td>
<td>115</td>
</tr>
<tr>
<td>β-Less Chloroplast</td>
<td>Mg²⁺</td>
<td>127</td>
</tr>
<tr>
<td>β-Less Chloroplast</td>
<td>Ca²⁺</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

*Control or reconstituted chromatophores (equivalent to 3–4 µg of bacteriochlorophyll/sample) were centrifuged at 90,000 × g, the pellets resuspended in 150 µl of 50 mM Tricine/NaOH, recentrifuged, and finally resuspended in 30 µl of the same buffer. This was done to remove residual MgCl₂ prior to assay.

*Assay mixtures contained 50 mM Tricine/NaOH (pH 8), 4 mM ATP, 2 mM MgCl₂, or 5 mM ATP and 5 mM CaCl₂. ATPase was measured by P₁ released after 8 min incubation at 35 °C. Other conditions were as described in Table I.

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![Fig. 2](image-url)  
**Fig. 2.** Mg²⁺-ATPase activity of β-less chromatophores reconstituted with varying amounts of isolated chloroplast β subunit. Mg²⁺-ATPase was measured using the coupled enzyme assay described under "Materials and Methods."
Reconstitution of $H^+$-ATPase

**FIG. 3.** Competition between the native and chloroplast $\beta$ subunits for binding to $\beta$-less chromatophores. $\beta$-Less chromatophores (equivalent to 4 $\mu$g of bacteriochlorophyll) were incubated with 23 $\mu$g of $R$. rubrum $\beta$ subunit together with the indicated amounts of the chloroplast $\beta$ subunit. ATP synthesis was measured as described under "Materials and Methods." The rate of ATP synthesis in the absence of chloroplast $\beta$ was 234 $\mu$mol/h/mg of bacteriochlorophyll. $\Delta$, ATP synthesis by chloroplast $\beta$ in the absence of $R$. rubrum $\beta$.

Explained by poor binding of the CF1 $\beta$ subunit.

The CF1 preparation lacking the $\delta$ and $\epsilon$ subunits was totally inactive in stimulating ATP synthesis in the $\beta$-less chromatophores and failed to compete with the $R$. rubrum $\beta$ for binding.

**Effects of Tentoxin and Oligomycin on Reconstituted Chromatophores**—Tentoxin is a highly specific inhibitor of ATP synthesis/hydrolysis in thylakoids and exerts its effect directly on CF1 (38). As shown in Table III, tentoxin strongly inhibited both ATP hydrolysis and synthesis by chromatophores reconstituted with the chloroplast $\beta$ but not by chromatophores containing the $R$. rubrum $\beta$ subunit. These results indicate that the chloroplast $\beta$ subunit is catalytically active and is responsible for conferring tentoxin sensitivity to the enzyme. Oligomycin usually has little or no effect on the chloroplast Fo-F1, but strongly inhibits the $R$. rubrum Fo-F1 (29). Interestingly, oligomycin (6 $\mu$m) inhibited both ATP synthesis and hydrolysis in hybrid chromatophores to a similar extent to that observed for the control or $R$. rubrum $\beta$-containing chromatophores (Table III).

**DISCUSSION**

The results presented in this paper demonstrate, for the first time, the feasibility of isolating the $\beta$ subunit from chloroplast Fo in an active form. Remarkably, CF1 $\beta$ subunit restored ATPase activity to $R$. rubrum chromatophores lacking the $\beta$ subunit. Under optimal conditions, the ATPase activity of the hybrid was more than 50% of that of chromatophores reconstituted with the native $\beta$ subunit. The sensitivity of this activity to tentoxin leaves little doubt that the chloroplast $\beta$ subunit was active in the hybrid enzyme. Moreover, the amount of CF1 $\beta$ subunit required to achieve maximum rates of ATP hydrolysis was only 3-fold higher than that required for the native $R$. rubrum $\beta$ subunit, and CF1 $\beta$ competed well with $R$. rubrum $\beta$ for binding to the $\beta$-less chromatophores.

A low rate of light-dependent ATP synthesis was also detected in chromatophores reconstituted with the CF1 $\beta$. Previously it was observed (18) that chromatophores reconstituted with EcF1 $\beta$ were also more active in ATP hydrolysis than in ATP synthesis relative to chromatophores containing $R$. rubrum $\beta$ subunits. The requirements for ATPase activity appear to be less stringent than those for ATP synthesis. The coupling between transmembrane proton flux and ATP synthesis must be very tight since the electrochemical proton gradient drives ATP synthesis. In contrast, ATP hydrolysis, an energetically favorable reaction, can apparently take place in a manner that is more loosely coupled to proton translocation. This conclusion is supported by the observation that the ATP-induced quenching of quinacrine fluorescence in chromatophores reconstituted with either EcF1 or CF1 is minimal, despite the fact that substantial ATPase activity is observed. The failure of the E. coli and chloroplast $\beta$ subunits to interact properly with $R$. rubrum $\beta$ subunits involved in a proton gating mechanism could explain the low ATP synthesis rates and poor ATP-induced generation of a proton gradient in these heterologous systems.

The native and hybrid enzymes differed substantially in their affinities for different metal ions as substrates, and in their sensitivity to the inhibitor tentoxin. These properties appear to be conferred upon the enzyme by the different $\beta$ subunits. The effect of tentoxin is particularly interesting, perhaps reflecting a direct interaction between the antibiotic and the chloroplast $\beta$ subunit. Our results do not, however, rule out the alternative possibility that tentoxin exerts its effect on the $\beta$ subunit via other $F_1$ subunits.

$R$. rubrum chromatophores containing the EcF1 $\beta$ subunits were considerably less sensitive to oligomycin than the native enzyme (18). Since EcF1 is normally insensitive to oligomycin, the result suggested that conferral of oligomycin sensitivity rests, at least in part, with the $\beta$ subunit. The apparent discrepancy between results obtained with EcF1 $\beta$ and those described here for CF1 $\beta$ subunit can be explained by invoking differences in the interactions between the different $\beta$ subunits with an oligomycin sensitivity conferring component of the $R$. rubrum Fo-F1 complex. Consistent with this explanation is the observation that reconstitution of EcF1 $\beta$ at pH 8 instead of at pH 6 results in a hybrid which is inhibited by oligomycin, although to a lower extent than chromatophores

**Table III**

<table>
<thead>
<tr>
<th>Chromatophores</th>
<th>$\beta$ Subunit</th>
<th>Inhibitor*</th>
<th>Mg$^{2+}$-ATPase $^{b}$</th>
<th>ATP synthesis $^{a}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>+Inh (%)</td>
<td>+Inh (%)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>None</td>
<td>Tentoxin</td>
<td>584 561 (96)</td>
<td>683 656 (96)</td>
</tr>
<tr>
<td>$\beta$-Less</td>
<td>$R$. rubrum</td>
<td>Tentoxin</td>
<td>367 413 (113)</td>
<td>463 546 (118)</td>
</tr>
<tr>
<td>$\beta$-Less</td>
<td>Chloroplast</td>
<td>Tentoxin</td>
<td>101 2 (2)</td>
<td>42 12 (29)</td>
</tr>
<tr>
<td>Control</td>
<td>None</td>
<td>Oligomycin</td>
<td>567 144 (25)</td>
<td>716 10 (1.4)</td>
</tr>
<tr>
<td>$\beta$-Less</td>
<td>$R$. rubrum</td>
<td>Oligomycin</td>
<td>404 114 (28)</td>
<td>377 5 (1.3)</td>
</tr>
<tr>
<td>$\beta$-Less</td>
<td>Chloroplast</td>
<td>Oligomycin</td>
<td>110 29 (26)</td>
<td>13 0 (0)</td>
</tr>
</tbody>
</table>

* Control or reconstituted chromatophores were preincubated with tentoxin (4 $\mu$m final concentration, 1 h room temperature) or oligomycin (6 $\mu$m final concentration, 30 min room temperature) where indicated.

$^b$ Rates are expressed as micromole/h/mg of bacteriochlorophyll. Other conditions were as described in Tables I and II.

* Inh stands for inhibitor.
reconstituted with either RrF₁β or CF₁β.²

The ATPase of chromatophores containing CF₁β was active without exposure of the membranes to either reducing conditions or to light. In contrast, the ATPase of chloroplast membranes is virtually inactive unless exposed to such conditions (37). This observation is consistent with evidence (38) that the γ and ε subunits are involved in regulation of the chloroplast H⁺-ATPase. The interactions between the CF₁β subunit and the smaller subunits of RrF₁ would appear then to differ from those of CF₁β with its homologous smaller subunits. No effect of reductants has been observed on the P-less enzyme from RrF₁, in contrast to those of CF₁, which differ from those of CF₁β in suggesting that all of the subunit and the smaller subunits of RrF₁ would appear then to differ from those of CF₁β with its homologous smaller subunits.

In summary, the CF₁β subunit can substitute for the native RrF₁β subunit to restore substantial rates of ATPase activity to the β-less enzyme from R. rubrum chromatophores. The hybrid enzyme is also partly coupled to H⁺-linked functions. These observations support other evidence including the overall structural similarities, amino acid sequence homologies and immunological cross-reactivities, in suggesting that all of the FₐFₐ subunits share a common ancestry and probably share the same basic mechanism of action. Differences in the properties of the enzymes from various sources, evident from their activation requirements, sensitivities to inhibitors, and from the lack of amino acid sequence homology between the smaller subunits, may have evolved to accommodate specific regulatory requirements for ATP synthesis/hydrolysis in the different systems.

Finally, the purification of the β subunit of CF₁ in active form will allow the study of its nucleotide binding properties, and raises the hope that the complete reconstitution of an organellar F₁ will be achieved.

REFERENCES

²Z. Gromet-Elhanan, unpublished results.