ATP Synthase Complex from Bovine Heart Mitochondria

PASSIVE H⁺ CONDUCTION THROUGH F₁ DOES NOT REQUIRE OLGOMYCIN SENSITIVITY-CONFERRING PROTEIN*

Michael J. Pringle§§, Mary K. Kenneally‡, and Saroj Joshi¶¶

From the *Boston Biomedical Research Institute, Department of Cell Physiology, Boston, Massachusetts 02114 and ‡Harvard Medical School, Department of Biological Chemistry and Molecular Pharmacology, Boston, Massachusetts 02115

Oligomycin sensitivity-conferring protein (OSCP) is a water-soluble subunit of bovine heart mitochondrial H⁺-ATPase (F₁-F₀). In order to investigate the requirement of OSCP for passive proton conductance through mitochondrial F₀, OSCP-depleted membrane preparations were obtained by extracting purified F₁-F₀ complexes with 4.0 M urea. The residual complexes, referred to as UF₀, were found to be deficient with respect to OSCP, as well as α, β, and γ subunits of F₁-ATPase, but had a full complement of coupling factor 6 as judged by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blotting techniques. These UF₀ complexes had no intrinsic ATPase activity and were able to bind nearly the same amount of F₁-ATPase in the presence of either OSCP or NH₄ ions alone, or a combination of the two. However, the preparations exhibited an absolute dependence on OSCP for conferral of oligomycin sensitivity to membrane-bound ATPase. The passive proton conductance in UF₀ proteoliposomes was measured by time-resolved quenching of 9-amino-6-chloro-2-methoxyacridine or 9-aminoacridine fluorescence following a valinomycin-induced K⁺-diffusion potential. The data clearly establish that OSCP is not a necessary component of the F₀ proton channel nor is its presence required for conductance blockage by the inhibitors oligomycin or dicyclohexylcarbodiimide. Furthermore, OSCP does not prevent or block passive H⁺ leakage. Comparisons of OSCP with the F₁-F₀ subunits from Escherichia coli and chloroplast lead us to suggest that mitochondrial OSCP is, both structurally and functionally, a hybrid between the b and δ subunits of the prokaryotic systems.

According to the chemiosmotic formalism, the energy required for converting ADP and phosphate into ATP is obtained by the dissipation of a transmembrane proton electrochemical gradient, where both processes are mediated by a multisubunit enzyme known as ATP synthase (1). While the transduction mechanism remains obscure it seems clear that both prokaryotic and eukaryotic forms of the enzyme the synthetic steps occur on an extrinsic portion of the complex known as F₁ (2, 3), and the proton conductance pathway is formed by a transmembrane portion known as F₀ (4, 5). In mammalian mitochondria (6, 7) and in Escherichia coli (8, 9), there is electron micrographic evidence suggesting that the F₁ and F₀ portions of the enzyme are physically linked by a group of subunits which are collectively referred to as the "stalk." Parts of the stalk interact with F₁ and are required for coupling proton conductance to ATP synthesis while other parts intercalate with F₀ subunits and may or may not constitute an integral part of the proton-conductance pathway.

Experimentally, the stalk proteins remain associated with the F₀ portion of the enzyme when the five subunits that comprise the extrinsic portion of F₀ are removed. Thus, in mammalian mitochondria, removal of F₁ leaves a membrane complex consisting of at least eight different proteins (10). This is a far more complex arrangement than in bacterial F₁ which consists of only three subunits designated α, β, and γ (11, 12). In the last few years much interest has been focused on distinguishing the stalk subunits that are required for interaction with F₁ from those that are essential for transmembrane proton conducton per se. To that end we have recently reported that F₀, a coupling factor presumed to be a stalk subunit of ATP synthase, is not essential for inhibitor-sensitive proton conductance through F₀ although it is an absolute requirement for energy coupling by the intact enzyme (13).

Oligomycin sensitivity-conferring protein (OSCP) is another component (molecular mass = 20,967 daltons) of mitochondrial ATP synthase that copurifies with the F₀ sector and, together with F₆, is considered to serve as a link between the catalytic (F₁) and proton-conducting (F₀) components (14). Both F₆ and OSCP have been implicated in the binding of F₁ to the membrane although the evidence concerning this aspect is controversial (15-17). The OSCP protein has no directly measurable biological activity. Its functional characterization has so far been based on its ability to associate with the F₁-F₀ complexes devoid of OSCP and to render the ATPase activity of the complex sensitive to inhibition by oligomycin, an antibiotic that binds to a specific site in F₆ (18). It has been reported that OSCP can interact with isolated F₁-ATPase via the α and β subunits (19) and confer partial cold stability to the enzyme in the process (20). It has further been shown that OSCP can also independently complex with membranes depleted of F₁ and OSCP (19), although

---

* This study was supported by United States Public Health Service Grant GM-26420.

† To whom correspondence should be addressed: Boston Biomedical Research Institute, Dept. of Cell Physiology, 20 Stamford St., Boston, MA 02114. Tel.: 017-742-2010. Fax: 017-520-0649.

‡ Present address: CIBA-Corning Diagnostics, 333 Coney St., East Walpole, MA 02032.

§§ To whom correspondence should be addressed: Boston Biomedical Research Institute, Dept. of Cell Physiology, 20 Stamford St., Boston, MA 02114. Tel.: 017-742-2010. Fax: 017-520-0649.

¶¶ To whom correspondence should be addressed: Boston Biomedical Research Institute, Dept. of Cell Physiology, 20 Stamford St., Boston, MA 02114. Tel.: 017-742-2010. Fax: 017-520-0649.

The abbreviations used are: F₁, coupling factor 1; F₆, coupling factor 6; F₀, membrane fraction of ATP synthase; ACMA, 9-amino-6-chloro-2-methoxyacridine; DCCD, dicyclohexyl carbodiimide; DTT, dithiothreitol; OSCP, oligomycin sensitivity-conferring protein; UF₀, particles obtained by treating F₁-F₀ complexes with urea; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine.
the functional significance of this association is not clear. Our previous report on the functional properties of trypsin-treated F₁, proteoliposomes showed that the surface-accessible segments of membrane-bound OSCP are not essential for inhibitor-sensitive passive proton conductance (21). These data, however, did not clarify whether trypsin-inaccessible (and presumably membrane-associated) segments of OSCP are involved in channeling protons through the proton pore, or in stabilizing the conductance apparatus of the F₁ channel per se.

In the present report we describe the isolation of an F₁₀ preparation obtained by treating purified F₁₀-OSCP complexes with urea and show that it has a full complement of F₁₀, a diminished level of F₁₀ subunits, but is highly deficient in OSCP. This selective removal of OSCP from the membrane, while maintaining its F₁₀ content, enables us to investigate the specific role of OSCP in mitochondrial energy transduction. The OSCP-depleted preparation is able to bind F₁₀-ATPase, provided NH₄⁺ ions or OSCP are present, although the complex only exhibits oligomycin-sensitive ATPase activity in the presence of OSCP. Most importantly, we find that proteoliposomes containing the urea-F₁₀ preparation (UF₀) are still able to conduct protons, and that this conductance is blocked by oligomycin and DCCD. These data, as an extension of our previous work on trypsinization of F₁₀, lead us to conclude that OSCP is not essential for binding F₁₀ to F₁₀, nor is it a necessary component of the proton-conducting pathway in mitochondrial F₁₀.

EXPERIMENTAL PROCEDURES

Materials

Acrylamide, N,N'-methylenebisacrylamide, nitrocellulose membranes (Bio-Rad), Freund's complete adjuvant (Calbiochem), Bacto Mycobacterium tuberculosis H37 RA (Difco), peroxidase-conjugated anti-rabbit IgG (Organon Pharmaceuticals), and purified phospholipids (Avanti Polar Lipids, Birmingham, AL) were all obtained from the indicated sources. ACMA was kindly provided by Dr. M. Ronjat from the Boston Biomedical Research Institute. All other chemicals were of reagent grade and were purchased from Sigma.

Biological Preparations

Bovine heart mitochondria (22), F₁₀-F₁₀ complexes (23), OSCP (24), F₁₀ (16), and F₁₀ (25) were prepared by published procedures. Treatment of F₁₀-F₁₀ with urea was carried out as described by Galante et al. (26) and afforded UF₀ complexes.

Antisera Preparations

Antisera to purified F₁₀ and OSCPs were raised by a single intradermal injection of 3-month-old female rabbits at multiple sites with a total of 100 µg of antigen mixed with an equal volume of complete Freund's adjuvant and Bacto M. tuberculosis H37 RA cells (1.9 mg/ml of final suspension). Monospecificity of the antisera was established by allowing the antisera to react with unfractionated mitochondrial membrane using the Western blotting technique (27).

Antisera to purified F₁₀ and OSCPs were raised by a single intradermal injection of 3-month-old female rabbits at multiple sites with a total of 100 µg of antigen mixed with an equal volume of complete Freund's adjuvant and Bacto M. tuberculosis H37 RA cells (1.9 mg/ml of final suspension). Monospecificity of the antisera was established by allowing the antisera to react with unfractionated mitochondrial membrane using the Western blotting technique (27).

Passive H⁺ Conduction through F₁₀ Does Not Require OSCP

Biological Assays

Reconstitution of Oligomycin-sensitive ATPase—Prior to reconstitution with depleted membranes, F₁₀ and OSCPs, stored as ammonium sulfate suspensions, were centrifuged, the pellets resuspended, and traces of salt removed by passage through a Sephadex G-25 column. Aliquots of UF₀ (160 µg) were incubated with 40 µg (3.0 units) of F₁₀, in a total volume of 300 µl containing 250 mM sucrose, 10 mM Tris-ISO (pH 8.5), 0.2% EDTA, and 5 mM DTT (STE-DTT buffer). In addition, some incubating mixtures contained OSCPs (6 µg) and/or NH₄⁺ ions (75 mM ammonium sulfate). After 15 min at 30 °C the samples were centrifuged to remove unbound F₁₀ and the sediments were resuspended in 300 µl of STE-DTT buffer in the absence of OSCP (6 µg) as indicated. The ATPase activity of the initial incubation mixtures, the resuspended particles, and the supernatant fraction, was assayed as described in the following paragraph. The extent of F₁₀ binding was estimated from the difference in the ATPase activity before and after centrifugation of the suspension (20). The ATPase activity of the resuspended particles was also measured in the presence of 1 µg of oligomycin in order to assess the sensitivity of the reconstituted particles for the inhibitor.

ATPase Activity—The ATPase activity was measured according to Tzagoloff et al. (29) by incubating F₁₀-ATPase or reconstituted F₁₀-OSCP in 0.5 ml of a buffer containing 50 mM Tris-HCl (pH 8.5), 2 mM MgCl₂, and 10 µM ATP at 30 °C. The rate of the reaction was monitored by adding 0.25 ml of 20% trichloroacetic acid. A 0.5-ml sample of deproteinized solution was assayed for inorganic phosphate according to Fiske and SubbaRow (30).

Proton Conductance Measurements

Measurements of H⁺ influx through UF₀ preparations were carried out by using the valinomycin-induced pH-dependent quenching of ACMA fluorescence in K⁺-loaded proteoliposomes containing UF₀ (88 OSCP) in a manner similar to that described previously (31). To prepare proteoliposomes, chloroform solutions of phosphatidylethanolamine/phosphatidylserine (7/3, w/w) were dried down to give 40 µg of phospholipid which was placed under high vacuum overnight. Aliquots of UF₀ were diluted to 2 mg/ml in 50 mM Tris acetate (pH 7.5) containing 250 mM sucrose and 1 mM DTT and incubated for 1 h at 30 °C in the presence or absence of OSCP (0.2 mg/ml). The reconstituted samples were spun down and homogenized with a suspension of phospholipid (see above) which had been sonicated to clarity at 20 mg/ml in a bath-type sonifier (Branson Cleaning Equipment Co.) in 10 mM Tricine/KOH (pH 8.0) containing 200 mM KCl and 1 mM DTT. The lipid/protein ratio was 20/1 (w/w) in each case. Protein-lipid suspensions were twice frozen in liquid nitrogen, thawed, and briefly sonicated (60 s). Proteoliposome aliquots containing 10 µg of protein were assayed in a Spex Industries, Inc. microfluorometer for valinomycin-induced fluorescence quenching in a buffer containing 10 mM Tricine (pH 7.0), 200 mM NaCl, 1 mM DTT, and 0.5 µM ACMA at 25.4 °C using excitation and emission wavelengths of 410 and 480 nm, respectively. In some experiments, the quenching assays were performed with 9-aminocaridine as the probe, as described previously (21, 32).

RESULTS

The UF₀ preparation obtained by urea treatment of purified F₁₀-OSCP was characterized with respect to (i) subunit composition, (ii) its ability to bind F₁₀ and OSCP, and (iii) its ability to conduct passive proton translocation through F₁₀ channels after reconstitution into proteoliposomes.

SDS-PAGE Characterization—Fig. 1 presents the SDS-PAGE pattern of various fractions obtained by treatment of F₁₀-OSCP complexes with urea as revealed by the Coomassie Brilliant Blue staining technique. Lane A is the gel pattern of a typical F₁₀-OSCP preparation that has a high energy-linked activity implying that the preparation employed in the present investigation has a full complement of its subunits including F₁₀-ATPase and the stalk components Fs and OSCP. The UF₀ preparation (lane B) obtained by treating F₁₀-OSCP twice with urea appears to contain only trace amounts of Fs, β, and γ subunits belonging to F₁₀-ATPase (compare lanes B and C) and no bands corresponding to OSCP (compare lane B with lanes A and E). However, all other proteins observed in F₁₀-OSCP (lane
Passive H+ Conduction through F0 Does Not Require OSCP

This study investigates the role of OSCP in the binding of F1 to OSCP-depleted submitochondrial particles (15, 18). In this context Sandri et al. (15) showed that subunit depletion treatments might have caused a perturbation in the local ionic environment near the F1 binding site(s) that could not be reversed by merely adding back the depleted factors.

It should be noted that the preparations employed in the earlier reports on establishing F1 binding requirements were subunit-depleted particles with varying degrees of subunit depletion (15, 18). It is not obvious for instance how well these were depleted of OSCP or whether the particles (or factors added for reconstitution) already had cation(s) in the suspending medium. In contrast to those reports the UF0 preparation employed in the present investigation was derived from purified F1-F0 complexes and was found to contain less than 200 pmol of OSCP/mg membrane fraction as described before. Furthermore, the coupling factors used in our experiments were carefully desalted to remove trace metal and NH4+ ion contaminants as described under "Experimental Procedures." The ability of OSCP-depleted membranes to bind F1 was assessed by incubating the UF0 preparations with desalted F1, OSCP, and NH4+ ions as indicated in Table I. It may be pointed out that the F1-ATPase activity did not undergo any significant change during the desalting or reconstituting steps. Therefore, the binding of F1 to the membrane could be estimated from the difference in ATPase activity of the supernatant before and after centrifugation of the suspension. It seems clear from the results presented in Table I that OSCP or NH4+ ions alone promote nearly equal binding of F1 (66 and 72%, rows 2 and 3, respectively) and that the combination

Table I

<table>
<thead>
<tr>
<th>Additions to UF0</th>
<th>Role of OSCP in binding F1 to UF0</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
</tr>
<tr>
<td>None</td>
<td>7</td>
</tr>
<tr>
<td>OSCP</td>
<td>66</td>
</tr>
<tr>
<td>NH4+</td>
<td>72</td>
</tr>
<tr>
<td>OSCP, NH4+</td>
<td>79</td>
</tr>
<tr>
<td>None + OSCP</td>
<td>7</td>
</tr>
<tr>
<td>NH4+ + OSCP</td>
<td>72</td>
</tr>
</tbody>
</table>

A similar to the present in the F0 fraction (lane B; see also lanes E and F). Those components missing from UF0 are clearly observed in the first urea extract (lane C) and faintly observed in the second extract (lane D). One should note that the first urea extract appears to be somewhat similar to untreated F1-F0 in SDS-PAGE (compare lane C with A). This is presumably due to a finite solubility of F1-F0 proteins in the extraction medium used in the present experiments. The important feature of the urea treatment procedure, however, is the generation of an F0 fraction that has no detectable OSCP but a full complement of F1 protein. To confirm this by a more sensitive technique, a parallel set of the samples shown (Fig. 1) were subjected to Western blotting using antisera to OSCP (Fig. 2, left panel) and F6 (Fig. 2, right panel). Both subunits are clearly present in purified ATP synthase (lane A), but OSCP is absent from urea-treated F1-F0 (lane B, left panel), whereas F6 is largely unaffected (lane B, right panel). The extracted OSCP is present in the two urea supernatants (lanes C and D, left panel), the first of which also shows that a small amount of F6 has been solubilized. The major effect, then, of treating F1-F0 with urea is to remove F1 and OSCP, and we refer to this preparation as a UF0 complex.

The OSCP content of UF0 complexes was found to be less than 200 pmol of OSCP/mg membrane fraction as judged by immunotitrations and silver staining techniques (data not shown). Assuming a molecular mass of 150,000 daltons for UF0 and a 1:1 stoichiometry of OSCP with respect to F1-ATPase in intact F1-F0 complexes (33, 34), the level of OSCP present in UF0 preparations approximates to 0% of that expected for undepleted F0 preparations. Should the stoichiometry of OSCP relative to F1 be 2 as proposed previously (35), or more, the OSCP content of UF0 will be even less than 3%.

Binding of F1 to OSCP-depleted Membranes—There is a discrepancy in the literature as to whether or not OSCP is necessary for binding F1-ATPase to the membrane sector of the enzyme. For example, the group that first proposed OSCP as the stalk linking F1 to F0 (36) reported later that F1 was able to bind directly to OSCP-deficient submitochondrial particles (18). In this context Sandri et al. (15) showed that the F1-ATPase activity did not undergo any significant change during the desalting or reconstituting steps. Therefore, the binding of F1 to the membrane could be estimated from the difference in ATPase activity of the supernatant before and after centrifugation of the suspension. It seems clear from the results presented in Table I that OSCP or NH4+ ions alone promote nearly equal binding of F1 (66 and 72%, rows 2 and 3, respectively) and that the combination

FIG. 1. SDS-PAGE profile of F1-F0 and its subfractions obtained as a result of treating F1-F0 with urea. Samples containing indicated amounts of protein were processed as described under "Experimental Procedures," and the electrophoresed gel was stained with Coomassie Brilliant Blue R-250. Lane A, 10 µg of F1-F0; lane B, 10 µg of UF0; lane C, 12.5 µg of urea extract I; lane D, 6 µg of urea extract II; lane E, 1 µg of OSCP; lane F, 1 µg of F6; lane G, 5 µg of F1; lane H, molecular weight markers (phosphorylase b, glutamic dehydrogenase, ovalbumin, lactate dehydrogenase, carbonic anhydrase, β-lactoglobulin, cytochrome c in order of decreasing molecular weight).

FIG. 2. Western blotting of F1-F0 and its subfractions obtained after treating F1-F0 with urea. Aliquots containing indicated amount of protein were subjected to SDS-PAGE, transferred to nitrocellulose membranes, and tested for immunoreactivity to anti-OSCP (left) or anti-F6 (right). Lane A, 5 µg of F1-F0; lane B, 5 µg of UF0; lane C, 5 µg of urea extract I; lane D, 5 µg of urea extract II; lane E, 0.5 µg of OSCP; lane F, 1 µg of F6.
nation of the two provides very little additional binding (79%, row 4). However, the ATPase activity of the resultant complex is inhibited by oligomycin only when OS is present in the F₁-binding medium (rows 2 and 4). In fact, OS could be added directly to the resuspended particles in order to restore oligomycin-sensitive ATPase activity (rows 5 and 6). In summary, our data suggest that OS is not an obligatory requirement for binding F₁ to OS-depleted membranes, although it can serve to do so, but it is absolutely essential for confering of oligomycin sensitivity to the bound ATPase.

Binding of OS to OS-depleted Membranes—To answer the converse question, i.e. whether F₁ is necessary for binding OS to the membrane, UF₀ complexes were incubated with or without OS, spun down, resuspended, and finally incubated with F₁-ATPase. The binding of OS to the membrane was determined by the ability of centrifuged suspensions to reconstitute oligomycin-sensitive ATPase activity following the addition of soluble F₁. The data on the ATPase activity of these complexes are presented in Table II. In the absence of OS the ATPase activity of UF₀ plus F₁ was only marginally sensitive to oligomycin (7–23% in three separate experiments, rows 1–3). However, when UF₀ complexes were incubated together with OS prior to the assay, the resulting ATPase activity of F₁ was strongly inhibited by oligomycin (86–90%, rows 4 and 5). In fact, the degree of inhibition obtained by adding F₁ to a UF₀-OS complex was as high (Table II, rows 4 and 5) as that obtained by reconstituting UF₀, OS, and F₁ prior to the assay (Table II, row 6). These data lead us to propose that OS is able to complex with the depleted membrane in the absence of α, β, and γ subunits of F₁ and that it can serve to restore significant oligomycin sensitivity to F₁ when the latter is added to UF₀-OS complexes.

Passive Proton Conductance in OS-depleted F₀ Segments—In order to examine the role of OS in passive proton conductance, aliquots of UF₀ were incorporated into liposomes in the absence or presence of OS, and these were examined for their ability to effect transmembrane proton conductance. The results are shown in Fig. 3. When oligomycin was added to potassium-loaded vesicles containing UF₀ a large decrease in ACMA fluorescence was observed (Fig. 3, lower left trace) indicating a rapid proton influx. A small additional quenching was observed when the protonophore carbonyl cyanide p-trifluoromethoxyphenylhydrazone was added, and this presumably reflects nonspecific proton conductance through vesicles containing no functional UF₀ complexes. The specificity of the proton influx accompanying valinomycin-induced K⁺ influx is demonstrated in the upper left trace of Fig. 3 which shows that the effect is almost totally blocked by 1 μg of oligomycin. On the other hand, carbonyl cyanide p-trifluoromethoxyphenylhydrazone is still able to elicit a nonspecific proton influx similar in magnitude to the total influx observed in the absence of oligomycin. Thus, UF₀ complexes, which we have shown to be devoid of measurable OS, are able to conduct protons in a manner which can be blocked by oligomycin. In separate experiments with 9-aminoacridine as the pH probe we were able to demonstrate that conductance through UF₀ could also be blocked by the other classical inhibitor DCCD. Thus, the rate of quenching of 9-aminoacridine fluorescence for UF₀ proteoliposomes was found to be 1.4%/min/μg protein while in the presence of 25 μM DCCD the rate dropped to 0.16%/min/μg protein.

Essentially the same behavior was observed with proteoliposomes containing a UF₀-OS recombinant (Fig. 3, right). If anything, the presence of OS appeared to have a slight inhibitory effect, i.e. the rate was marginally slower, although the quenching (i.e. conductance) rates in Fig. 3 were too fast for quantitative analysis. Again, oligomycin was able to almost completely block the conductance (Fig. 3, upper right trace). Thus, the data obtained by using ACMA or 9-aminoacridine as the pH probes clearly establish that OS is not a necessary component of the F₀ proton channel nor is its presence required for conductance blockade by the inhibitors oligomycin and DCCD.

DISCUSSION

Mitochondrial F₀ is an integral membrane complex whose precise subunit composition and stoichiometry remain unknown. It is empirically defined as that portion of the ATP synthase which remains bound to the mitochondrial membrane after removal of the soluble catalytic five-subunit sector designated F₁. Functionally, F₀ has been shown to contain subunits that allow passive transmembrane proton conductance which may be blocked by the classical mitochondrial inhibitors oligomycin and DCCD. The interactions between the membrane sector and the F₁ complex are mediated by the subunits of F₀ which are collectively referred to as the stalk since they can be visualized as such by electron microscopy (6, 7, 36). The composition, stoichiometry, and function of this stalk are not completely known, but work from this and other laboratories show that it includes the OS, coupling factor 6, and probably a 24-kDa protein (4, 10, 21). Data on the stoichiometry of OS relative to F₁ are equivocal with estimates of either one (33, 34) or two copies (35) per F₁ complex. A priori, the function of the stalk proteins may be to mediate interactions between F₁ and F₀ or to constitute part of the proton-conducting apparatus of F₀ or both.

Binding of F₁ to UF₀—It has been shown by others and confirmed by us (4, 15, 16) that both OS and F₁ are essential for restoring energy-linked activity to complexes reconstituted from F₁ and F₀ when the latter have been specifically depleted of OS and F₁. As far as binding of F₁ is concerned, we have shown previously (21) that when trypsin-accessible segments of OS are removed from F₀, F₁ (in ammonium sulfate) is still able to rebind to F₀, but the reconstituting complexes are cold-labile and the ATPase activity shows poor sensitivity to inhibition by oligomycin. In the present work we have demonstrated by immunological methods that after treatment of purified F₁-F₀ results in the selective removal of F₁ and OS with very little effect on the F₀ subunit. These UF₀ complexes differ somewhat from the trypsinized F₀ preparation we described previously (21) in that they are depleted of the entire OS protein rather than

<table>
<thead>
<tr>
<th>Table II Binding of OS to UF₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment</td>
</tr>
<tr>
<td>-------------</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
</tbody>
</table>

Downloaded from http://www.jbc.org by guest on October 16, 2017
just the extrinsic segments. The UFo complexes, therefore, represent a useful preparation for studying the role of OSCP in binding F1 to the purified membrane sector. The data in Tables I and II show that the binding of F1 to UFo is possible in the presence of NH4 ions alone (Table I, row 3; Table II, rows 1–3) but the resultant complex shows no sensitivity to oligomycin (Table I, row 3; Table II, rows 1–3). This is in agreement with the work of Sandri et al. (15) who showed that cations such as NH4, Rb+, and Cs+ could effectively permit binding of F1 to submitochondrial particles depleted of F1, OSCP, and F0. However, in contrast to the former authors, we find that F1 binding to UFo could take place even in the complete absence of cations provided that OSCP was present (Table I, row 2). The cation-induced binding of F1 is known to involve prevention of coulombic repulsion between negative charges on the membrane surface and F1-ATPase. It is conceivable that F1 and OSCP, both bearing a net positive charge, can mimic the role of cations in facilitating binding of F1-ATPase. Thus, UFo preparations, both bearing a net positive charge, can mimic the role of cations in facilitating binding of F1-ATPase. Therefore, UFo in the absence of cations, OSCP could form an obligatory link between F1 and the membrane sector.

Role of OSCP in Passive Proton Conductance—The main focus of the present study was to examine the proton-conducting ability of OSCP-depleted particles reconstituted into phospholipid vesicles. The data clearly show that such particles are able to conduct protons, leading us to the conclusion that OSCP is not part of the conductance pathway per se. Furthermore, it is clear that OSCP is required for oligomycin sensitivity of membrane-bound ATPase but not for oligomycin-induced blocking of passive proton conductance. Therefore, it would appear that OSCP has no role at the level of F0, but is presumably involved in transmitting oligomycin binding effects from F0 to the active site(s) on F1. However, this still leaves open the question as to whether, in the intact F0-F1 complex, protons are channeled through OSCP, F0, or other subunits to promote the catalytic events on F1.

Comparison between Mitochondrial OSCP and Prokaryotic H+-ATPases Subunits—It is well established that the catalytic activities of prokaryotic H+-ATPase, in contrast to those of the mitochondrial enzyme, are not inhibited by oligomycin. It may be noted that subunit 6 of mitochondrial H+-ATPase has two loci in its gene known as oli2 and oli4 which are known to be essential for oligomycin-sensitive ATPase activity (37). The bacterial homologue of mitochondrial subunit 6 is subunit α which is coded by the uncB gene that lacks the oli4 locus (38). Therefore, the lack of oligomycin sensitivity of catalytic activities of bacterial H+-ATPase is presumably related to deletion of oli4 locus in the uncB gene rather than to the absence of OSCP. In fact, from an evolutionary standpoint one would expect OSCP or a prototype(s) of OSCP to be conserved since it has been established that OSCP is absolutely essential for all coupled reactions of respiration-driven ATP synthesis by mitochondria (24). In this context a comparative analysis of the primary structure and predicted hydropathy profiles of OSCP and E. coli H+-ATPase subunits reveal similarities between OSCP and bacterial α (39) as well as β subunits (40).

It has been reported that the primary structures of OSCP and bacterial α subunits show homologies in the N- and C-terminal regions (39). Functionally, this is reflected in the observation that both proteins bind to α and β subunits of F1-ATPase, and both are required for integration of F1 and F0 segments. However, whereas bacterial (41) and chloroplast (42) β subunits prevent proton leakage through F0-proteoliposomes, OSCP exhibits only a marginal regulatory effect on conductance through UFo. A similar analysis of the predicted hydropathy profiles of OSCP and E. coli H+-ATPase subunits reveal similarities between OSCP and bacterial β (39) as well as β subunits (40).
OSCP may be involved in regulation of proton flow and transmission of conformational changes occurring in the F₀ segment to the catalytic site(s) in the F₁ segment. Further studies aimed at defining the physical interaction between OSCP and specific F₁ subunits are currently in progress. These should help to ascertain the mechanism whereby OSCP mediates the functional coupling of F₁ to F₀.

Acknowledgments—We wish to thank Drs. D. Rao Sanadi and J. Badway for comments and Angela DiPerri for secretarial help.

REFERENCES

ATP synthase complex from bovine heart mitochondria. Passive H+ conduction through F0 does not require oligomycin sensitivity-conferring protein.

M J Pringle, M K Kenneally and S Joshi


Access the most updated version of this article at http://www.jbc.org/content/265/13/7632

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/265/13/7632.full.html#ref-list-1