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Rapid Report

ATP synthase complex from bovine heart mitochondria: the oligomycin sensitivity conferring protein is essential for dicyclohexyl carbodiimide-sensitive ATPase

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The requirement of bovine heart mitochondrial oligomycin sensitivity conferring protein (OSCP) in conferring dicyclohexylcarbodiimide (DCCD)-sensitivity to membrane-bound F_1 was investigated by using OSCP-depleted membrane fraction (UF_0) of ATP synthase. The ATPase activity of UF_0-F_1 was completely insensitive to DCCD while that of UF_0-F_1-OSCP was inhibited 95% by 16 μM DCCD. Both UF_0-F_1 and UF_0-F_1-OSCP complexes bound 5 nmol [^{14}C]DCCD/mg UF_0, and all the radioactivity was found to be associated with the DCCD-binding proteolipid. The data suggest that OSCP may be necessary for transmitting not only energy-linked signals, but also signals induced by F_0 inhibitory ligands in mitochondrial energy transduction.

The ATP synthases form a class of enzymes that consist of a water-soluble extrinsic part, F_1, that carries the catalytic centers for interconversion of ATP and ADP, and a membrane-integrated part, F_0, that constitutes a trans-membrane proton channel (see Refs. 1, 2 for recent reviews). Resolution and reconstitution studies have demonstrated that the activities of membrane-bound F_1 and F_0-proteoliposomes can be blocked by F_0 inhibitory ligands, e.g., DCCD (pro- as well as eucaryotes), oligomycin and venturicidin (eucaryotes), whereas that of soluble F_1 is comparatively insensitive to these inhibitors (see Ref. 3 for review). In agreement with these data, the binding sites for all three inhibitors have been found to reside on a subunit of F_0 [4–6]. The identity of individual subunit(s) that are needed for conferring sensitivity to F_0 inhibitors and insights into the mechanisms whereby the inhibitor induced changes in F_0 are propagated to the catalytic centers in F_1 still remain to be obtained.

The bacterial F_0 has the simplest structure of all the F_0s and consists of three subunits *a* (*unc*B), *b* (*unc*F) and *c* (*unc*E) [1,2]. Analyses of deletion mutants and reconstitution studies with F_0 subcomplexes formed by purified subunits demonstrate that all three subunits are indispensable for the formation of an active H^+ channel as well as for DCCD-sensitive ATPase activity [1]. By comparison, the mitochondrial F_0 has a far more complex organization, and the functional role of many of its components is still unclear. OSCP is a soluble subunit of mitochondrial ATP synthase that is located at the interface between the catalytic and proton-translocating domains [7]. Depletion-reconstitution and genetic approaches have established that OSCP is indispensable for coupling the energy of H^+ translocation through F_0 to the synthesis of ATP by F_1 [8,9]. OSCP has no intrinsic catalytic activity and it is neither required for passive H^+ conductance through isolated F_0 proteoliposomes nor for blocking of the H^+ conductance by oligomycin [10]. However, as the name signifies, OSCP is responsible for conferring to the mitochondrial F_1 the sensitivity to inhibition by the classic F_0 inhibitor, oligomycin [7]. Thus, OSCP is not only an energy transfer factor but it is also involved in transmitting oligomycin-induced conformational changes from F_0 to F_1 that ultimately lead to altered binding

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Abbreviations: DCCD, dicyclyohexyl carbodiimide; F_1, soluble part of ATP synthase; F_0, membraneous part of ATP synthase; OSCP, oligomycin sensitivity conferring protein; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate.

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affinities of the catalytic sites for ATP and ADP [11,12]. Whether OSCP is instrumental in propagating changes induced by other inhibitory ligands pertaining to F_0 has, however, not yet been investigated.

In the present communication we present data on the requirement of OSCP for conferring to mitochondrial F_1 sensitivity to inhibition by DCCD, and discuss the relevance of these observations in the context of the proposed homology of OSCP to subunits b and δ of *Escherichia coli* ATP synthase [13].

Isolation of F_0-F_1 [14], UF_0 [10], F_1-ATPase [15] and OSCP [8] from bovine heart mitochondria, reconstitution of UF_0 with F_1 and OSCP, and ATPase activity measurements in the reconstituted complexes [10] were carried out by published procedures. Protein determinations were made with bovine serum albumin as a standard [16].

Preparations to be modified with DCCD were suspended at 1 mg/ml in 50 mM Tris-acetate (pH 7.5) and incubated with DCCD for 30 min at room temperature. Prior to SDS-PAGE, unbound DCCD was removed from the DCCD-modified preparations by incubating protein samples for 1 h at $-20\,^{\circ}C$ with 10 volumes of cold acetone, followed by a 20 min centrifugation at 20000 rpm in a Sorvall SS-34 rotor. Acetone was removed by aspiration, sediments were dried under a stream of nitrogen, and resuspended at 1 mg/ml in 4 × sample buffer [17]. Resolution of various polypeptides was carried out on slab gels (16 × 14 × 0.075 cm) containing a 12–20% linear acrylamide gradient [17]. Bisacrylamide was replaced by *N,N'*-diallyltartardiamide in the separating gel in order to facilitate direct counting of protein-bound [^{14}C]DCCD after SDS-PAGE. To measure the incorporation of DCCD, slab gels were sliced (1.5 or 3 mm thick) with a sharp razor blade and each slice was incubated for 1 h at 37 °C with 1.5 ml of 5% sodium periodate. Following complete depolymerization of the gel, 10 ml of liquiscint was added and the samples were counted.

For Western blot analysis, polypeptides separated by SDS-PAGE were transferred electrophoretically (50 mA, 16 h at room temperature) to nitrocellulose using 20 mM sodium phosphate buffer (pH 7.0). Further processing of the blots was as described previously [18].

Molecular weight markers (Diversified Biotech, Newton, MA), peroxidase-conjugated $F(ab')_2$ fragments of goat anti-rabbit IgG (Cappel Laboratories, West Chester, PA), and nitrocellulose (Bio-Rad, Richmond, CA) were obtained as indicated. All other chemicals were of reagent grade and were purchased from Sigma, St. Louis, MO.

Isolation of OSCP-depleted ATP synthase preparation. In order to investigate the role of OSCP in conferring DCCD-sensitivity to membrane bound F_1-ATPase, a preparation that is specifically depleted of this coupling factor was isolated. This was achieved by extracting ATP synthase preparation twice with 4.0 M urea [10]. As we have shown previously, this procedure yields a membrane fraction, UF_0, that contains no more than 3% of the amount of OSCP expected in undepleted F_0. In order to verify depletion of OSCP in our preparation and to evaluate their suitability for the proposed studies, we tested the ability of UF_0-F_1 complexes to reconstitute oligomycin sensitive ATPase. Data presented in Table I demonstrate that while the ATPase activity of undepleted F_0-F_1 is inhibited 96% by 1 μg oligomycin (line 1), that catalyzed by UF_0-F_1 complex is essentially insensitive (line 2) unless OSCP is present during reconstitution (line 3). This demonstrates that UF_0 is highly deficient in OSCP.

TABLE I

UF_0-F_1 complex requires OSCP for restoration of oligomycin sensitive ATPase

Aliquots of UF_0 were reconstituted with F_1 and OSCP as described in the text. ATPase activity was measured at 30 °C for 5 min using 100 μg aliquots of undepleted F_0-F_1 or 20 μg aliquots of reconstituted complexes. Where indicated, 1 μg oligomycin was added during the assay

	ATPase (μmol/min/mg)		% Oligomycin sensitivity
	oligomycin		
	(−)	(+)	
(1) Undepleted F_0-F_1	2.30	0.09	96
(2) UF_0 + F_1	4.38	3.68	16
(3) UF_0 + F_1 + OSCP	5.05	0.60	88

DCCD-sensitivity of ATPase activity of UF_0-F_1 complexes. The requirement of OSCP for conferring DCCD sensitivity of membrane bound F_1 was investigated by reconstituting OSCP-depleted F_0 preparations with F_1, incubating with DCCD and measuring the ATPase activity of reconstituted complexes. Data presented in Table II show that the ATPase activity of undepleted F_0-F_1 complex is somewhat inhibitable by 1.0 μM DCCD (lines 1, 2), and 95% inhibition is achieved at 10 μM DCCD (lines 3, 4). The ATPase activity of the complex formed of OSCP-depleted F_0 and F_1, however, is essentially insensitive to DCCD (lines 5–8). Increasing the inhibitor concentration by a factor of 5 over that needed for inhibiting undepleted F_0-F_1 had no measurable inhibitory effect on the ATPase activity. The inhibition of ATPase activity by DCCD could, however, be restored by including OSCP during reconstitution. Data presented in lines 9–12 demonstrate that up to 97% inhibition of ATPase activity could be obtained in complexes containing OSCP. These data provide strong evidence to suggest that OSCP is necessary for conferral of DCCD-sensitivity to membrane bound F_1. In this respect OSCP resembles subunit *b* of bacterial H^+-ATPase.

DCCD binding of UF_0-F_1 complexes. It is well established that DCCD binds covalently to the DCCD-binding protein, a proteolipid subunit of ATP synthase, and blocks passive H^+ fluxes through isolated F_0 proteoliposomes. Since OSCP is not needed for passive H^+ fluxes or blockage of the conductance by DCCD [10], one would not expect OSCP to be necessary for binding of DCCD to the F_0 fraction. However, since our present data demonstrated the requirement of OSCP for conferring DCCD-sensitivity to membrane bound F_1, it was considered important to compare DCCD binding pattern of UF_0-F_1 complexes in the absence and presence of OSCP.

Data presented in Fig. 1 confirm that DCCD binds covalently to only one polypeptide in UF_0-F_1 complex and this pattern does not change even upon inclusion of OSCP in the complex (compare lanes 2 and 3 in panel B). There was no detectable binding of DCCD to any other polypeptide as judged by the autoradiograph of an electrophoresed sample as well as by direct counting of 3 mm wide slices of the gel for [^{14}C]DCCD incorporation. The bound radioactivity corresponded to 5 nmol DCCD per mg UF_0 and showed no appreciable change in the presence of OSCP. Thus, OSCP does not seem to affect modification of F_0-F_1 by DCCD although it is absolutely essential for conferral of DCCD-sensitivity of membrane bound F_1.

The identity of polypeptide modified by DCCD in the present studies with the authentic DCCD-binding proteolipid subunit was established by allowing it to react to a high titer mono-specific anti-DCCD binding protein in Western blots (panel C, lanes 1–3). It may be pointed out that the DCCD-binding proteolipid, following modification with [^{14}C]DCCD, moved with a different mobility in SDS-PAGE compared to its unmodified counterpart (Fig. 1 A–C, compare lane 1 with 2 and 3). The observed change in relative mobility of DCCD-binding protein was not an artifact of acetone precipitation of ATPase complexes, but was associated specifically with modification of this subunit by DCCD. The explanation for a DCCD-induced change in the mobility of the DCCD-binding protein is unclear, however.

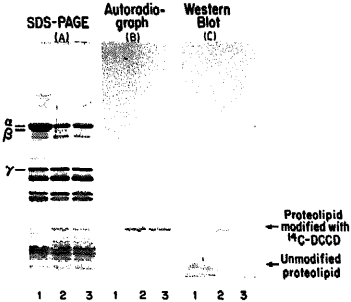

Fig. 1. OSCP does not affect DCCD binding of UF_0-F_1. Samples containing 300 μg UF_0, 75 μg F_1 and 12 μg OSCP were reconstituted in a volume of 300 μl as described in the text and in the legend to Table I. To the reconstituted complexes was added 1 μl of 5 mM [^{14}C]DCCD and incubations were resumed for 30 min at room temperature. A 0.24 ml aliquot of each sample was then withdrawn, precipitated with acetone and processed for SDS-PAGE in triplicate as described in the text. Two sets of gels were stained with Coomassie blue of which one was subjected to autoradiography. Proteins from a parallel set were electrotransferred for Western analysis using anti-DCCD-binding protein. Lane 1, 30 μg of undepleted F_0-F_1; 2, 30 μg of UF_0-F_1 + DCCD; 3, 30 μg of UF_0-F_1-OSCP + DCCD. (A) Coomassie staining; (B) autoradiographs; (C) Western blotting analysis.

It is well established that OSCP is a subunit of mitochondrial ATP synthase and is crucial for coupling the electrochemical energy of H^+ gradient to the synthesis of ATP [6,8]. Depletion-reconstitution studies have clearly demonstrated that OSCP is not needed for functional activities of isolated F_0 or F_1 subcomplexes [10]. There are proposals that it may be involved in 'signal transduction' between the H^+ translocating and catalytic segments of the enzyme [6,19]. The eponymous activity of OSCP is to confer to F_1 the susceptibility to inhibition by oligomycin, an inhibitory ligand of F_0 [20]. Elegant studies of Penefsky and Cross et al. have shown that oligomycin-induced changes in F_0 are transmitted to F_1, resulting in altered affinities of F_1 for ADP and ATP [11,12]. Taken together, these observations imply that OSCP may be involved in propa-

TABLE II

UF_0-F_1 complex requires OSCP for restoration of DCCD-sensitive ATPase

Reconstitution of UF_0 with the coupling factors and assay for ATPase were according to the conditions described in Table I and in the text. Incubation of the complexes with DCCD was for 30 min at room temperature prior to ATPase activity measurements

	ATPase (μmol/min/mg)	% DCCD-sensitivity
(1) Undepleted F_0-F_1	2.31	
(2) Undepleted F_0-F_1 + 1 μM DCCD	1.70	26
(3) Undepleted F_0-F_1 + 10 μM DCCD	0.10	95
(4) Undepleted F_0-F_1 + 100 μM DCCD	0.10	95
(5) UF_0-F_1	4.38	
(6) UF_0-F_1 + 50 μM DCCD	4.34	< 1
(7) UF_0-F_1	3.45	
(8) UF_0-F_1 + 16 μM [^{14}C]DCCD	3.39	3
(9) UF_0-F_1-OSCP	4.15	
(10) UF_0-F_1-OSCP + 50 μM DCCD	0.70	83
(11) UF_0-F_1-OSCP	3.88	
(12) UF_0-F_1-OSCP + 16 μM [^{14}C]DCCD	0.13	97

gating oligomycin induced changes between F_0 and F_1 segments. Our present data, establishing the requirement of OSCP for DCCD sensitive ATPase, suggest that OSCP may provide a general pathway for communication of signals induced by inhibitors of F_0 in mitochondrial energy transduction.

Until now, OSCP has been considered to be unique to mitochondrial ATP synthase since comparative analyses of its primary structure and those of *unc* gene products of *E. coli* have revealed comparatively weak homologies with subunits δ and *b* [2,13]. Functional studies using trypsin-treated F_0 from mitochondria and *E. coli*, however, have revealed striking similarities between the roles of OSCP and subunit *b* [14,21,22]. For example, the data showed that while the extrinsic (trypsin-accessible) segments of both proteins are required for interactions of F_0 and F_1 and for inhibitor-sensitive ATPase and energy-linked reactions, they are not necessary for inhibitor sensitive H^+ conductance through isolated F_0. Our present data demonstrating requirement of OSCP for reconstitution of DCCD-sensitive ATPase provide further evidence favoring functional similarities between mitochondrial OSCP and bacterial subunit *b*.

The data presented here are consistent with the hypothesis that OSCP has a more crucial role in energy coupling than to simply confer oligomycin sensitivity to membrane-bound F_1. A detailed knowledge of the structure–function relationships of OSCP, therefore, is of utmost importance to elucidate the molecular basis of role of this protein in energy transduction.

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