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The Oligomycin Sensitivity Conferring Protein (OSCP) of Beef Heart Mitochondria: Studies of Its Binding to F_1 and Its Function[1,2]

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Abstract

The binding of "oligomycin sensitivity conferring protein" (OSCP) to soluble beef-heart mitochondrial ATPase (F_1) has been investigated. OSCP forms a stable complex with F_1, and the F_1 · OSCP complex is capable of restoring oligomycin- and DCCD-sensitive ATPase activity to F_1- and OSCP-depleted submitochondrial particles. The F_1 · OSCP complex retains 50% of its ATPase activity upon cold exposure while free F_1 is inactivated by 90% or more. Both free F_1 and the F_1 · OSCP complex release upon cold exposure a part—probably 1 out of 3—of their β subunits; whether α subunits are also lost is uncertain. The cold-treated F_1 · OSCP complex is still capable of restoring oligomycin- and DCCD-sensitive ATPase activity to F_1- and OSCP-depleted particles. OSCP also protects F_1 against modification of its α subunit by mild trypsin treatment. This finding together with the earlier demonstration that trypsin-modified F_1 cannot bind OSCP indicates that OSCP binds to the α subunit of F_1 and that F_1 contains three binding sites for OSCP. The results are discussed in relation to the possible role of OSCP in the interaction of F_1 with the membrane sector of the mitochondrial ATPase system.

Key Words: Oligomycin-sensitive ATPase; F_1-ATPase; F_1-subunit; oligomycin sensitivity conferring protein; trypsin digestion; reconstitution.

Introduction

"Oligomycin sensitivity conferring protein" (OSCP) was purified in David Green's laboratory by MacLennan and Tzagoloff (1968). Together with F_6 (Fessenden-Raden, 1972), OSCP is required for the interaction of the

[1] This paper is dedicated to the memory of David E. Green—scholar, pioneer, visionary.

[2] Abbreviations: DCCD, *N,N'*-dicyclohexylcarbodiimide; OSCP, oligomycin sensitivity conferring protein; SDS, sodium dodecylsulfate.

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catalytic (F_1) and proton-translocating (F_0) moieties of the mitochondrial ATPase to yield a membrane-bound, oligomycin- and DCCD-sensitive ATP synthase (Kagawa and Racker, 1966). F_6 alone can promote the binding of F_1 to the membrane sector of F_0, but both F_6 and OSCP are required for the conferral of oligomycin and DCCD sensitivity (Vàdineanu *et al.*, 1976; Russell *et al.*, 1976; Norling *et al.*, 1978; Sandri *et al.*, 1981). OSCP has been found associated both with the membrane sector of F_0 (Tzagoloff *et al.*, 1968) and with F_1 (Van de Stadt *et al.*, 1972), suggesting that it may form a separate link between the two, parallel to F_6 (Norling *et al.*, 1978). The present paper describes the results of experiments relating to the binding of OSCP to soluble F_1, with a discussion of the significance of these results for the function of OSCP.

We have reported (Hundal and Ernster, 1979) that OSCP can form a stable complex with soluble F_1 which can restore oligomycin- and DCCD-sensitive ATPase activity to OSCP- and F_1-depleted submitochondrial particles. In contrast to free F_1, which is inactivated upon cold exposure (Pullman *et al.*, 1960; Penefsky and Warner, 1965), the F_1 · OSCP complex retained 50% of its ATPase activity even after prolonged cold treatment (Hundal and Ernster, 1979). Both free F_1 and the F_1 · OSCP complex underwent dissociation in the cold, leading to a partial loss of α and/or β subunits. The data to be presented here confirm the loss of β subunits, while the loss of α subunits appears less certain. It is shown that the cold-treated F_1 · OSCP complex, which contains a deficient supplement of β subunits, is capable of restoring oligomycin- and DCCD-sensitive ATPase activity to F_1- and OSCP-depleted submitochondrial particles.

Mild trypsin treatment of soluble mitochondrial F_1 results in a digestion of the α subunit to a polypeptide (α') with an apparent molecular weight close to that of the β subunit (Hundal and Ernster, 1981; Skerret *et al.*, 1981; Di Pietro *et al.*, 1983). The enzyme so modified has the ability to bind to F_1-depleted submitochondrial particles, giving rise to an oligomycin- and DCCD-sensitive ATPase, but has no (Leimgruber and Senior, 1976; Pedersen *et al.*, 1981) or only limited (Hundal and Ernster, 1979) capacity to restore F_1-dependent energy-linked functions. We have found (Hundal *et al.*, 1983) that α'-F_1 lacks the ability to bind OSCP but is still capable of binding to F_1-depleted particles and exhibiting oligomycin- and DCCD-sensitive ATPase activity provided the particles contain their native complement of OSCP. Results presented here show that bound OSCP efficiently protects soluble F_1 against trypsin-induced modification of its α subunit. The data strongly suggest that OSCP binds to the α subunit of F_1.

The results are discussed in relation to the possible role of OSCP as a structural and functional link between the F_1 and F_0 components of mitochondrial ATPase. The available information suggests a similarity between the

function of OSCP in mitochondria and that of the δ subunit of F_1 in *E. coli.* This is in accordance with the recently established structural homology between the two polypeptides (Grinkevich *et al.*, 1982; Walker, *et al.*, 1982; Ovchinnikov, *et al.*, 1984).

A brief account of the present results has been given at a recent symposium (Norling *et al.*, 1984).

Materials and Methods

Submitochondrial Particles

Submitochondrial particles were prepared from beef-heart mitochondria by sonication in the presence of EDTA ("EDTA particles") as described earlier by Lee and Ernster (1967). F_1-depleted particles were prepared by treating EDTA particles with Sephadex and urea (Racker and Horstman, 1967), and F_1- and OSCP-depleted particles by subsequent treatment with ammonia (Tzagoloff *et al.*, 1968). These preparations will be referred to as "ESU particles" and "ESUA particles," respectively.

Preparation of F_1 and OSCP

F_1 was purified from beef-heart mitochondria according to Horstman and Racker (1970). The specific activity was 80–100 μmol ATP hydrolyzed min^{-1} · mg $protein^{-1}$. The protein was stored as a 50% saturated $(NH_4)_2SO_4$ precipitate at 4°C. OSCP was prepared as described by Russell *et al.* (1976).

Preparation of F_1 · OSCP Complex

F_1 · OSCP complex was prepared by incubating F_1 and OSCP in a molar ratio of 1:4 for 10 min at 30°C in a medium containing 100 mM sucrose, 10 mM Tris-sulfate, pH 8, and 0.25 mM EDTA.

In experiments where F_1 · OSCP complex and free OSCP were separated on a sucrose gradient, a molar ratio F_1:OSCP of 1:6 was used. The linear sucrose gradient (5–15%) containing 10 mM Tris-SO_4, pH 8, and 0.25 mM EDTA was prepared according to Van de Stadt *et al.* (1972). The gradient was prepared in a final volume of 4.2 ml, and the samples were layered on top of the gradient. The gradients were centrifuged at 20°C for 2.5 hr at 48,000 rpm in an SW 56 rotor.

Labeling of F_1 with [^{14}C]-DCCD

F_1 (2 mg/ml) was incubated with 0.2 mM [^{14}C]-DCCD in 50 mM Tris-Cl, pH 6.7, and 2 mM EDTA for 4 hr at 25°C. This treatment resulted in approximately 90% inhibition of the ATPase activity.

Trypsin Treatment of F_1 and F_1 · OSCP Complex

Trypsin treatment was performed by incubating F_1 or F_1 · OSCP (1.3 mg/ml) with 5 μg trypsin/mg F_1 or F_1 · OSCP in a medium containing 10 mM Tris-SO_4, pH 8, and 0.25 mM EDTA at 30°C. After 5 min trypsin inhibitor (5 g/g trypsin) was added.

ATPase Assay

ATPase activity was measured at 30°C by coupling the reaction to the pyruvate kinase and lactate dehydrogenase systems and measuring the oxidation of NADH spectrophotometrically at 340 nm. The composition of the reaction mixture was as described by Glaser *et al.* (1980).

Reconstitution of Oligomycin-Sensitive ATPase

Reconstitution of oligomycin-sensitive ATPase was performed by incubating ESU or ESUA particles (3 mg/ml) with F_1 or F_1 · OSCP in a medium containing 100 mM sucrose, 10 mM Tris-SO_4, ph 8, and 0.25 mM EDTA, at 25°C for 30 min. The protein concentration ratio between the particles and F_1 or F_1 · OSCP was 15:1. Aliquots were taken and the ATPase activity was determined in the absence and presence of 3 μg oligomycin.

Polyacrylamide Gel Electrophoresis

SDS slab-gel electrophoresis was performed on a 15% polyacrylamide gel according to Laemmli (1970). Gel electrophoresis in the absence of SDS was carried out in a 7% polyacrylamide gel as described by Andreu *et al.* (1973). The gels were stained with Coomassie blue, destained, and scanned at 560 nm.

Protein Determination

Protein was estimated either by the biuret method or according to Lowry *et al.* (1951).

Results

Complex Formation Between F_1 and OSCP

Figure 1 shows the results of an experiment in which F_1 and OSCP were incubated in a molar ratio of 1:6 and the F_1 · OSCP complex was separated from excess OSCP by centrifugation on a sucrose gradient, as described in Materials and Methods. Fractions 6–8 were pooled and run on an SDS

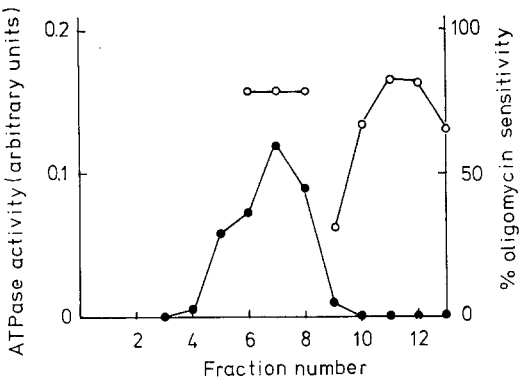

Fig. 1. Separation of F_1 · OSCP and free OSCP on a linear sucrose gradient. F_1 and OSCP in a 1:6 molar ratio were loaded on a sucrose gradient, 5–15%, in 200 μl aliquots. Fractions were tested for ATPase activity (●) and oligomycin sensitivity (○). Oligomycin sensitivity was tested by binding 50 μl aliquots of the various fractions to 200 μg ESUA particles. To those samples which had no ATPase activity, i.e., which contained free OSCP, F_1 (13 μg) was also added to test oligomycin sensitivity.

polyacrylamide gel (Fig. 2) with OSCP (lane a) and F_1 (lane b) as references. The isolated F_1 · OSCP complex (lane c) shows four bands, of which three correspond to the α, β, and γ subunits of F_1 and one corresponds to OSCP. The δ and ϵ subunits of F_1 were not detectable with the amount of protein used.

Properties of F_1 · OSCP Complex: Cold Stability

Exposure of soluble F_1 to cold led, as expected, to a decrease of the ATPase activity, the extent of which was 90–95% after 4 hr at 0°C (Fig. 3). In contrast, as reported earlier (Hundal and Ernster, 1979) the isolated F_1 · OSCP complex was resistant to cold inactivation to an extent of 50%. This 50% protection of the enzyme by OSCP was observed consistently in a large number of experiments and was maintained even after prolonged exposure to cold. OSCP alone had no appreciable effect on the ATPase activity of F_1.

As shown in Fig. 4, the K_m for ATP of cold-treated F_1 · OSCP complex was the same as for isolated F_1.

The addition of Mg^{2+} (0.5–1.5 mM) considerably increased the rate of cold inactivation of F_1 (Fig. 5). The same phenomenon was found for the F_1 · OSCP complex. However, OSCP still had some stabilizing effect on F_1, since after 4 hr of cold treatment, the F_1 · OSCP complex retained approximately 25% of its activity, whereas F_1 incubated under the same conditions had less

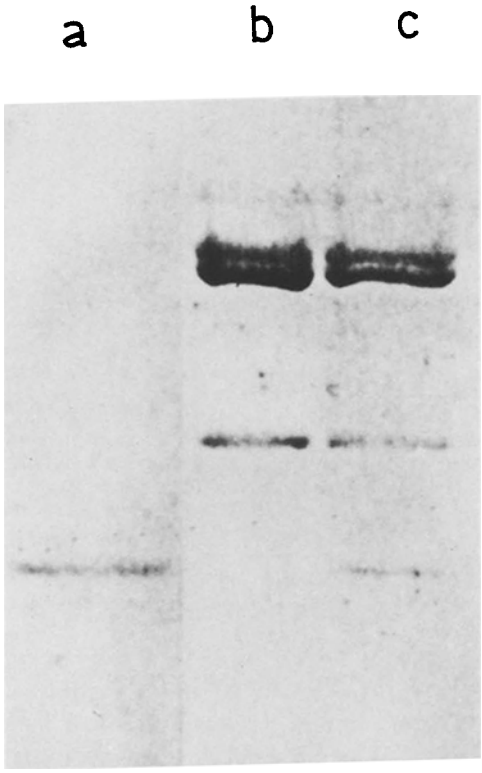

Fig 2. SDS slab-gel electrophoresis of (a) 4 μg OSCP, (b) 16 μg F_1, and (c) 16 μg F_1 · OSCP. F_1 · OSCP was isolated on a sucrose gradient. (Fractions 6–8 in Fig. 1.)

than 2% of its original activity. The effect of Mg^{2+} was not influenced by ATP or ADP. Effects similar to that of Mg^{2+} were obtained with Ca^{2+} and Mn^{2+} (not shown).

Effect of Trypsin on the F_1 · OSCP Complex

As reported earlier (Hundal and Ernster, 1981), mild trypsin treatment of soluble F_1 results in a selective digestion of the α subunit to a polypeptide (α′) with an apparent molecular weight close to that of the β subunit. This effect is illustrated in Figs. 6a, b. We have also reported (Hundal *et al.*, 1983) that trypsin-treated F_1 lacks the ability to bind OSCP, suggesting that OSCP interacts with F_1 via the α subunit. As shown in Fig. 6c, when the F_1 · OSCP complex was formed prior to trypsin treatment, no digestion of the α subunit was seen as revealed by SDS gel electrophoresis of the trypsin-treated F_1 ·

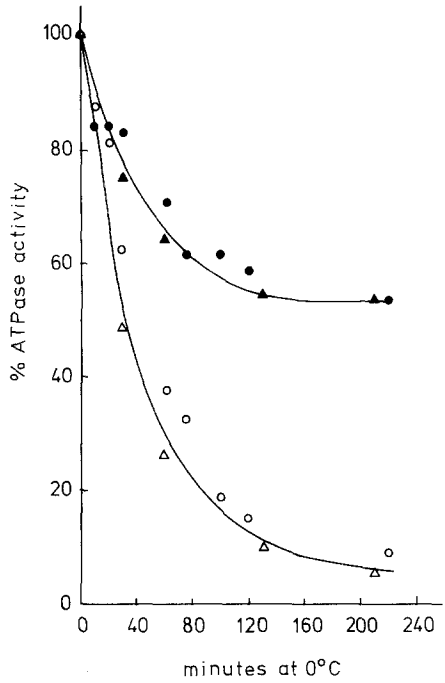

Fig 3. Effect of OSCP on the cold inactivation of F_1-ATPase. F_1 and F_1 · OSCP from sucrose gradients, 5–15%. F_1 (○, △); F_1 · OSCP (●, ▲). Circles and triangles refer to two independent experiments.

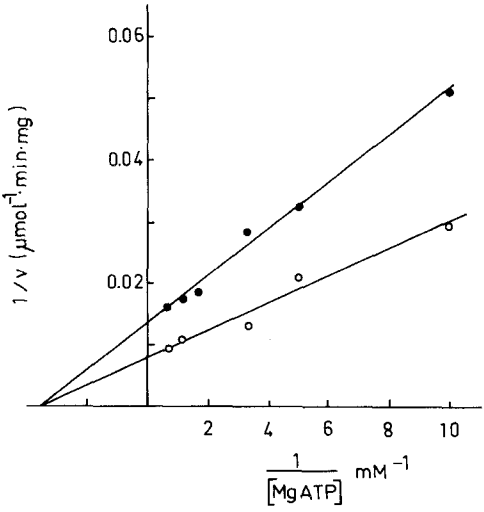

Fig. 4. Dependence of the ATPase activities of F_1 and F_1 · OSCP on ATP concentration. F_1 after incubation at room temperature for 6 hr (○); F_1 · OSCP after incubation at 0°C for 6 hr (●).

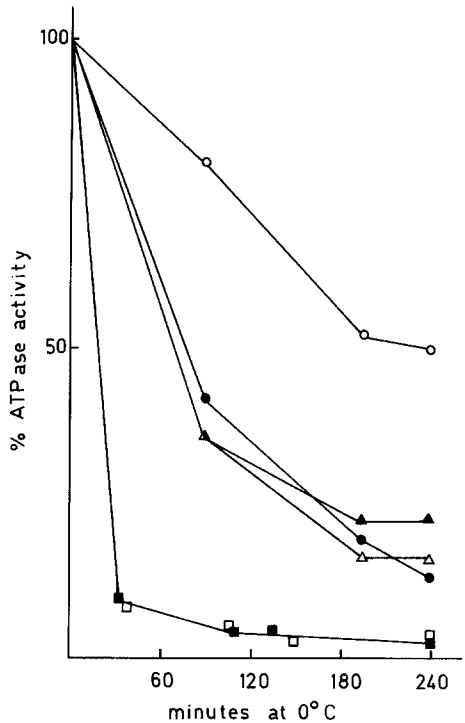

Fig. 5. Effect of Mg^{2+} on the partial cold stabilization of F_1 by OSCP. F_1 and F_1 · OSCP complex were incubated in the cold in the absence and presence of Mg^{2+} · F_1 (●); F_1 + 0.5 mM Mg^{2+} (□); F_1 + 1.5 mM Mg^{2+} (■); F_1 · OSCP (○); F_1 · OSCP + 0.5 mM Mg^{2+} (△); F_1 · OSCP + 1.5 mM Mg^{2+} (▲).

OSCP complex. At the same time, the OSCP band of the F_1 · OSCP complex became diffuse as compared to the control (Fig. 6d). Under the same conditions, free OSCP was extensively digested by trypsin and was no longer visible on the gel.

Stoichiometry of F_1 to OSCP

From the relative staining of the α and β subunits seen in Fig. 6c it seems that OSCP protects all three α subunits against trypsin digestion. Indeed, as seen in Fig. 7, 3 moles of OSCP per mole of F_1 were needed to obtain maximal OSCP effect measured either as cold stabilization of soluble F_1 or conferral of oligomycin sensitivity on F_1 added to ESUA particles.

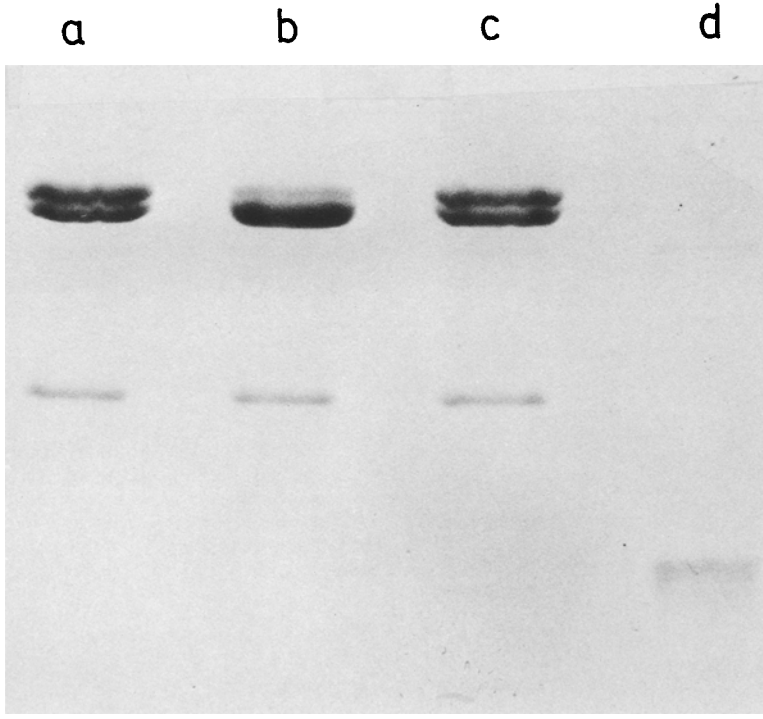

Fig. 6. Effect of trypsin on F_1 and F_1 · OSCP. Coomassie blue staining of SDS-polyacrylamide gel of (a) F_1, (b) trypsin-treated F_1, (c) trypsin-treated F_1 · OSCP, and (d) OSCP.

Dissociation of F_1 and F_1 · OSCP in the Cold

Polyacrylamide gel electrophoresis of F_1 in the absence of SDS showed a single band upon Coomassie blue staining (Fig. 8). Upon cold exposure resulting in 90% inactivation of the ATPase activity, the enzyme was split into two fragments, one with about the same migration as the native F_1 (fragment I) and one which migrated more toward the anode (fragment II). Cold-treated F_1 · OSCP showed the same electrophoretic pattern as did cold-treated F_1, even though it retained about 50% of its ATPase activity. As reported earlier (Hundal and Ernster, 1979) the residual ATPase activity was found exclusively in fragment I. For comparison, Fig. 8 also shows the electrophoretic patterns of the purified α and β subunits. It can be seen that the position of fragment II coincided with that of the β subunit. The purified α subunit showed a somewhat diffuse migration pattern, which overlapped partly with that of fragment I.

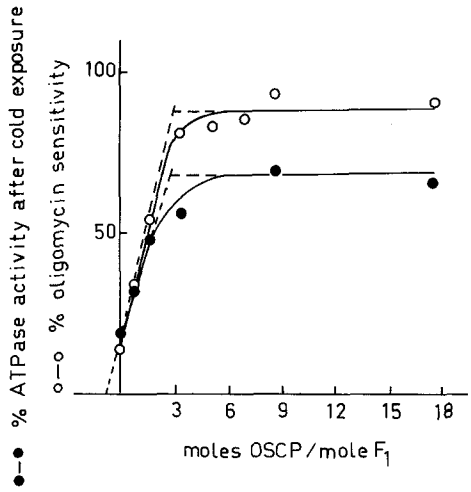

Fig. 7. Stoichiometry of F_1:OSCP interaction. F_1 · OSCP complex was formed as described in Materials and Methods by incubating a constant amount of F_1 (0.25 mg/ml) with increasing amounts of OSCP. Reconstitution of oligomycin-sensitive ATPase was performed by adding aliquots from the different incubations to ESUA particles as described in Materials and Methods. Oligomycin sensitivity was measured (O). The remaining F_1:OSCP samples were incubated at 0°C for 3 hr and the ATPase activity was measured (●).

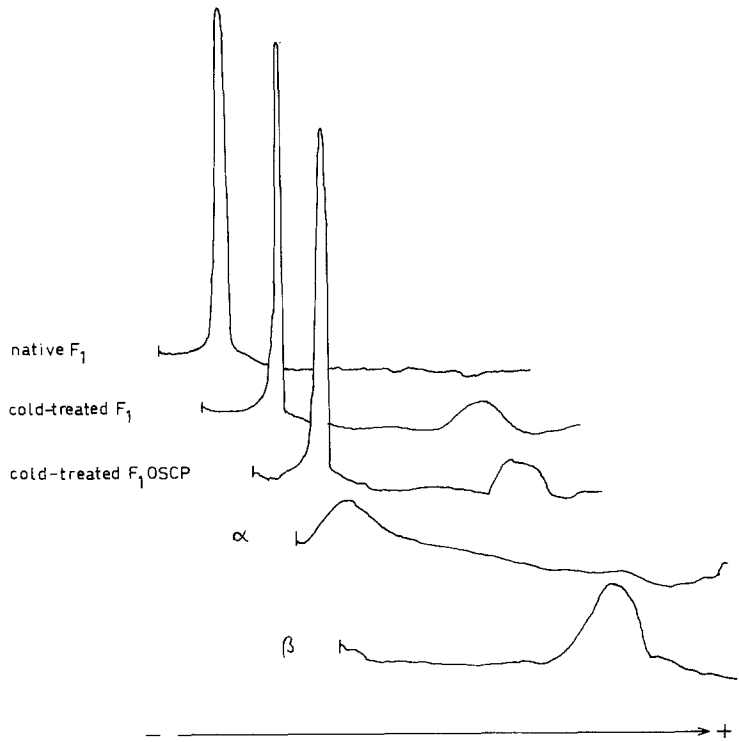

Fig. 8. Dissociation pattern of F_1 and F_1 · OSCP after cold treatment. Polyacrylamide gel electrophoresis (in the absence of SDS) of native F_1, cold-treated F_1, cold-treated F_1 · OSCP, and isolated α and β subunits.

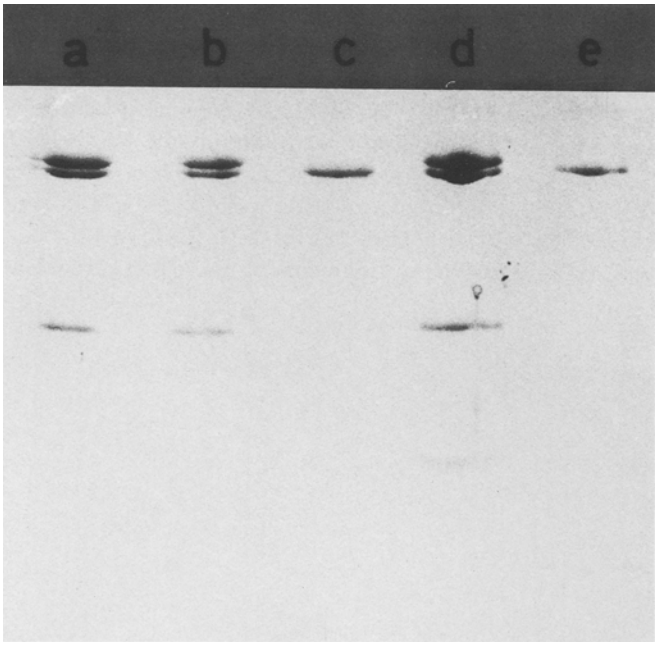

Fig. 9. SDS polyacrylamide gel electrophoresis of fragments I and II from cold-treated preparations of F_1 and F_1 · OSCP. (a) native F_1; (b) fragment I from cold-treated F_1; (c) fragment II from cold-treated F_1; (d) fragment I from cold-treated F_1 · OSCP; (e) fragment II from cold-treated F_1 · OSCP.

Table I. Reconstitution of Oligomycin-Sensitive ATPase with F_1, F_1 · OSCP, or Cold-Treated F_1 · OSCP and ESU/ESUA Particles[a]

Components	Total activity of added F_1 (μmol · min^{-1})	Total activity after reconstitution (μmol · min^{-1})	(%)	Oligomycin and DCCD sensitivity (%)
Experiment 1				
ESU particles + F_1	1.40	1.34	96	≥97
Experiment 2				
a. ESUA particles + F_1 · OSCP	1.62	0.54	33	≥90
b. ESUA particles + cold-treated F_1 · OSCP	0.99	0.36	36	≥90

[a]Reconstitution of oligomycin-sensitive ATPase and formation of the F_1 · OSCP complex was performed as described in Material and Methods. In experiment 2b, F_1 · OSCP complex was incubated at 0°C for 2.5 hr before reconstitution with ESUA particles.

SDS-polyacryamide gel electrophoresis of the isolated fragments (Fig. 9) revealed that fragment I contained subunits α, β, and γ, whereas fragment II contained only the β subunit. The pattern was the same in the case of F_1 and the F_1 · OSCP complex. OSCP in the latter was found in fragment I.

It is known that DCCD binds to the β subunit of F_1-ATPase (Pougeois *et al.*, 1979). When F_1 was incubated with [^{14}C]-DCCD and subsequently run on polyacrylamide gel in the absence of SDS, a single peak of radioactivity was obtained (Fig. 10a). After cold exposure (Fig. 10b), two radioactive peaks were found, corresponding to fragments I and II. These results further

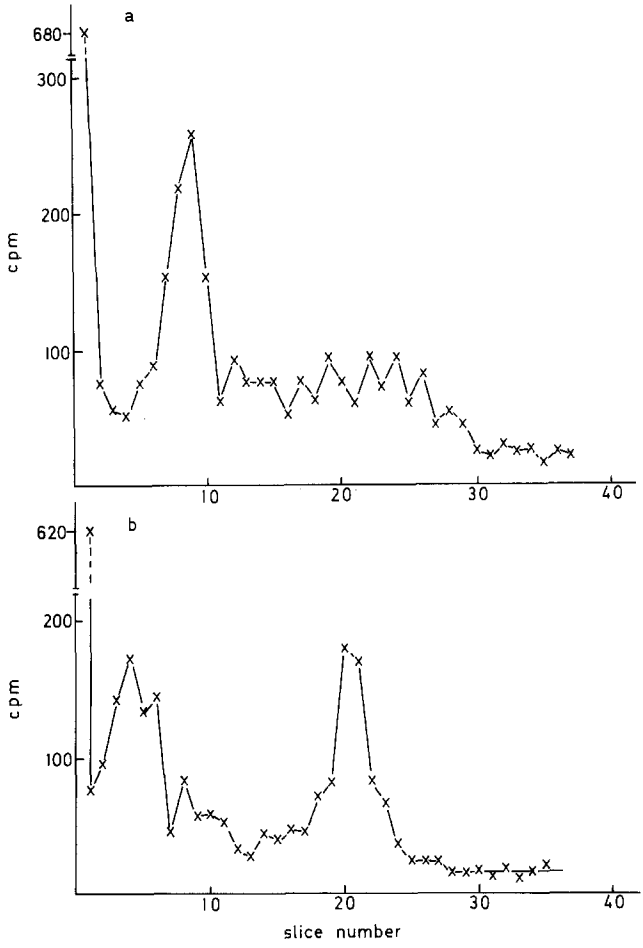

Fig. 10. Recovery of [^{14}C]-DCCD label in (a) native F_1 and (b) cold-treated F_1 after electrophoresis on a polyacrylamide gel in the absence of SDS.

demonstrate that both fragments contain the β subunit. Rough quantitative estimates of the surfaces under the two peaks, after correction for the average baselines, indicate that less than one-half, possibly one-third, of the total β subunit content of F_1, i.e., probably one out of three β subunits, was released from F_1 upon cold exposure.

Effect of Cold Treatment of F_1 and F_1 · OSCP Complex on the Reconstitution of Oligomycin- and DCCD-Sensitive ATPase

In Table I, exp. 1 it is shown that F_1 reconstituted with ESU particles exhibited the same specific activity as soluble F_1 and acquired virtually complete oligomycin and DCCD sensitivity. On the other hand, when the F_1 · OSCP complex was reconstituted with ESUA particles (experiment 2a), only 30 to 40% of the added ATPase activity was recovered after reconstitution. The recovered activity was over 90% sensitive to oligomycin and DCCD. Likewise, the partially cold-inactivated F_1 · OSCP complex (experiment 2b), which is deficient in β subunit(s) (see Figs 8 and 9), showed over 90% oligomycin and DCCD sensitivity when rebound to ESUA particles. The specific activity again was decreased to 30 to 40% upon reconstitution, similar to the case of non-cold-treated F_1 · OSCP complex.

Discussion

The work presented in this paper was performed with the aim of acquiring a better insight into the mode of interaction of the F_0 and F_1 components of mitochondrial ATPase and especially into the role of OSCP in this interaction. We have earlier reported (Hundal and Ernster, 1979) that OSCP can form a complex with soluble F_1 which renders F_1 partially cold-stable. The complex retained approximately 50% of its ATPase activity upon cold exposure, whereas free F_1 under the same conditions loses more than 85% of its activity (Fig. 3). Free F_1 and the F_1 · OSCP complex showed the same dissociation pattern after cold treatment and were converted into a form which contained a diminished number of β subunits (Figs. 8, 9). Whether α subunits also are released upon cold exposure is uncertain because of the difficulties in obtaining a separation of the cold-treated F_1 · OSCP complex from isolated α subunit with the method employed (Fig. 8). Begusch and Hess (1979) and Doster *et al.* (1980) have earlier reported a release of α subunit upon cold exposure of yeast F_1.

The cold-treated F_1 · OSCP complex was capable, just as the native complex, of rebinding to F_1- and OSCP-depleted particles, giving rise to an oligomycin- and DCCD-sensitive ATPase (Table I).

This modified enzyme may provide a valuable tool for assessing the need

of a complete set of β subunits for various functions of the F_0F_1-ATPase such as ATP synthesis and ATP-driven energy-linked functions.

A stoichiometry of 3 moles OSCP per mole F_1 was found to be needed for maximal cold stabilization and for conferral of complete oligomycin and DCCD sensitivity in reconstitution experiments with F_1- and OSCP-depleted particles (Fig. 7). Dupuis *et al.* (1983) have arrived at a value of 1.1 ± 0.5 moles of OSCP per mole of F_1, based on extrapolation of OSCP titration curves using low concentrations of OSCP. The low stoichiometry obtained by these authors may reflect a high-affinity binding of OSCP to one α subunit rather than the total number of binding sites. The same may be true for estimates reported by Liang and Fisher (1983b).

We have found that mild trypsin treatment of mitochondrial F_1 causes the loss of a small segment of the α subunit (Hundal and Ernster, 1981) and that this modification results in a lack of ability of F_1 to bind OSCP (Hundal *et al.*, 1983). Thus OSCP seems to require an intact α subunit for binding to F_1. When OSCP was bound to F_1 prior to trypsin treatment, no digestion of the α subunit could be seen on a polyacrylamide gel (Fig. 6), indicating that OSCP binds to the α subunit and in so doing protects it against proteolysis. From the same experiments it appears that, conversely, F_1 protects OSCP against total digestion, since the trypsin-treated F_1 · OSCP complex contained OSCP which was still detectable on the gel. Under the same conditions free OSCP was completely cleaved into small peptides. The finding that OSCP seems to fully protect the α subunits of F_1 against trypsin digestion further supports the conclusion that F_1 contains three binding sites for OSCP.

As pointed out in earlier reports (Hundal *et al.*, 1983; Norling *et al.*, 1984) and further underlined by the present data, our results reveal a functional similarity between mitochondrial OSCP and the δ subunit of *E. coli* F_1. Dunn *et al.* (1980) have shown that mild trypsin treatment of *E. coli* F_1 results in a partial digestion of the α subunit and that the enzyme so modified can no longer bind its δ subunit. Thus, both the *E. coli* δ subunit and mitochondrial OSCP require an intact α subunit for binding. Parallel to the present studies, the primary structure of beef heart OSCP has been determined (Grinkevich *et al.*, 1982; Ovchinnikov *et al.*, 1984) and has revealed a striking homology between this polypeptide and the δ subunit of *E. coli* F_1 (Walker *et al.*, 1982; Ovchinnikov *et al.*, 1984). Thus, OSCP may be regarded as a counterpart of the *E. coli* δ subunit both structurally and functionally. Recent analysis of the OSCP structure has also revealed a homology between a certain segment of this polypeptide and the hydrophobic portion of the *b* subunit of *E. coli* F_0 (Grinkevich *et al.*, 1984a). This would suggest that OSCP is a "hybrid" between the δ and *b* subunits of the *E. coli* F_0F_1-ATPase and may explain why in mitochondria OSCP can be found associated with

both F_0 and F_1, while in the *E. coli* ATPase the δ subunit remains with F_1 when F_0 and F_1 are separated. Another striking difference between the mitochondrial and bacterial ATPases is that trypsin-modified mitochondrial F_1 can be rebound to F_0, whereas trypsin-modified bacterial F_1 cannot. This difference may be accounted for by the presence in mitochondria of F_6, which does not seem to occur in bacteria. As suggested by recent observations, the role of F_6 may not be simply that of a connecting link between F_1 and F_0 but rather that of a positive effector of a binding site on the membrane sector of F_0 for F_1 (Sandri *et al.*, 1981; Norling *et al.*, 1984; Sandri *et al.*, 1984). A possible role of F_6 in the catalysis of P_i-ATP exchange by rat-liver F_0F_1-ATPase has also been indicated (Liang and Fisher, 1983a). Structural information now forthcoming regarding F_6 and its relationship to other ATPase subunits (Grinkevich *et al.*, 1984b) may eventually shed light on its function.

When this manuscript was completed, a paper by Williams *et al.* (1984) appeared which deals with the temperature-dependent dissociation of rat-liver F_1-ATPase. It is shown that the inactivation of rat-liver F_1 is greatly accelerated by Mg^{2+}, which is in accordance with our findings (Hundal and Ernster, 1979; and the present paper, Fig. 5). The Mg^{2+}-induced cold inactivation of rat-liver F_1 is partially reversed by raising the temperature to 20°C. At 37°C, the cold-treated rat-liver F_1 can be separated into a soluble and an insoluble fraction, containing β, γ, δ, ϵ and α, γ subunits, respectively. Both fragments are catalytically inactive. Whether OSCP can protect rat-liver F_1 against cold inactivation and whether a similar dissociation of cold-treated beef-heart F_1 occurs upon heating to 37°C, are interesting questions that may be worth investigating.

Acknowledgments

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