

# Lack of ability of trypsin-treated mitochondrial $F_1$ -ATPase to bind the oligomycin-sensitivity conferring protein (OSCP)

Torill Hundal, Birgitta Norling and Lars Ernster

*Department of Biochemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden*

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Soluble beef-heart mitochondrial  $F_1$ -ATPase modified in its  $\alpha$ -subunit by mild trypsin treatment ( $\alpha'$ - $F_1$ ) can no longer bind oligomycin-sensitivity conferring protein (OSCP) but is still capable of binding to  $F_1$ -depleted submitochondrial particles, giving rise to a maximally oligomycin-sensitive ATPase, provided the particles contain their native complement of OSCP. When OSCP is removed from the particles,  $\alpha'$ - $F_1$  can still bind to the particles, but added OSCP induces only a low degree of oligomycin sensitivity. The possible role of OSCP in the functional coupling of the catalytic ( $F_1$ ) and  $H^+$ -translocating ( $F_0$ ) moieties of mitochondrial ATPase is discussed. The results suggest a functional similarity between the OSCP component of mitochondrial ATPase and the  $\delta$ -subunit of *E. coli* ATPase, which is in accordance with the structural homology recently found to exist between the two polypeptides.

<i>Oligomycin-sensitive ATPase</i>	<i><math>F_1</math>-ATPase</i>	<i><math>F_1</math>-subunit</i>	<i>Oligomycin-sensitivity conferring protein</i>
	<i>Reconstitution</i>	<i>Trypsin digestion</i>	

## 1. INTRODUCTION

The interaction of the catalytic ( $F_1$ ) and the  $H^+$ -translocating ( $F_0$ ) components of mitochondrial ATPase involves the participation of two polypeptides,  $F_6$  [1] and OSCP [2], which are generally believed to have no counterparts in chloroplasts and bacteria.  $F_6$  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 sensitivity [3–6]. OSCP has been found associated both with the membrane sector of  $F_0$  [7] and with  $F_1$  [8], and it may form a separate link between the two, parallel to  $F_6$  [6]. Soluble  $F_1$  can form a complex with OSCP [9] which is capable of restoring oligomycin-sensitive ATPase activity to particles depleted of  $F_1$  and OSCP.

**Abbreviations:**  $F_0$  and  $F_1$ , proton-translocating and catalytic moieties of the mitochondrial ATPase system; OSCP, oligomycin-sensitivity conferring protein;  $F_6$ , coupling factor 6; SDS, sodium dodecyl sulfate

Mild trypsin treatment of soluble mitochondrial  $F_1$  results in a selective digestion of the  $\alpha$ -subunit to a polypeptide ( $\alpha'$ ) with an apparent  $M_r$ -value close to the  $\beta$ -subunit [10–12]. The enzyme so modified has the ability to bind to  $F_1$ -depleted submitochondrial particles, giving rise to an oligomycin-sensitive ATPase, but has no [13,14] or only limited [10] capacity to restore  $F_1$ -dependent energy-linked functions. As shown here, trypsin-treated  $F_1$  lacks the ability to bind OSCP, but is still capable of binding to  $F_1$ -depleted particles and exhibiting a high degree of oligomycin-sensitive ATPase activity provided the particles contain their native complement of OSCP. The results are discussed in relation to the role of OSCP in the interaction of the  $F_0$  and  $F_1$  components of the mitochondrial ATPase system. The available data suggest a similarity between the function of OSCP in mitochondria and that of the  $\delta$ -subunit of  $F_1$  in *E. coli*, in accordance with the recently established structural homology between the two polypeptides [15].

## 2. MATERIALS AND METHODS

### 2.1. *Submitochondrial particles*

'EDTA particles' were prepared by sonication of beef heart mitochondria in a medium containing EDTA as in [16]. Particles depleted of  $F_1$  were prepared by treating the EDTA-particles with Sephadex and urea [17], and particles depleted of both  $F_1$  and OSCP were obtained by treating the  $F_1$ -depleted particles with ammonia [7]; these preparations will be referred to as 'ESU particles' and 'ESUA particles', respectively.

### 2.2. *Preparation of $F_1$ and OSCP*

$F_1$  was purified from beef-heart mitochondria according to [18]. The specific activity was 80–100  $\mu\text{mol ATP hydrolyzed} \cdot \text{min}^{-1} \cdot \text{mg protein}^{-1}$ . The protein was stored as a 50% saturated  $(\text{NH}_4)_2\text{SO}_4$  precipitate at 4°C. OSCP was prepared as in [4].

### 2.3. *Trypsin treatment*

Trypsin treatment was performed by incubating  $F_1$  (1.7 mg/ml) in a medium containing 100 mM sucrose, 10 mM Tris- $\text{SO}_4$  (pH 8), 0.25 mM EDTA (STE-buffer) and trypsin (5  $\mu\text{g}/\text{mg } F_1$ ) at 30°C. After 5 min, trypsin inhibitor (5 g/g trypsin) was added.

### 2.4. *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 in [19].

### 2.5. *Reconstitution of oligomycin-sensitive ATPase*

This was done by incubating  $F_1$  (30  $\mu\text{g}$ ) either with ESU particles (420  $\mu\text{g}$  protein) or with ESUA particles (420  $\mu\text{g}$  protein) and OSCP (6.2–12.3  $\mu\text{g}$ ) in 100  $\mu\text{l}$  STE buffer at 25°C for 30 min. Aliquots of 50–75  $\mu\text{g}$  particle protein were taken and ATPase activity was determined in the absence and presence of 3  $\mu\text{g}$  oligomycin.

### 2.6. *Binding experiments using sucrose gradient technique*

$F_1$ ·OSCP complex was prepared as in [9] by in-

cubating  $F_1$  (150  $\mu\text{g}$ ) and OSCP (67  $\mu\text{g}$ ) in 300  $\mu\text{l}$  STE buffer at 30°C for 10 min. The  $F_1$ ·OSCP complex was separated from excess OSCP on a linear sucrose gradient (5–15%) according to [8]. The gradient was prepared in 4.2 ml final vol. The samples were layered on top of the gradients and centrifuged for 2.5 h at 48000 rev./min in an SW 56 rotor. After puncturing the tubes at the bottom, fractions of 0.3 ml were collected and assayed for ATPase activity. The same method was used in the experiments with trypsin-treated  $F_1$ .

### 2.7. *Polyacrylamide gel electrophoresis and protein determination*

SDS slab-gel electrophoresis was performed on a 15% polyacrylamide gel as in [20]. The gels were stained with Coomassie blue.

Soluble protein was determined as in [21], and particulate protein by the biuret method, using in both cases bovine serum albumin as standard.

## 3. RESULTS AND DISCUSSION

$F_1$ ·OSCP complex, prepared by incubating  $F_1$  and OSCP in an approximate molar ratio of 1:8, and separated from excess OSCP on a sucrose gradient (section 2), revealed a Coomassie blue staining pattern as shown in fig.1, lanes 1–3, representing increasing amounts of the ATPase-containing fraction; lane 4 shows purified OSCP. It is seen that OSCP is associated with the fraction containing  $F_1$ , thus demonstrating a complex formation between  $F_1$  and OSCP.

Fig.1, lanes 5–7 show Coomassie blue staining patterns of a preparation similar to those in lanes 1–3, except that trypsin-treated  $F_1$  was used. Evidently, no OSCP is associated with the fraction containing ATPase activity, indicating that the trypsin-modified  $F_1$  is incapable of binding OSCP.

We have reported that the binding of OSCP renders  $F_1$  resistant to cold-inactivation to an extent of about 50% [9]. Fig.2 shows that incubation with OSCP did not stabilize trypsin-treated  $F_1$  against cold, in accordance with the finding that no complex was formed between the modified enzyme and OSCP (cf. fig.1). Modification of the  $\alpha$ -subunit per se, or trypsin inhibitor when added prior to trypsin, did not affect the cold-sensitivity

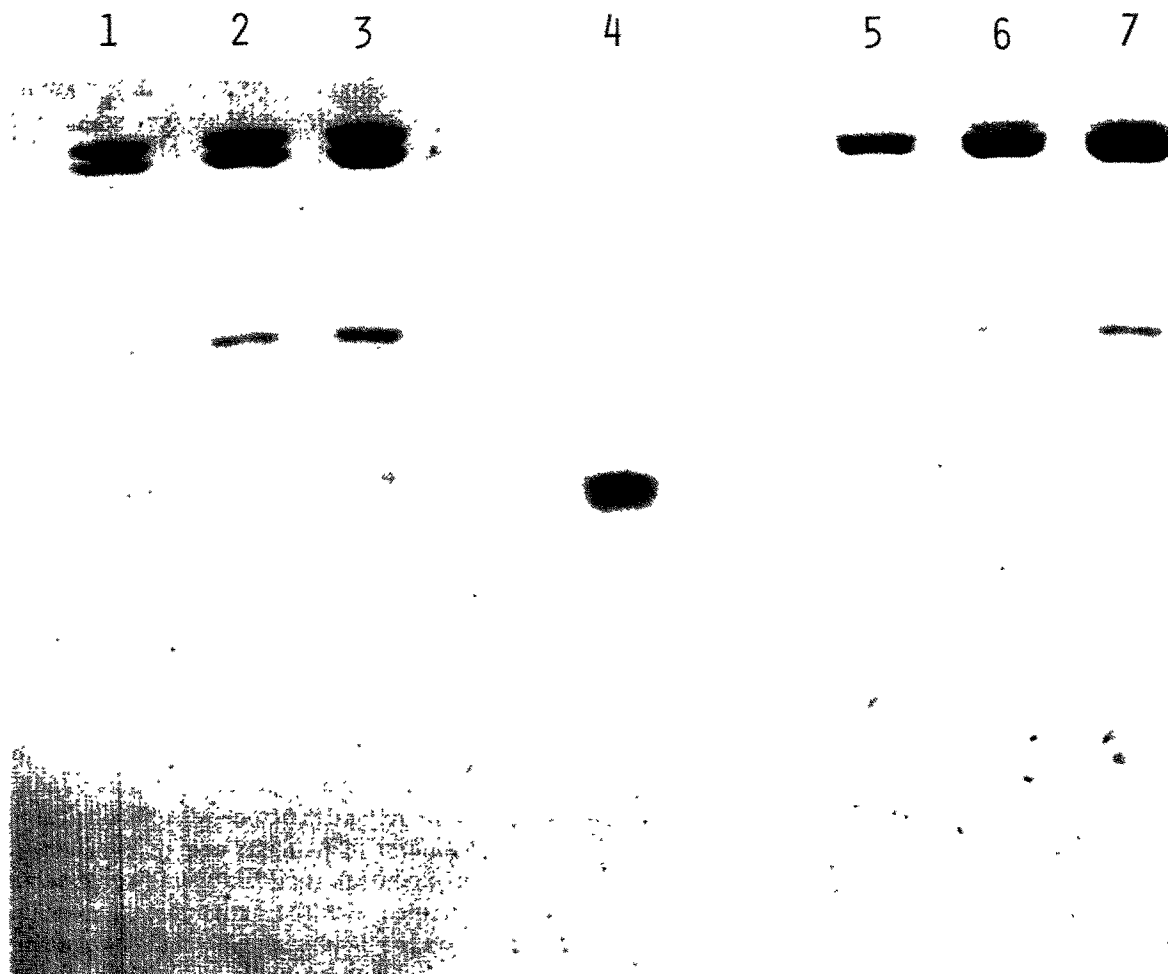


Fig.1. Inability of trypsin-treated  $F_1$  to bind OSCP.  $F_1$  + OSCP or trypsin-treated  $F_1$  + OSCP were incubated at a molar ratio of 1:8 and centrifuged on a sucrose gradient as in section 2. The fractions containing ATPase activity were subjected to electrophoresis on a 15% SDS-polyacrylamide gel. Lanes 1-3 show increasing amounts (10, 20, 30  $\mu$ g) of the active fraction collected from the gradient loaded with  $F_1$  + OSCP; lane 4, purified OSCP (4  $\mu$ g); lanes 5-7, increasing amounts (10, 20, 30  $\mu$ g) of the active fraction collected from the gradient loaded with trypsin-treated  $F_1$  + OSCP.

of  $F_1$  either in the presence or the absence of OSCP.

Table 1 describes the results of experiments involving a reconstitution of oligomycin-sensitive ATPase using normal and trypsin-treated  $F_1$  in combination with either ESU particles or ESUA particles and purified OSCP. It was reported that  $F_1$  makes up about 10% of the total protein of various types of submitochondrial particles [3,22] including EDTA particles [23]. In the reconstitution experiments in table 1 an excess of ESU or ESUA particles to  $F_1$  (14:1) was used, in order to

ensure maximal oligomycin sensitivity.

As reported [10], trypsin-modified  $F_1$  can acquire a high degree (75-95%) of oligomycin sensitivity when combined with  $F_1$ -depleted (ESU) particles, in a fashion similar to native  $F_1$  (table 1, exp.1). In the case of  $F_1$ - and OSCP-depleted (ESUA) particles, incubation with  $F_1$  alone resulted in a low degree (about 20%) of oligomycin sensitivity, which probably was due to residual OSCP [24] (table 1, exp.2).

Incubation of ESUA particles with  $F_1$  plus OSCP resulted as expected [2] in a high degree of

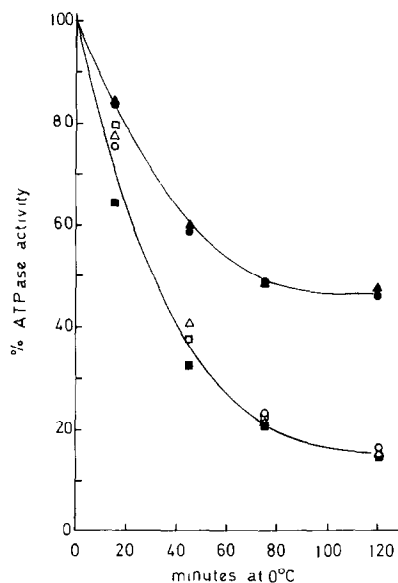


Fig. 2. Lack of ability of OSCP to stabilize modified  $F_1$  against cold-inactivation: (○—○)  $F_1$ ; (●—●)  $F_1$  + OSCP; (△—△)  $F_1$  + trypsin inhibitor + trypsin (trypsin inhibitor added first); (▲—▲)  $F_1$  + trypsin inhibitor + trypsin + OSCP; (□—□) trypsin-treated  $F_1$ ; (■—■) trypsin-treated  $F_1$  + OSCP. In all experiments the concentration of  $F_1$  of trypsin-treated  $F_1$  was 0.18 mg/ml and those containing OSCP, the molar ratio of  $F_1$ :OSCP was 1:2.5. Trypsin treatment of  $F_1$  was performed as in section 2. The different samples were incubated at 0°C in 10 mM Tris-Ac (pH 8) and 0.25 mM EDTA, and ATPase activity was measured at times indicated in the figure.

oligomycin sensitivity (table 1, exp.2). The same was found when ESUA particles were incubated with a preformed  $F_1$ ·OSCP complex (table 1, exp.3). When trypsin-treated  $F_1$  was incubated with ESUA particles in the presence of OSCP, the oligomycin sensitivity of the ATPase activity was about 40% (table 1, exp.2); i.e., about twice as high as in the absence of OSCP, but clearly lower than in the case of normal  $F_1$ . This oligomycin sensitivity was not increased by increasing the molar ratio of OSCP to  $F_1$ .

These results show that the trypsin-modified  $F_1$ , although it cannot bind OSCP, can interact with  $F_0$ , giving rise to an oligomycin-sensitive ATPase, and that this interaction involves OSCP. In the case of ESU particles, which contain their native complement of OSCP, this interaction results in a

high degree of oligomycin sensitivity, comparable to that found with native  $F_1$ . This suggests that, in this case, the binding of OSCP to  $F_1$  is not essential for oligomycin sensitivity. However, when OSCP is removed from  $F_0$ , reconstitution of a highly oligomycin-sensitive ATPase requires a binding of OSCP to both  $F_1$  and the membrane sector of  $F_0$  in order that OSCP can acquire its correct position and confer a high degree of oligomycin sensitivity. These concepts are schematically illustrated in fig.3.

It was reported [13,14] that trypsin-modified  $F_1$  cannot reconstitute  $F_1$ -dependent energy-linked functions, although some reconstitutive activity could be demonstrated in our laboratory [10]. It appears, thus, that a binding of OSCP to  $F_1$  may be more essential for the interaction of  $F_1$  and  $F_0$  in exhibiting energy-linked functions than for the conferral of oligomycin-sensitivity. Modification of *E. coli*  $F_1$  by trypsin has likewise been reported to abolish the ability to reconstitute  $F_1$ -dependent energy-linked functions [25].

The modification of mitochondrial  $F_1$  by mild trypsin treatment is similar to that earlier described for *E. coli*  $F_1$ , in that it consists of a digestion of the  $\alpha$ -subunit to a polypeptide with  $M_r$  close to that of the  $\beta$ -subunit [25]. In the case of the *E. coli*  $F_1$  it has been shown that the modified  $\alpha$ -subunit when combined with the native  $\beta$ - and  $\gamma$ -subunits cannot bind the  $\delta$ -subunit; i.e., that the modification of the enzyme probably results in a dissociation of the  $\delta$ -subunit. These results thus suggest a functional similarity between the OSCP compo-

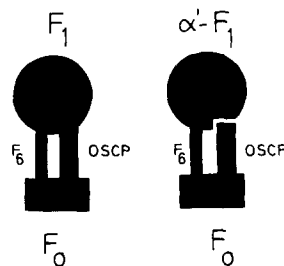


Fig. 3. Scheme illustrating the effect of mild trypsin treatment on the ability of mitochondrial  $F_1$  to interact with  $F_0$ . The term  $F_0$  is used to denote the total oligomycin-sensitive ATPase minus  $F_1$ ; i.e., the membrane sector +  $F_6$  + OSCP. The trypsin-treated  $F_1$  is denoted as  $\alpha'$ - $F_1$ . For further explanation, see text.

Table 1

Reconstitution of oligomycin-sensitive ATPase by means of  $F_1$  or trypsin-treated  $F_1$  and  $F_1$ -depleted (ESU) or  $F_1$ - and OSCP-depleted (ESUA) submitochondrial particles

Exp. no.	Components	OSCP: $F_1$	Oligomycin sensitivity of the reconstituted ATPase activity
		Molar ratio	(%)
1	ESU + $F_1$		95
	ESU + trypsin-treated $F_1$		88
2	ESUA + $F_1$		23
	ESUA + $F_1$ + OSCP	3.7	87
	ESUA + $F_1$ + OSCP	5.5	81
	ESUA + $F_1$ + OSCP	7.7	75
	ESUA + trypsin-treated $F_1$		22
	ESUA + trypsin-treated $F_1$ + OSCP	3.7	39
	ESUA + trypsin-treated $F_1$ + OSCP	5.5	41
	ESUA + trypsin-treated $F_1$ + OSCP	7.7	42
3 <sup>a</sup>	ESUA + $F_1$ ·OSCP		82
	ESUA + trypsin-treated $F_1$ + OSCP		22

<sup>a</sup> In this experiment  $F_1$  and trypsin-treated  $F_1$  were incubated with OSCP, then separated on a sucrose gradient (see fig.1)

nent of mitochondria and the  $\delta$ -subunit of *E. coli*  $F_1$ , the binding of both polypeptides being dependent on an intact  $\alpha$ -subunit. Such a functional similarity would be in accordance with recent data on the primary structure of beef-heart OSCP [26] which have revealed a striking homology with the  $\delta$ -subunit of *E. coli*  $F_1$  [15]. Whether the two polypeptides bind directly to the  $\alpha$ -subunit of  $F_1$ , or whether the failure of trypsin-modified  $F_1$  to bind them is a secondary consequence of a modification of the quaternary structure of the enzyme as a whole, remains to be elucidated.

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