INTERACTION BETWEEN COUPLING FACTORS OF OXIDATIVE PHOSPHORYLATION AND SUBMITOCHONDRIAL PARTICLES

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Received August 14, 1969

SUMMARY. Coupling factor F_3 does not bind to submitochondrial particles (A particles) unless F_1 is simultaneously present. The same seems to be true for X, a coupling factor isolated together with F_1.

The isolation of a soluble ATPase(F_1-X) with a high coupling activity, resulting from association with another coupling factor (X), has been previously reported (Vallejos et al. 1968). It was suggested that X may be a purified form of F_3[++].

In this paper we report a study of the binding of F_1, F_3 and F_1-X to submitochondrial particles and of the interaction between F_1 and X.

Methods. Ammonia-treated particles (A particles) were prepared from heavy beef heart mitochondria as described by Fessenden and Racker (1966), coupling factors F_1 and F_1-X were prepared as described by Vallejos et al. (1968) and F_3 as described by Fessenden and Racker (1967).

Gel filtration in Sephadex G-75 was carried out by a small scale adaptation of the desalting technique of Determann (1968). In our case the purpose was to separate large molecules like F_1 (MW 284000 (Penefsky and Warner (1965)), that were excluded from Sephadex G-75 from those that were not(MW less than 70.000) and to

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[++] A coupling factor described by Racker (1962).

use this method for studing the interaction of F_1 and X. The Sephadex G-75 gel was equilibrated with 0.25 M sucrose, 10 mM Tris-SO_4 pH 7.5, 1 mM EDTA (STE medium). Then it was centrifuged for 20 min at 1000 g in a tube closed with a nylon net (Determann 1968) to drain off the void volume. The sample (2 mg of protein/ml) in STE medium was layered on top of the gel and after 10 min equilibration, centrifuged for 20 min at 1000 g. The filtrate was collected for assay. All manipulations were carried out at 4° unless otherwise stated.

The binding of coupling factors to A particles was studied by the following method. A particles alone or with coupling factors were preincubated 5 min at 25°. Unbound coupling factors

TABLE 1

GEL FILTRATION OF COUPLING FACTORS

Additions to A particles + F_1	P_i-ATP exchange activity (nmoles ATP /15 min)
F_1-X (10 µg)	470
F_1-X (10 µg) gel filtered	435
F_1-X cold treated (10 µg)	400
F_1-X cold treated (10 µg) gel filtered	110
F_3 (100 µg)	400
F_3 (100 µg) gel filtered	100

2 mg of factor was filtered through Sephadex G-75 as described in Methods. F_1-X cold treated was obtained by keeping F_1-X for 1 h 30 min at 0° in STE containing 0.2 M KBr, before layering on the gel. Pi-ATP exchange activity of A particles was measured as previously described (Vallejos et al., 1968) in the presence of a saturating amount of F_1 (0.8 unit). It was 930 nmoles ATP /15 min. The stimulation due to X or F_3 over this value is reported.

were separated from A particles by dilution and centrifugation. The pellet of A particles plus bound factors was resuspended and its Pi-ATP exchange activity determined with or without addition of F_1. Details are given in Table II.

Soluble protein was determined spectrophotometrically (Warburg and Christian (1941)) and insoluble protein by the biuret method (Szarkowska and Klingenberg (1963)).

Results and Discussion. The interaction of F_1 and X was explored by gel filtration in Sephadex G-75. Table 1 shows that when F_1-X was filtered through Sephadex G-75 the specific coupling activity of X remained nearly the same, that is, X was excluded from the gel together with F_1. Exclusion of F_1 from the Sephadex G-75 was assessed by measuring the specific ATPase activity of the filtrate which was the same as that of the sample applied (not shown, experiment done at 10°). Penefsky and Warner (1965) showed that cold treatment of F_1 in the presence of KBr leads to depolymerization of F_1 into subunits of a molecular weight of 30.000. When F_1-X was incubated at 0° for 1.5 h in STE medium containing 0.2 M KBr and then filtered only a small fraction of X coupling activity was recovered in the filtrate (Table 1). F_3, like cold treated F_1-X was also retained by Sephadex G-75, suggesting that its molecular weight is below 70.000.

The fact that X is excluded from the gel only when native F_1 is present would suggest that they are closely associated. An alternative, but unlikely explanation, would be to suppose that X is a large molecule (NW 70.000) that is excluded from Sephadex G-75 and that it depolymerizes in the same conditions as F_1 does but without loss of activity.

Table II line 2 shows that when A particles were preincubated

with F_1 then diluted and centrifuged to eliminate unbound F_1, its Pi-ATP exchange activity increased twice with respect to the control (line 1). When F_3 was also present in the preincubation mixture (Table II, line 3) a stimulation of nearly 10 times was observed which was further increased by adding F_1 to the assay system. However, when F_1 was omitted from the preincubation medium, F_3 failed to stimulate the Pi-ATP exchange even with F_1 present in the assay mixture (Table II, line 4).

Preincubation of A particles with F_1-X, as with F_3 plus F_1, resulted in A particles with an exchange activity higher than

TABLE II

BINDING OF COUPLING FACTORS TO A PARTICLES

Preincubation system Additions to A particles		P_i-ATP exchange (nmoles ATP/15 min) Additions	
		None	F_1
1	None	27	160
2	F_1	77	185
3	F_3, F_1	227	577
4	F_3	53	149
5	F_1-X	116	431
6	F_1-X cold treated	20	175
7	F_1, F_1-X cold treated	154	490

A particles (4 mg) were preincubated alone or with F_1 (374 µg), F_1-X cold treated or not (500 µg) and F_3 (2 mg) in 0.4 ml of 0.25 M sucrose, 5 mM Tris SO_4 pH 7.5, 0.5 mM EDTA and 1.5 mM $MgCl_2$ at 25° for 5 min. Then 1.7 ml of cold 0.25 M sucrose was added and the tubes were centrifuged 30 min at 105.000 g. Pellets were homogenized in 0.8 ml of 0.25 M sucrose and the P_i-ATP exchange activity of the particles was measured as previously described (Vallejos et al. 1968) in aliquots of 0.5 mg of protein with or without the addition of 0.8 unit of F_1. Cold treatment of F_1-X was at 4° for 20 h in the STE medium.

that observed by preincubation with F_1 alone (Table II, line 5). The exchange activity was also further increased by adding F_1 to the assay medium.

Preincubation of A particles with cold treated F_1-X, which is without F_1 activity (Vallejos et al., 1968), completely eliminated the stimulatory effect due to X (Table II, line 6). However, this stimulatory effect was fully restored by adding native F_1 together with the cold treated F_1-X to the preincubation system (Table II, line 7).

The Pi-ATP exchange activity of A particles preincubated in the different conditions stated in Table II and measured in the presence of a saturating amounts of F_1-X was about 500 nmoles ATP /15 min except when they were preincubated with cold treated F_1-X. In this case the activity did not go beyond the value obtained with F_1 alone. The same interference was observed by preincubation with cold treated F_1.

Coupling factors present in the preincubation system of Table II were in excess of the saturating amounts. The exchange activity of A particles before centrifugation was the same as that obtained after centrifugation and determined in the presence of F_1 (second column of Table II) with the exception of the experiments shown in lines 4 and 6 which gave 50 and 42 nmoles ATP /15 min respectively. This observation implies that there are two types of binding of F_1 to submitochondrial particles. Some F_1 was strongly bound to the particles increasing its exchange activity and was not removed by washing and centrifugation, some portion of the F_1 interacted weakly with the particles, remained in the supernatant when the particles were centrifuged off and had to be added back to the assay medium to achieve maximum activity. This interpretation is in agreement with a similar

suggestion by Bulos and Racker (1968) who studied the binding of F_1 to TUA-particles by following ATPase activity.

It can also be concluded from the data in Table II that the order of addition of coupling factors to submitochondrial particles is important. F_3 will not bind to its active site in A particles if F_1 is not present simultaneously. Addition of F_1 to A particles after removing unbound F_3 does not result in the high exchange activity induced by F_3 plus F_1.

The same explanation seems to be true with X, but in this case the interference by cold treated F_1 weakens the argument. Attempts are being made to separate X from F_1 in order to study the binding with X alone.

It should be pointed out that the coupling activity of F_3 is only slightly higher than that of X (unpublished observations).

Acknowledgments. I am grateful to Professor A.O.M. Stoppani for his encouragement and support and to Professor B.C. Chappell for reading the manuscript. These studies were supported by grants of the Consejo Nacional de Investigaciones Científicas y Técnicas (Argentina) and The Fund for Overseas Research Grants and Education, Inc. (N.York, USA).

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