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A soluble ATPase with high coupling activity

In 1958 PULLMAN, PENEFSKY AND RACKER, described the isolation from beef heart mitochondria of a soluble ATPase (called F_1) that is able to stimulate oxidative phosphorylation in deficient sub-mitochondrial particles[1]. Recently, the existence of several coupling factors required in addition to F_1 for maximal stimulation of phosphorylation and related reactions has been described[2,3]. The present communication reports the isolation from beef-heart mitochondria of two soluble ATPase preparations with coupling activity. One of these resembles F_1 in that in the absence of other coupling factors or oligomycin it has relatively little coupling activity with the deficient submitochondrial particles used in the present experiments (*cf.* refs. 2, 4). The other has a high coupling activity on its own, probably due to the presence, in close association with the F_1, of one or more additional factors.

An acetone powder of beef-heart mitochondria was suspended in water and the suspension sonically disintegrated (2 min in the Mullard apparatus at maximum output). The powder was recovered by centrifugation at 38000 × *g* for 15 min, and the extraction with water repeated three times. Each extract was cleared by centrifugation at 105000 × *g* for 30 min, precipitated with $(NH_4)_2SO_4$ at 50 % saturation, and the precipitate suspended in 0.25 M sucrose–1 mM EDTA–10 mM Tris sulphate (pH 7.4). The suspension was centrifuged at 38000 × *g* for 15 min. The supernatant contained the coupling factor that will be referred to as F_1-X in this paper. The specific ATPase activity was much higher with the fourth aqueous extract of the acetone powder than with the earlier extracts. From the $(NH_4)_2SO_4$ precipitates which had oligomycin-sensitive ATPase activity, an oligomycin-insensitive ATPase was solubilized by keeping for 2 min at 65° in the presence of 4 mM ATP. Since this ATPase had the properties of F_1 as described by PENEFSKY *et al.*[4] and PULLMAN *et al.*[5] it will be referred to under this name. Both F_1-X and F_1 contained a Mg^{2+}-dependent, oligomycin-insensitive, dinitrophenol-stimulated and cold-labile ATPase (*cf.* ref. 5).

Table I shows that F_1 alone doubled or trebled the P:O ratios of submitochondrial particles with succinate as substrate. The effects of F_1 and oligomycin are additive (Expt. 1). The activity of F_1 is cold-labile (Expt. 2). Similar effects on the ATP-dependent reduction of NAD^+ by succinate (Table II) and on the P_i–ATP exchange reaction (Table III) were observed. F_1-X stimulated the P:O ratio (Table I, Expt. 3), the ATP-dependent reduction of NAD^+ by succinate (Table II), and the P_i–ATP exchange reaction much more than F_1. The effects of F_1-X shown in Tables I–III are comparable with those reported for a mixture of F_1, F_2 and F_4 by FESSENDEN AND RACKER[2] or for a mixture of F_1 and F_3 by FESSENDEN, DANNENBERG AND RACKER[10]. In the presence of F_1-X, oligomycin had no effect on the P:O ratio (Table I, Expt. 3).

Table I, Expt. 4, shows that the relative coupling activity of different preparations of F_1-X correlated quite closely with the ATPase activities. Both coupling and ATPase activities were inactivated to the same extent by cold treatment. On the other hand, cold-treated F_1-X was effective in increasing the coupling activity of F_1 (Table I, Expt. 5 and Table II) or of sub-optimal amounts of F_1-X (Table III).

TABLE I

STIMULATION OF P:O RATIOS BY OLIGOMYCIN, F_1 AND F_1-X

0.5 mg Submitochondrial particles (A particles[2] in Expts. 1, 2 and 4 or EDTA particles[6] in Expts. 3 and 5) were preincubated at room temperature with the additions indicated in a final vol. of 0.3 ml containing 2 μmoles $MgSO_4$, 2 μmoles ATP and 30 μmoles potassium phosphate (pH 7.4). After 5 min the reaction was started by adding 0.2 ml containing 1 μmole $MgSO_4$, 0.5 μmole ATP, 16 μmoles glucose, 2.5 μmoles Tris sulphate (pH 7.4), 0.25 μmole EDTA, 10 μmoles sodium succinate, 0.5 mg bovine serum albumin, 100 Cori units of hexokinase and 300000 counts per min of carrier-free $^{32}P_i$. The reaction was carried out for 20–30 min at 25° and stopped with 0.5 ml of 10% trichloroacetic acid. Oxygen consumption was determined manometrically and the $^{32}P_i$ incorporated into glucose 6-phosphate according to NIELSEN AND LEHNINGER[7]. ATPase activity of F_1-X was determined by the method of PULLMAN *et al.*[5]. The amounts of F_1 and F_1-X refer to protein determined spectrophotometrically[8].

Expt. No.	*Addition to particles*	*Glc-6-P (μmoles)*	*P:O*
1	None	0.37	0.09
	F_1 (37 μg)	0.85	0.18
	Oligomycin (0.05 μg)	0.84	0.17
	Oligomycin (0.05 μg) + F_1 (37 μg)	1.42	0.31
2	None	0.10	0.05
	F_1 (120 μg)	0.26	0.14
	F_1 (120 μg), cold-treated**	0.08	0.05
3	None	0.18	0.04
	Oligomycin (0.05 μg)	1.17	0.24
	Oligomycin (0.1 μg)	1.25	0.26
	F_1-X (120 μg)	3.31	0.68
	F_1-X (120 μg) + oligomycin (0.05 μg)	3.31	0.66
	F_1-X (120 μg) + oligomycin (0.1 μg)	3.38	0.66
	F_1 (88 μg)	0.45	0.10
4	None	0.31	0.08
	F_1-X Prep. I* (182 μg)	1.75	0.46
	F_1-X Prep. I* (365 μg)	2.43	0.53
	F_1-X Prep. I* (730 μg)	2.13	0.53
	F_1-X Prep. II* (128 μg)	2.49	0.54
	F_1-X Prep. III* (21 μg)	1.50	0.32
	F_1-X Prep. III* (42 μg)	2.45	0.52
	F_1-X Prep. III* (81 μg)	2.45	0.52
5	None	0.16	0.04
	F_1 (18 μg)	0.35	0.08
	F_1 (72 μg)	0.41	0.09
	F_1-X (500 μg), cold-treated**	0.28	0.06
	F_1 (72 μg) + F_1-X (500 μg), cold-treated**	1.91	0.47

* These three preparations, which were made from the second, third and fourth aqueous extracts of the acetone powder, had the following ATPase activities (expressed as μmoles P_i/mg protein per min): Prep. I, 4.1; Prep. II, 21; Prep. III, 32.

** 2 h at 0°.

It appears then that F_1-X contains one or more cold-stable coupling factors as well as the cold-labile F_1. Table II shows that if F_2 is in F_1-X it cannot account for the coupling activity. Indeed, F_2 has been reported to be without effect on the reduction of NAD^+ by succinate[10]. F_2 could also not replace cold-treated F_1-X in experiments similar to those of Table I, Expt. 5.

TABLE II

EFFECT OF F_1 AND F_1-X ON THE ATP-DEPENDENT REDUCTION OF NAD^+ BY SUCCINATE

0.5 mg A particles were preincubated at room temperature (21°) with the additions indicated in a vol. of 0.6 ml containing 150 μmoles Tris sulphate (pH 7.4), 10 μmoles $MgSO_4$, 30 μmoles sodium succinate and 0.5 mg bovine serum albumin. After 5 min 3 μmoles KCN and 3 μmoles NAD^+ were added and 1–2 min later the reaction was started with 4 μmoles ATP. The final vol. was 3 ml. Changes in absorbance at 340 mμ were followed spectrophotometrically. F_2 was a succinate dehydrogenase preparation, kindly supplied by Mr. W. P. ZEYLEMAKER (*cf.* ref. 9).

Additions to A particles	*NADH formation (nmoles/mg per min)*
None	1.2
F_1 (37 μg)	3.6
F_1-X (96 μg)	21.4
F_1-X (96 μg) cold-treated*	2.6
F_1-X (96 μg) cold-treated* + F_1 (37 μg)	21.0
F_2 (236 μg)	2.6
F_1 (37 μg) + F_2 (236 μg)	4.8

* 150 min at 0°.

TABLE III

EFFECT OF F_1-X ON THE $^{32}P_i$–ATP EXCHANGE REACTION IN A PARTICLES

0.5 mg of particles (A particles in Expt. 1, EDTA particles in Expt. 2) were preincubated at 25° with the additions as in Table I. The reaction was started by adding 0.2 ml containing 8 μmoles $MgSO_4$, 8 μmoles ATP and carrier-free $^{32}P_i$ (242000 counts/min). After 15 min at 25° it was stopped with 0.05 ml of 50% trichloroacetic acid and [^{32}P]ATP determined according to NIELSEN AND LEHNINGER[7].

Expt. No.	*Additions to particles*	*nmoles [^{32}P]ATP/mg*
1	None	26
	F_1-X (56 μg)	923
	F_1-X (84 μg)	1190
	F_1-X (56 μg) cold-treated*	184
	F_1-X (17 μg)	100
	F_1-X (17 μg) + F_1-X (56 μg) cold-treated*	725
2	None	81
	F_1 (60 μg)	200
	F_1-X (65 μg)	1970

* 150 min at 0°.

As little as 42 μg of F_1-X were found maximally effective (Table I, Expt. 4). This may be compared with 40 μg F_1 + 200 μg F_2 + 300 μg F_3 used by FESSENDEN, DANNENBERG AND RACKER[10] in comparable experiments. This shows that in F_1-X the other factor(s) must be present in high concentrations, perhaps as a complex with F_1.

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Laboratory of Biochemistry, R. H. VALLEJOS
B. C. P. Jansen Institute, E. C. SLATER
University of Amsterdam, Amsterdam (The Netherlands)

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