AN OXIDATIVE PHOSPHORYLATION COUPLING FACTOR WHICH ACTIVATES REVERSED ELECTRON TRANSFER

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Received September 14, 1964

Previous communications from this laboratory described a soluble protein factor which stimulated ATP-dependent NAD reduction by succinate, and net esterification of P_i coupled to NADH oxidation, without affecting the rate of oxidation (1,2). The factor has now been purified approximately 100-fold by isoelectric precipitation, ammonium sulfate fractionation, and chromatography on DEAE-cellulose. The factor also stimulates $^{32}P_i$-ATP exchange in depleted particles. The specific activity of the factor in the exchange reaction increased parallel to the stimulation of NAD reduction. A third activity associated with the fraction is ATPase, which is also purified roughly 100-fold, although the degree of purification at the intermediate stages did not follow the pattern observed with the other two activities. The fourth activity associated with the enzyme is the stimulation of net phosphorylation coupled to the oxidation of succinate and NADH (Table I). In the early stages of purification, the preparation occasionally had ADP-ATP exchange activity, which was eliminated completely in the final stages. These assays were kindly carried out by Dr. R. Glaze and Dr. C. Wadkins.

Since the purified factor catalyzes oxidative phosphorylation coupled to the aerobic oxidation of succinate and stimulates reversed electron transfer, it may act as a coupling factor at two or more sites of phosphorylation in the respiratory chain. However, since Ernster (3) and

Table I

RESTORATION OF PHOSPHORYLATION COUPLED TO OXIDATION OF SUCCINATE AND NADH

Substrate	Factor μg/mg particle	ΔP_i μmoles	P/O
Succinate	0	3.0	0.33
"	9	4.0	0.46
"	17	4.5	0.55
NADH	0	3.2	0.41
"	9	4.5	0.64
"	17	4.3	0.65

Oxidation was measured using conventional manometry and phosphorylation by the net disappearance of phosphate. The mitochondrial particles were depleted of endogenous coupling factor by washing with 1.0 M urea-0.5 mM ATP. The assay medium contained 0.2 M sucrose, 10 mM Tris-H_2SO_4 (pH 7.5), 5 mM K phosphate (pH 7.5), 3 mM $MgSO_4$, 10 mM glucose, 1.5 mM ADP, 1.5 mg particles, 5 mg hexokinase (Type III), and substrate. The substrate was either 5 mM succinate or an NADH generating system containing 23 mM ethanol, 0.17 mM NAD, 5 mM semicarbazide, and 0.5 mg yeast alcohol dehydrogenase.

Snoswell (4) have demonstrated the transfer of energy via a nonphosphorylated high-energy intermediate from one site of phosphorylation to another, it is not possible to decide with the present knowledge whether the enzyme is a multisite or site-specific factor.

Since the coupling factor has some properties in common with the cold-labile ATPase or F_1 (5,6), a more detailed study of its properties was undertaken. Table II shows the effect of incubation temperature on the activities of our purified coupling factor. The ATPase activity was partially cold labile; but the ability to stimulate net phosphorylation, $^{32}P_i$-ATP exchange, and ATP-dependent NAD reduction by succinate in depleted particles was cold stable. Exposure to 30° stimulated the ATPase activity 2- to 3-fold but decreased the $^{32}P_i$-ATP exchange and NAD reduction activities. Our data are consistent with the conclusions from Racker's laboratory (5,6) that the ATPase is an aberrant activity, and in our preparation, only this aberrant activity is cold labile.

Table II

EFFECT OF STORAGE TEMPERATURE ON ACTIVITIES OF COUPLING FACTOR

	μmoles Δ Phosphate	μmoles/min/mg		
		$^{32}P_i$-ATP exchange	NAD reduction by succinate	ATPase
Control	0.72	1.6	1.1	19
45 min at 0°	0.78	1.5	1.1	8.7
45 min at 30°	0.84	1.1	0.75	48

The coupling factor, purified on a DEAE-cellulose column, was incubated in the eluting medium (approx. 0.1 M Tris-H_2SO_4 - 0.1 mM EDTA) at 0° and 30°. Phosphorylation was measured by the esterification of $^{32}P_i$. The esterified phosphate was separated on a reversed phase partition column (7). The conditions for measuring $^{32}P_i$-ATP exchange, ATP-dependent NAD reduction, and ATPase activity were similar to published methods (1,5).

A natural ATPase inhibitor which confers cold stability on ATPase as well as the activities related to phosphorylation coupling has been described recently (8). The inhibitor could be dissociated from the ATPase by heating at 65° in the presence of ATP and subsequently fractionated with ammonium sulfate. Such treatment increased the ATPase activity of our preparation several-fold, but ATP-dependent NAD reduction by succinate was unaffected. The heated enzyme showed increased cold lability with respect to ATPase activity, but the ATP-dependent NAD reduction was still cold stable. It would appear that the cold stability of the latter is not related to the presence of an inhibitor.

Fig. 1 shows the effect of recycling the heat and cold treatment on ATPase activity. After the activity is enhanced about 5-fold by heating, storage at 0° produces decline. The ATPase is reactivated by a second heat treatment, and the cold inactivation persists after the second heating.

These data suggest that the factor described here is closely related to F_1 or cold-labile ATPase (5,6). The low ATPase and cold stability of coupling activity in our preparation can be explained on the basis of differences in the isolation methods. The original procedure described for

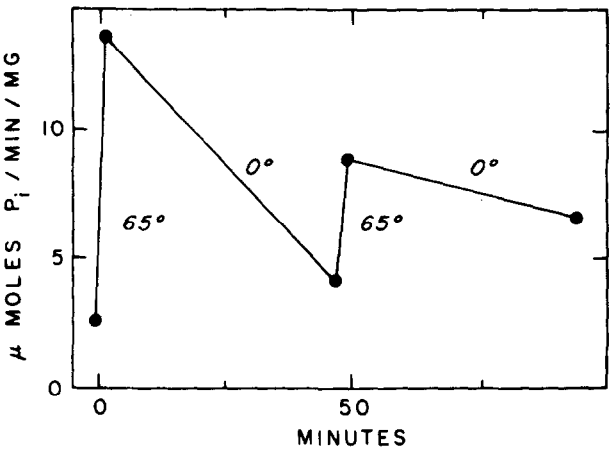

Fig. 1. Reversible thermal activation and inactivation of ATPase. The factor was heated at 65°, cooled, and precipitated with an equal volume of saturated ammonium sulfate. The heat treatment was repeated after the sample had stood for 40 min. at 0°.

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the isolation of F_1 includes protamine precipitation and heating at 65° in order to remove inert protein (5).

This factor also appears to be closely related to, if not identical with, the Site II specific factor from Green's laboratory (9). The latter also stimulates phosphorylation coupled to the oxidation of both succinate and NADH and stimulates ATP-dependent NAD reduction by succinate. The factor appears to be different from the factor described by Hommes (10), since the lag in ATP-dependent NAD reduction by succinate was not eliminated.

The demonstration that a nonphosphorylated high-energy intermediate generated at one site can be utilized at another (3,4), and the present observation that the same functional phosphorylation coupling factor could be isolated with somewhat different properties (5,9), emphasize the need for caution in the interpretation of data concerning the site specificity or multisite participation of coupling factors in oxidative phosphorylation.

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