A RECONSTITUTED SYSTEM OF OXIDATIVE PHOSPHORYLATION*

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It was recently reported (Racker, 1963) that exposure of submitochondrial particles to a sequential treatment with trypsin, urea, and sonic oscillation yielded a factor (F_o) that conferred oligomycin sensitivity on soluble ATPase.

The demonstration of the requirement for multiple factors at the first two sites of oxidative phosphorylation in beef-heart mitochondria (Racker and Conover, 1963) has led to repeated attempts to reconstruct, with the various factors, a soluble system that catalyzes oxidative phosphorylation. These attempts have been unsuccessful until recently, when two problems were resolved. One was the conferral of oligomycin sensitivity on ATPase by additions of preparations of F_o to the ATPase (Racker, 1963), and the second was the successful stabilization of coupling factor 2 (Penefsky et al., 1960).

It will be shown below that both oxidative phosphorylation and a P_i^{32}-ATP exchange reaction were catalyzed in the presence of F_o, coupling factor 1 (F_1), and coupling factor 2 (F_2). As can be seen from Table I, the three factors were required for exchange activity, and there was little effect from the addition of various other coupling factors or of serum albumin. Addition of oligomycin or 2,4-dinitrophenol abolished the exchange.

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Table I

RECONSTITUTION OF THE P_i^{32}-ATP EXCHANGE REACTION

The complete system contained, in a volume of 0.5 ml, 640 μg of F_0, 50 μg of F_1, 632 μg of F_2, and 30 μmoles of P_i; after 5 minutes at 25°, 10 μmoles of ATP, 10 μmoles of $MgSO_4$, and P_i^{32} (1.2×10^5 cpm) were added and the final volume adjusted to 0.6 ml. After 15 minutes at 30° the mixture was analyzed for ATP^{32} (Conover et al., 1963).

	mμmoles of ATP^{32} formed/ mg /15 min
Complete system	260
F_2 omitted	35
F_1 omitted	16
F_0 omitted	16
Complete system + F_4 (160 μg)	270
Complete system + bovine serum albumin (1 mg)	271
Complete system + F_3 (460 μg)	258
Complete system + oligomycin (1.6 μg)	16
Complete system + dinitrophenol (0.5 mM)	16

Crude "soluble" preparations of F_0 were prepared by exposure of T-U-particles to sonic oscillation and centrifugation for 1 or 2 hours at 100,000 x g. The supernatant solution catalyzed the oxidation of DPNH and of succinate, but no phosphorylation took place. On addition of F_1, F_2, and a phosphate acceptor system, oxidative phosphorylation was catalyzed by the complete system, as shown in Table II. It can be seen that oxidation of succinate was not affected when F_1 or F_2 was omitted, but that omission of either fraction virtually eliminated phosphorylation. Crude mitochondrial extracts replaced F_2, but partially purified preparations gave better P:O ratios. Even at best the P:O ratio was low, but phosphate esterification was completely eliminated in the presence of dinitrophenol.

It is apparent that the crude F_0 preparation not only contains the entire chain of respiratory catalysts but must also contain other coupling factors; for example, F_4, which is required for oxidative phosphorylation (Conover et al., 1963).

Table II

OXIDATIVE PHOSPHORYLATION IN THE RECONSTITUTED SYSTEM

The complete system contained, in a final volume of 0.6 ml, 30 μmoles of K phosphate buffer (pH 7.4), 10 μmoles of succinate, 20 μmoles of glucose, 5 units of hexokinase, 2 μmoles of $MgSO_4$, 2 μmoles of ATP, 500 μg of bovine serum albumin, 1.1 mg of F_0, 32 μg of F_1, 632 μg of F_2, and 1.2×10^5 cpm of P_i^{32}. Measurements of oxidation were carried out manometrically as described previously (Conover et al., 1963), and P^{32}-labeled glucose-6-P was determined in the water phase after isobutanol extraction (Lindberg and Ernster, 1956).

	μatoms O	μmoles glucose-6-P^{32}	P:O
Complete system	3.1	0.25	0.08
F_1 omitted	3.8	0.02	0.005
F_2 omitted	3.1	0.05	0.016
F_1 and F_2 omitted	2.9	0.00	0
Complete system with crude extract (450 μg) replacing the F_2 preparation	3.1	0.11	0.035
Complete system + dinitrophenol (0.5 mM)	3.2	0.00	0

These experiments demonstrate a reconstitution of oxidative phosphorylation with partially purified factors from mitochondria. Although the F_0 solution used in the experiment shown in Table II was obtained after 60 minutes of centrifugation at 100,000 x g, the question of "true solubility" may be raised. As reported previously (Racker, 1963), F_0 activity was readily precipitated from solution at very low salt concentrations and is considered to be a form of structural protein to which many catalysts are firmly bound. However, in contrast to submitochondrial particles, F_0 preparations were found to be highly sensitive to treatment with trypsin. It may therefore be concluded that although the F_0 preparation is still very crude, it represents the most highly degraded form of an actively phosphorylating respiratory chain that has been obtained thus far.

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