A RÔLE FOR COENZYME A IN PHOSPHORYLATIONS ASSOCIATED WITH ELECTRON TRANSPORT

Sir:

Submitochondrial particles from sonic extracts of rat liver mitochondria[1,2] catalyze oxidative phosphorylation but with considerably lower efficiency than that of intact mitochondria or particles obtained by digitonin[3] or alcohol treatment[4] of mitochondria. Phosphorylation accompanying oxidation of D,L-β-hydroxybutyrate by the particles required the addition of DPN,[5] ATP, KF and Mg^{++}. Addition of a boiled extract of the 105,000 $\times$ *g* supernatant fraction from mitochondrial sonic extracts to the complete system enhanced phosphate esterification without increasing oxygen consumption, to result in an increased P:O ratio. Stimulation of phosphorylation was abolished by treating the heat-stable fraction with activated charcoal (Nuchar) or Dowex 1, suggesting the role of a nucleotide-like cofactor. Of the known nucleotides tested, the mono-, di- and triphosphates of cytosine, inosine, guanosine or uridine did not enhance phosphate uptake. Crude fractions of yeast nucleotides[6] stimulated in proportion to their content of coenzyme A.

TABLE I

EFFECT OF COENZYME A CONCENTRATION ON OXIDATIVE PHOSPHORYLATION[a]

CoA added, *M*	ΔP, μmole	ΔO, μatom	P:O ratio
0	0.4	4.4	0.09
3.3×10^{-6}	0.9	4.5	.20
8.3×10^{-6}	1.3	4.1	.33
1.7×10^{-5}	1.7	4.4	.37
3.3×10^{-5}	2.2	4.3	.50
6.7×10^{-5}	2.8	4.6	.61

[a] Rat liver mitochondria, suspended in 2 ml. of water per g. of original tissue, were treated in the Raytheon 10 kc. sonic oscillator for 20 sec. The sonic extracts were centrifuged at 25,000 $\times$ *g* for 10 min., and the resulting supernatant fluid was centrifuged at 105,000 $\times$ *g* for 40 min. The pellet was washed once with water and the washed particles were suspended in 0.5 ml. of water per g. of original tissue. The incubation mixture contained 50 μmoles of tris buffer (*p*H 7.4), 12 μmoles of potassium phosphate (*p*H 7.4), 15 μmoles of $MgCl_2$, 10 μmoles of KF, 2 μmoles of ATP, 5 μmoles of DPN, 40 μmoles of D,L-β hydroxybutyrate, excess yeast hexokinase (Sigma) and glucose and 0.2 ml. of enzyme in a final volume of 3.0 ml. Reaction carried out at 30° for 15 min. following the addition of hexokinase.

Purified coenzyme A[7] in concentrations from 3.3×10^{-6} *M* to 6.7×10^{-5} *M* markedly stimulated phosphate uptake without affecting oxygen consumption when D,L-β-hydroxybutyrate was the substrate (Table I). Increasing the CoA concentration above 6.7×10^{-5} *M* did not produce any further increase of the phosphorylation. Addition of CoA stimulated oxidative phosphorylation when either D(−)β-hydroxybutyrate, ethanol + yeast alcohol dehydrogenase, DPNH or succinate were used as substrate. L(+)β-Hydroxybutyrate was not oxidized by the sonic preparation in the presence or absence of CoA.

TABLE II

EFFECT OF THE OXIDATION–REDUCTION STATE OF COENZYME A[a]

Conditions	ΔP, μmole	ΔO, μatom	P:O ratio
Control	3.4	5.2	0.65
+ CoA (6.7×10^{-5} *M*)	5.0	5.4	.93
+ oxidized CoA (6.7×10^{-5}*M*)	3.6	5.5	.66
+ oxidized and rereduced CoA (6.7×10^{-5}*M*)	4.8	5.8	.84
+ CoA + oxidized CoA (6.7×10^{-5} *M*)	4.9	5.5	.91

[a] Conditions as in Table I. The oxidation state of the CoA solutions was followed by the sulfhydryl determination described by Grunert and Phillips.[8]

Coenzyme A promoted phosphorylation only when added in the reduced form (Table II). When the –SH group was oxidized by exposure to oxygen at room temperature for several hours the stimulatory effect was abolished. Recovery of the –SH function by sodium amalgam reduction of the oxidized CoA was associated with recovery of the ability to enhance phosphate esterification. Oxidized CoA did not affect the stimulation by the reduced form.

The same concentrations of glutathione, thioglycolate, homocysteine, cysteine, cysteine ethyl ester or N-acetylcysteine were ineffective. Concentrations of CoA which were active in increasing the phosphate uptake did not inhibit the ATPase activity of the particles, ruling out the possibility that CoA simply prevented ATP breakdown.

Although the heat-stable factor in the supernatant fraction has not been identified as CoA, no further stimulation of phosphorylation was obtained with this fraction in the presence of CoA. The extent of the stimulation of phosphorylation by CoA was quite variable from preparation to preparation, but the effect always was observed.

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(1) W. C. McMurray, G. F. Maley and H. A. Lardy, *J. Biol. Chem.* (in press); W. C. McMurray and H. A. Lardy, Am. Chem. Soc., Div. Biol. Chem., New York, Sept. 1957, 51C.

(2) W. W. Kielley and J. R. Bronk, *Biochim. Biophys. Acta*, **23**, 448 (1957); Am. Chem. Soc., Div. Biol. Chem., New York, Sept. 1957, 51C.

(3) C. Cooper and A. L. Lehninger, *J. Biol. Chem.*, **219**, 489 (1956).

(4) D. Ziegler, R. Lester and D. E. Green, *Biochim. Biophys. Acta*, **21**, 80 (1956).

(5) The following abbreviations are used: DPN, diphosphopyridine nucleotide; DPNH, reduced diphosphopyridine nucleotide; ATP, adenosine triphosphate; CoA, coenzyme A.

(6) Generously supplied by Dr. Sam Morell of Pabst Laboratories.

(7) Pabst Laboratories, Lot 410. This lot contains very little glutathione.

(8) R. R. Grunert and P. H. Phillips, *Arch. Biochem. Biophys.*, **30**, 217 (1951).

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ALKALI METAL TETRACHLOROBORATES

Sir:

Salts of fluoboric acid are legion, but analogous chloroborates have not been established[1] until re-

(1) Complexes of boron trichloride with various chlorine compounds have been discussed in a review by D. R. Martin, *J. Phys. Chem.*, **51**, 1400 (1947).