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 × *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 × *g* for 10 min., and the resulting supernatant fluid was centrifuged at 105,000 × *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.

We thank Mr. Robert Traut for assistance in early phases of this study. This work was supported by The American Cancer Society and the United States Public Health Service.

(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).

cently when Lappert[2] prepared pyridinium tetrachloroborate. Lappert suggested that the existence of pyridinium chloroborate and the non-existence of the alkali metal chloroborates may be due to a required stabilization of the complex anion by large cations. We have now found that potassium, rubidium and cesium chloroborates can be formed.

The alkali metal chloroborates were prepared by heating 10 to 30 g. samples of the metal chlorides with 60 g. of boron trichloride in a 145-ml., "Hastelloy C" lined pressure vessel to temperatures of 400–500° for about $1/2$ hour, and then cooling the vessel over a period of eight hours. Complete reaction at 400° was not effected; typical empirical compositions of the products were $3.3KCl \cdot BCl_3$, $2RbCl \cdot BCl_3$ and $1.7CsCl \cdot BCl_3$. The order of reactivity of the chlorides appeared to be Cs > Rb > K. Cesium and rubidium chlorides underwent essentially complete reaction at 500°; the solid products showed weight increases that indicated BCl_3/MCl ratios of 0.95 to 1.12. These products were usually contaminated with 3–8% extraneous material derived from the reactor.

Anal. Calcd. for $CsBCl_4$: Cs, 46.4; B, 3.78; Cl, 49.7. Found: Cs, 44.07; B, 3.13; Cl, 45.51; atomic ratios: Cs, 1.0; B, 0.88; Cl, 3.9.

X-Ray analysis of the cesium chloroborate showed the lines of a new component; the pattern could be indexed as rhombohedral[3] with $a_0 = 10.0 \pm 0.5$ Å. and $\alpha = 24° \pm 30'$. Potassium chloride at 500° consistently yielded a mixture of the chloroborate (~65%) and unreacted chloride. Reaction of boron trichloride (250% excess) with potassium fluoborate at 400° and ~100 atm. also gave potassium chloroborate, along with boron trifluoride, in about a 30% conversion. In contrast to the above halides, sodium chloride showed no evidence of reaction with boron trichloride.

All of the chloroborates reacted exothermally with water. The resulting clear solutions gave an immediate precipitate when aqueous silver nitrate was added. The precipitate, however, continued to form over a short period of time. This behavior suggested that the hydrolysis of the chloroborate anion might be sufficiently slow to permit purification of the salts by rapid solution and crystallization. However, this procedure yielded only gross mixtures of the metal chloride, boric acid and possibly a metal borate. The ease of hydrolysis of the chloroborates seems surprising in view of the stability of boron trichloride–trimethylamine[4] toward attack by water and by alcohols; however, proton attack of the negatively charged chloroborate anion should occur more readily than proton attack of the neutral amine adducts.[5] The relatively higher order of stability of the amine adduct was indicated in partial displacement of Cl^- in BCl_4^- (as $KBCl_4$) by trimethylamine at 150° to give boron trichloride-trimethylamine.

The formation of chloroborates only by alkali metal ions of low polarizing power and the extreme hydrolytic instability suggest that the stability of the chloroborate lattice is marginal. Crude measurements of the dissociation pressures of $KBCl_4$, $RbBCl_4$ and $CsBCl_4$ showed average values of 3 atm. at 225, 275 and 370°, respectively, and approximate heats of dissociation in the range 8–15 kcal. The corresponding fluoborates have dissociation pressures of <1 mm. at these temperatures and heats of dissociation of about 27–29 kcal.[6] Thus the chloroborates are thermodynamically more prone toward dissociation than the fluoborates.

(2) M. F. Lappert, *Proc. Chem. Soc. (London)*, 121 (1957).

(3) $CsBF_4$ appears to be dimorphic and one form is reportedly rhombohedral. H. S. Booth and D. R. Martin, "Boron Trifluoride and its Derivatives," John Wiley and Sons, Inc., New York, N. Y., 1949, p. 108.

(4) E. Wiberg and W. Sütterlin, *Z. anorg. Chem.*, **202**, 35 (1931).

(5) The fluoroborate anion also is attacked more readily by water than is boron trifluoride–trimethylamine. However, in contrast to the facile hydrolysis of the chloroborate anion, hydrolysis of the fluoborate anion is difficult to force to completion.

(6) J. H. de Boer and J. A. M. van Liempt, *Rec. trav. chim.*, **46**, 124 (1927).

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PHOSPHORYLATION OF SERINE IN RAT LIVER[1]

Sir:

We wish to report that serine is converted to O-phosphorylserine in rat liver both *in vivo* and *in vitro*.

Eight rats were injected intravenously with L-serine-3-C^{14} and killed 30 minutes later. Serine and phosphorylserine were isolated from a picric acid extract of the pooled livers by a combination of ion exchange and paper chromatography.[2]

From Table I it may be seen that the specific activity of the isolated phosphorylserine is about one-

TABLE I

ISOTOPE CONCENTRATION OF RAT LIVER CONSTITUENTS AFTER INJECTION OF L-SERINE-3-C^{14}[a]

Compd. isolated	μM. per g. liver (wet wt.)	C.p.m. per μM.[b]
Free serine	1.1	351
Phosphorylserine	0.04	100
Protein serine	...	15.1

[a] 10.8 μM. per 100 g. body wt. of L-serine-3-C^{14} containing 4.67×10^4 c.p.m. per μM. were injected in the tail vein. [b] Counted in a proportional flow counter on stainless steel planchets of 1.54 cm.^{2} area, corrected to 5 mg. per planchet (M. L. Karnovsky, *et al.*, *Anal. Chem.*, **27**, 852 (1955)).

fourth that of the free liver serine and much higher than that of protein serine or any other liver constituent isolated, *e.g.*, phosphorylethanolamine, choline, etc. In contrast the concentration of phosphorylserine is quite low, only one-thirtieth that of free serine.

In further experiments, rat liver homogenates or cellular fractions have been incubated one hour with L-serine-3-C^{14}. Phosphorylserine was isolated with

(1) This work was supported by grants from the Nutrition Foundation, Inc., and the William Milton Fund of Harvard University.

(2) Details of these procedures will be reported elsewhere.