THE UNCOUPLING OF OXIDATIVE PHOSPHORYLATION BY BASIC PROTEINS, AND ITS REVERSAL WITH POTASSIUM[1]

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Basic proteins, such as protamine (salmine), have been shown to inhibit the oxidation and reduction of exogenous cytochrome c by mitochondria or preparations from mitochondria (Smith and Conrad, 1961; Person and Fine, 1961; Machinist, et al, 1962). Machinist, et al (1962) have recently proposed that endogenous cytochrome c is bound to phospholipids in a pore in the mitochondrial membrane. Exogenous cytochrome c can enter this pore and transfer electrons to or from the endogenous cytochrome. Protamine is visualized as binding to the outer part of the pore, blocking this traffic, and producing the noted inhibitions.

As part of an inquiry into the reasons for the RNase (ribonuclease) uncoupling of mitochondria (Hanson, 1959), we have studied the effect of basic proteins on oxidative phosphorylation by corn shoot mitochondria. The addition of protamine sulfate to the Warburg vessel resulted in lowered phosphorylation with very little effect on substrate oxidation (Table I). Polylysine was effective in reducing oxidation, but phosphorylation declined more rapidly, reducing the P/O ratio.

Mitochondria were preincubated with protamine and polylysine, reisolated, and oxidative phosphorylation determined as described for RNase studies (Hanson, 1959). At ice temperatures polylysine produced small inhibitions of oxidation and phosphorylation, but protamine was without significant effect

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TABLE I

INHIBITORY EFFECT OF BASIC PROTEINS ON OXIDATIVE PHOSPHORYLATION

Basic Protein	Q_{O_2} (N)	P/O
none	1074	2.09
0.1 mg protamine	980	2.05
0.2 mg protamine	930	0.65
none	482	2.20
0.1 mg protamine	483	1.86
0.1 mg polylysine	341	1.67

Mitochondria were isolated and oxidative phosphorylation determined with succinate-pyruvate (upper experiment) and α-ketoglutarate (lower experiment). Vessel additives and procedures are those described by Hanson (1959) except that cytochrome c was omitted in both experiments, and Tris (tris(hydroxymethyl)aminomethane) was substituted for K in all neutralizations in the lower experiment. Indicated amounts of protamine sulfate (Nutritional Biochemicals Corp.) or polylysine (Mann Research Laboratories) were contained in a total volume of 2.5 ml. Q_{O_2} (N) = ul O_2/hr/mg N.

(Table II). Preincubation at 37°C, however, produced marked inhibitions with both proteins, reminiscent of that produced by RNase. Unlike RNase, however, the protamine inhibition is difficult to demonstrate if determinations of

TABLE II

THE EFFECT OF PREINCUBATION TEMPERATURE ON THE UNCOUPLING DUE TO BASIC PROTEINS

Preincubation additives	Temperature: Ice Q_{O_2} (N)	Ice P/O	37°C Q_{O_2} (N)	37°C P/O
none	617	1.85	500	1.78
0.1 mg/ml polylysine	536	1.57	370	1.14
0.2 mg/ml protamine	675	1.72	340	1.09

Two milliliters of washed mitochondria (about 1 mg N) were suspended in 0.5 M sucrose and 0.01 M potassium phosphate, final pH 6.8, and basic protein added to concentrations indicated. After 15 minutes of preincubation at indicated temperatures, 25 ml of isolation medium (0.5 M sucrose, 0.067 M phosphoric acid, 0.005 M EDTA, neutralized with Tris to pH 7.0) were added, the mitochondria reisolated, and the tube and pellet rinsed in 0.5 M sucrose. The Warburg vessels contained the Tris-neutralized α-ketoglutarate medium of Table I.

oxidative phosphorylation are made in the presence of the potassium ion, which we ordinarily used to neutralize the isolation and reaction mediums. With a Tris-neutralized system the inhibition is readily discernible, but the addition of potassium chloride or nitrate will restore phosphorylation and accelerate oxidation (Table III). Comparable results have been obtained with polylysine.

TABLE III

REVERSAL OF PROTAMINE INHIBITION OF PHOSPHORYLATION BY KCl

Preincubation	Q_{O_2} (N)		P/O	
	-K	+K	-K	+K
37° for 15 min.	785	903[a]	2.25	2.20
+0.2 mg/ml protamine - SO_4	762	801[a]	1.59[b]	2.05[a]

Preincubation and determination of oxidative phosphorylation as in Table II, with and without 0.08 M KCl in the vessel. Mean values from 5 experiments. Analysis of variance shows increases due to K marked "a" are significant at the 1% level; the decrease in P/O due to protamine marked "b" is also significant at this level. Other comparisons fail to attain this statistical significance.

Gamble (1957) found several neutral salts to reverse protamine aggregation of mitochondrial fragments. Person and Fine (1961) noted that inhibition of cytochrome oxidase by protamine was more pronounced in Tris than in phosphate buffer. Machinist, et al. (1962) report high potassium phosphate concentrations to prevent the protamine inhibition of cytochrome oxidase, cytochrome c reductase, and succinoxidase. We suggest that the potassium, rather than the phosphate, may be the important ion in these experiments.

We believe our results indicate that basic proteins are doing something in addition to preventing passage of substances into the membrane. The requirement for incubation in the warm to produce inhibition suggests that penetration into certain susceptible sites is involved. Basic proteins, such as RNase, do penetrate plant membranes (McLaren, et al. 1960). The sites, whatever they are, are certainly implicated in oxidative phosphorylation, for phosphorylatation is inhibited at protamine concentrations that do not affect electron transfer.

The nature of the negative sites is not known, but it is possible that they ordinarily bind magnesium or potassium; we are currently investigating this possibility.

The action of potassium ion in reversing the P/O inhibition cannot be explained at present. Potassium may serve to displace the protamine, being more effective than Tris for some steric reason. If so, this effect of potassium may be quite important physiologically, serving to maintain the mitochondrion *in situ* from inhibition by various cytoplasmic cations. The increase in $Q_{O_2(N)}$ with potassium may be related to the activity of cytochrome oxidase (Miller and Evans, 1956).

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