A NEGATIVELY CHARGED PORE IN THE MITOCHONDRIAL MEMBRANE AS A SITE FOR CYTOCHROME C FUNCTION[1]

J. M. Machinist, M. L. Das, F. L. Crane, and E. E. Jacobs
Department of Biological Sciences, Purdue University, Lafayette, Indiana, and Hansen Laboratories of Physics, Stanford University, Palo Alto, California

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Inhibition of reduced cytochrome c oxidase by protamine and other cationic proteins has been demonstrated by Smith and Conrad (1956) and Person and Fine (1961). Minnaert and Smith (1961) have also observed protamine inhibition of succinic cytochrome c reductase.

We have found that the closed succinoxidase system of beef heart mitochondria, in which the cytochrome c is held in a lipid bound form, is only partially inhibited by protamine, whereas inhibition of cytochrome c oxidase and succinic cytochrome c reductase is essentially complete. Thus, the addition of protamine to mitochondria which are in the partially open form, in that they can interact with external cytochrome c, converts the mitochondria into a closed system. This system is capable of carrying out the oxidation of succinate by means of endogenous cytochrome c, but incapable of oxidation or reduction of external cytochrome c.

Table I shows the effects of protamine on the three activites of beef heart mitochondria.

It is also observed that protamine inhibition of these three activities is blocked by high phosphate levels in the reaction mixture. Data from these experiments are summarized in Table II.

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Table I

Effect of protamine on electron transport activities in beef heart mitochondria at low phosphate levels

	Additions			
Assay	None	Cyt c	Protamine	Cyt c + Protamine
"Succinoxidae	0.23	0.35	0.12	0.13
#Cyt c oxidase	0.03	0.15	0.03	0.03
*Succinic Cyt c reductase		0.24		0.01

"Complete reaction mixture contained 80 umoles sodium succinate (0.2M, pH 6.6), 130-140 umoles potassium phosphate (0.05M, pH 7.4), and beef heart mitochondria (3-5 mg protein). Cytochrome c (0.025 umoles) and protamine sulfate (2.0 mg) were then added to the mixture individually and together. Final reaction volume, 3.2 ml.

#100 umoles ascorbate (0.5M, pH 7.3) added in place of sodium succinate. Both activities expressed as uAO_2/min/mg protein.

*Complete reaction mixture contained 10 umoles sodium succinate (0.1M, pH 6.6), 20 umoles potassium phosphate (0.01M, pH 7.0), 0.05 umoles cytochrome c, 1.0 mg protamine sulfate, potassium cyanide (10-3M), and beef heart mitochondria (0.03-0.05 mg protein) in a total volume of 3.0 ml. Activity expressed as umoles cyt c/min/mg protein.

In studies of the complexes formed between purified phospholipids and cytochrome c, we find that electrostatic bonding between the amino groups of cytochrome c and the phosphate groups of the phospholipids is primarily responsible for the comples formation. Thus, the complex formed between phospholipids of the cephalin group (phosphatidyl ethanolamine and phosphatidyl serine) contain 32 moles of phospholipid per mole of cytochrome c, a ratio which corresponds well with the 32 free amino groups of cytochrome c. Secondary bonding of phospholipids probably account for higher ratios reported by Reich and Wanio 1961. Destruction of the free amino groups of the lysine

Table II

Effect of protamine on electron transport activities in beef heart mitochondria at high phosphate levels

	Additions			
Assay	None	Cyt c	Protamine	Cyt c + Protamine
Succinoxidase	0.08	0.11	0.08	0.10
Cyt c oxidase	0.10	0.11	0.10	0.11
Succinic Cyt c reductase		0.02		0.02

The conditions were the same as listed in Table I, except that the concentration of potassium phosphate (0.5M) was increased to 1400-1475 umoles in the succinoxidase and cyt c oxidase assays and to 750 umoles in the succinis cytochome c reductase assay.

residues and terminal amino groups of cytochrome c by treatment with nitrous acid prevents formation of an isooctane soluble phospholipid-cytochrome c complex. The presence of high concentration of phosphate buffer or of protamine in the mixture of phospholipid and cytochrome c also prevents formation of the isooctane soluble complex, whereas the preformed phospholipid-cytochrome c combination is not destroyed by treatment with phosphate buffer or protamine. Protamine also forms an isooctane soluble complex with phospholipids.

We propose from this evidence that cytochrome c is bound in a negatively charged pore in the mitochondrial membrane with the negatively charged phospholipids predominating at the surface of the pore. External cytochrome c can enter this pore and transfer electrons to or from the internal cytochrome c. Protamine added to mitochondria is bound in the outer part of the pore and prevents entry of added cytochrome c, thus causing inhibition of cytochrome c

reductase and cytochrome c oxidase. The added protamine, however, cannot disrupt the tightly bound cephalin-cytochrome c complex endogenous to the mitochondria so that succinoxidase activity is only partially inhibited by the protamine. Some of the bound cytochrome c is released upon treatment with protamine and this is correlated to a partial inhibition of succinoxidase activity. We interpret this effect as evidence that part of the cytochrome c is bound in a less stable complex such as that formed when lecithin combines with cytochrome c.

The fact that high concentration of phosphate tends to prevent protamine inhibition of cytochrome c oxidase (Person and Fine, 1961) is consistent with this interpretation of the mechanism of protamine inhibition, since the presence of phosphate will tend to prevent formation of the phospholipid-protamine complex at the pore surface. Jacobs and Crane (1961) have also shown that both intact mitochondria and mitochondria depleted of endogenous cytochrome c catalyze the rapid oxidation of tetrachlorohydroquinone in the presence of polylysine or protamine which indicates that protamine inhibits interaction of cytochrome c with oxidase and not the interaction of oxidase with oxgen.

We feel that effects of other ions on the rates of mitochondrial electron transport may be more clearly understood when interpreted on the basis of charged pore systems in mitochondrial membranes with their precise reactions determined to a large extent by the type of phospholipid exposed.

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