THE EFFECTS OF CATIONIC PROTEINS OF RABBIT POLYMORPHONUCLEAR LEUKOCYTE LYSOSOMES ON THE RESPIRATORY ACTIVITY OF LIVER MITOCHONDRIA*

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Summary: The cationic proteins of lysosomes of rabbit polymorphonuclear leukocytes exert a biphasic effect (stimulation at low concentrations: inhibition at high concentrations) on the State 4 respiration of rat liver mitochondria. Inhibition is the only effect observed on State 3 respiration. When separated into two fractions of circa 8000 and 4000 molecular weight, each containing three components, both fractions can stimulate State 4 mitochondrial respiration but neither is capable of complete inhibition of State 3 activity.

The lysosomes of rabbit polymorphonuclear leukocytes (PMN) contain six electrophoretically-identifiable, highly cationic proteins of low molecular weight, which are rich in arginine and cystine and low in lysine (1-4). The cationic proteins have been implicated in several biological activities of PMN. They are known to exert potent cytotoxic actions against bacteria as well as host tissue cells (5,6). The mechanisms of these actions are not fully known, although there is evidence that the antibacterial activity of cationic proteins may be mediated by damage to the cell membrane and/or an inhibition of aerobic respiration (6). Histones and other polycations have long been known to exert profound effects on mitochondrial respiration (7), and the possibility exists that host tissue damage by PMN may also be mediated through an effect of their lysosomal cationic proteins on mitochondria. For this reason we have investigated the effects of the cationic proteins on respiratory processes, using liver mitochondria as a representative system.

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METHODS

The isolation of lysosomes from PMN of peritoneal exudates, and the acid extraction of the cationic proteins therefrom, have been described (1). Permeation chromatography of the acid extract through Sephadex G-25 freed the cationic proteins of extraneous proteins and other cell components. Liver mitochondria were isolated from mature rats by the method of Johnson and Lardy (8) and estimations of respiratory rates were performed according to Estabrook (9). Respiration measurements were made with a GME oxygraph fitted with a Clark electrode. Mitochondrial proteins were determined by the biuret procedure (10); cationic proteins were determined by the Lowry method (11).

RESULTS AND DISCUSSION

The ratio of cationic to mitochondrial proteins as well as the integrity and respiratory state of the mitochondria each have a demonstrable influence on the capacity of PMN cationic proteins to affect mitochondrial respiration. In Figure 1 (Curve A), the addition of cationic proteins is clearly stimulatory to State 4 succinoxidase activity; stimulations of 70, 136 and 70% were observed at protein ratios of 2.2, 4.4, and 6.5, respectively. However, under State 3 conditions (Curve B) the concentration maximally stimulatory to State 4 activity now exhibits no stimulation of respiration; the onset of respiratory inhibition is immediate.

Figure 2 presents a comparison of the time course of inhibition of succinate (Curve A) and glutamate (Curve C) oxidations by the cationic proteins. Inhibition of glutamate oxidation is complete in less than half the time required for full inhibition of succinate oxidation. The approach to complete inhibition is apparently not reversed by ADP, cytochrome c or succinate; separate experiments also establish that neither oligomycin nor DNP affect the inhibition. When the mitochondria are rendered uncoupled (Curve B), addition of the cationic proteins results in an instanteous inhibition of respiration. When obtained Traces A and C were thought to reflect a greater sensitivity of the oxidation

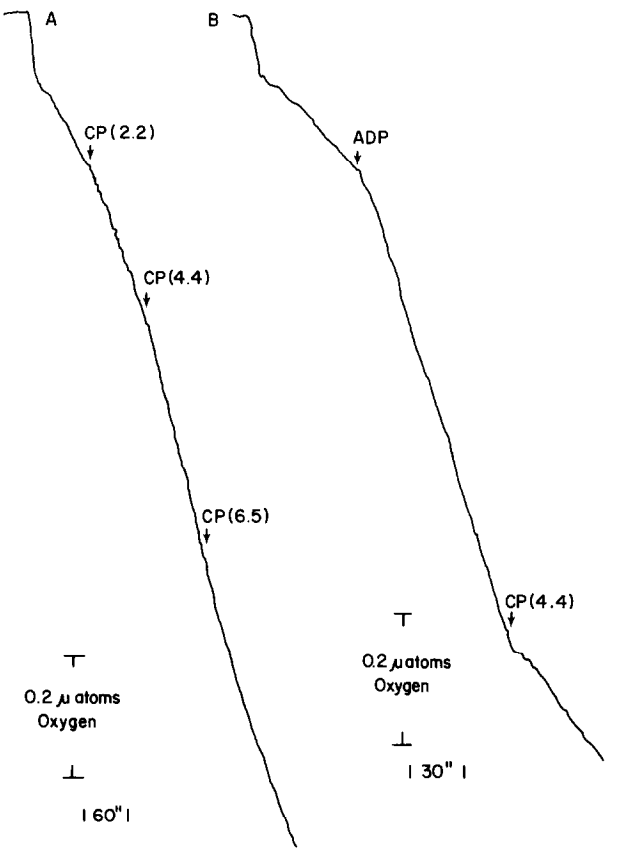

Figure 1 Effects of Respiratory State on Character of Effect of Cationic Proteins on Mitochondria

Conditions: Each system contained 400 μmoles sucrose, 67 μmoles potassium phosphate, pH 7.4, 13 μmoles $MgCl_2$, 33 μmoles KCl, 20 μmoles succinate, 3 μmoles ADP (where indicated). Curve A - 3.2 mg mitochondrial protein (acceptor control ratio = 4.8); 3.7 ml; 30°. Curve B - 7.7 mg mitochondrial protein (acceptor control ratio = 3.4); 3.6 ml; 30°. Protein Ratios (μg cationic proteins x μg mitochondrial protein^{-1} x 10^3) indicated in parentheses.

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of NAD-linked substrates to inhibition by cationic proteins. However, a further study of this point indicated that the respiratory rates supported by succinate versus pyruvate or glutamate were all similarly affected by a given

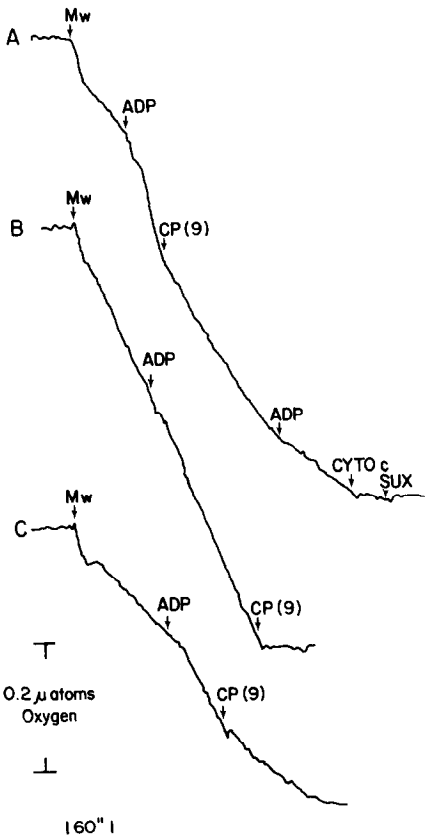

Figure 2 Time course of Inhibition of Mitochondrial Respiration by Cationic Proteins.

Conditions: Basic system and conditions as indicated in Figure 1, 3.8 mg mitochondrial protein (acceptor control ratio (succinate) = 3.4). Curve A - 20 μmoles succinate, 0.5 mg cytochrome c, where indicated; Curve B - 20 μmoles succinate, mitochondria preincubated 20 minutes at 37°, in absence of substrate; Curve C - 50 μmoles glutamate.

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level of cationic proteins. The results of a typical experiment employing two levels of cationic proteins are given in Table 1 (Experiment 1). Similar results were also obtained with uncoupled mitochondria when they were supple-

mented with NAD in the oxidation of substrates requiring that cofactor.* Thus, we believe the apparent greater sensitivity of NAD-linked substrate oxidations (as in Figure 2) most likely stems from the loss of NAD from the mitochondria as membrane integrity is lost.

The cationic protein mixture can be separated by permeation chromatography (1,2) into two fractions in each of which there are three components of the same approximate size. Fractions 1 and 2 so derived contain components of approximately 8000 and 4000 M.W., respectively. The two fractions contain different entities as evidenced by profound differences in their respective contents of arginine and several non-polar amino acids (1,2). Like the parent mixture, both Fraction 1 and Fraction 2 can stimulate State 4 respiration; the stimulations occur in the same range of protein ratios and are of the same magnitude as those observed for the six component mixture. However, neither of the fractions is capable of complete inhibition of State 3 electron transport (Table 1). The maximal extents of inhibition of State 3 respiration found for Fractions 1 and 2 were 27% and 65%, respectively. With uncoupled mitochondria, the relative magnitudes of the effects of the two fractions are reversed.* In these circumstances, the maximum inhibition of succinoxidase by Fraction 2 is again 60 to 65%, but, now Fraction 1 can completely inhibit succinoxidase at protein ratios of 3 to 5.

These experiments establish that the basic proteins of PMN lysosomes have a potent capacity to affect the respiratory activity of isolated liver mitochondria. In general the lysosomal cationic proteins act on mitochondria very much as do histones. The concentrations of lysosomal proteins which stimulated respiration in Figure 1 range from 2.4 to 7.1 x 10^{-7}M (assuming an average molecular weight of 8000); this range is of the magnitude at which Schwartz observed stimulation of respiration of liver mitochondria by histones from cardiac muscle and thymus (7). Johnson et al (12) found also that histone

*R. Penniall and H. I. Zeya, unpublished data.

Table 1

Inhibition of State 3 Respiration by the Lysosomal Cationic Protein Mixture and Subfractions of the Mixture

Conditions: Experiment 1 - 668 μmoles sucrose, 107 μmoles potassium phosphate, pH 7.4, 21 μmoles $MgCl_2$, 53 μmoles KCl, 10 μmoles substrate, 2 μmoles ADP, 3.5 mg mitochondrial protein (acceptor control ratio (succinate) = 5.4), 4.5 ml final volume, 30°; Experiments 2 and 5 - basic system as for experiment 1, except 5 μmoles ADP, 7.2 mg mitochondrial protein (acceptor control ratio (glutamate) = 7.1), 4.8 ml total volume, 30°; Experiments 3, 4, 6 and 7 - basic system as given in Figure 1, 1.8 mg and 0.9 mg mitochon drial protein (acceptor control ratio = 3) employed in experiments # 3, 6 and # 4, 7, respectively, 3.7 ml total volume, 30°.

Cationic Proteins	Experiment	Substrate	Protein Ratio	Per Cent Inhibition
Complete Mixture	1	Succinate	4	23%
			8	70%
		Pyruvate-Malate	4	27%
			8	68%
Fraction 1	2	Glutamate	8	27%
		Succinate	8	27%
	3	Succinate	18	*22%
	4	Succinate	18	*27%
Fraction 2	5	Succinate	10	53%
	6	Succinate	33	58%
			44	63%
	7	Succinate	22	*65%

*Denote experiments wherein additional cationic protein had no discernible effects.

f2a at protein ratios of 5 to 7, affords a stimulation of State 4 respiration of liver mitochondria, of about the magnitude we have observed in this work. However, while Schwartz and his collaborators found also that histone f2a (the most active histone) had no effect on State 3 respiration at concentrations stimulatory to State 4 respiration (12), we have found repeatedly that the action

of the lysosomal cationic proteins is facilitated in the presence of added ADP (Figure 1).

We believe the action of lysosomal cationic proteins on mitochondria is divisible into two parts: a. Effects of the proteins on mitochondrial membrane integrity; b. An ability, following the penetration of the proteins, to stop electron transport. The cationic protein mixture, as well as Fractions 1 and 2 as mentioned, each stimulate State 4 respiration of mitochondria. On the other hand, each of these entities is inhibitory in the presence of ADP. In addition, uncoupled mitochondria are far more swiftly affected than are intact mitochondria. These observations suggest that the integrity of one or both mitochondrial membranes (presumably the inner membrane) determines the nature and the time course of the effects of the cationic proteins on respiration. It suggests also that ADP may induce a conformational state of the mitochondrial inner membrane favorable to penetration of the cationic proteins. The stimulation of respiration which is demonstrable with both the large and small size components of the cationic protein mixture is an evanescent phenomenon; frequently we have found it impossible of demonstration, at appropriate protein ratios, with mitochondria seemingly as intact as other preparations with which the phenomenon can be seen. In this regard it should be noted that very decided effects of histones on mitochondrial membranes were reported by Schwartz et al at concentrations of histones which were also stimulatory to respiration and ATPase activity (13). We believe that our failure to detect stimulation of State 4 respiration by the cationic proteins with some mitochondrial preparations must indicate in those instances subtle changes in membrane integrity which are not reflected by alterations in acceptor control and P:O ratios.

The ability of the cationic proteins to completely inhibit mitochondrial electron transport is presently under study. Apparently, like other polycations (14) the lysosomal cationic proteins possess a potent capacity to inhibit rat liver cytochrome oxidase. Preliminary experiments indicate that, at concentrations approximately equivalent to the cytochrome a_3 content in the system,

the cationic protein mixture can completely inhibit a soluble rat liver cytochrome oxidase preparation*.

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