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STUDIES ON THE CYTOCHROME OXIDASE. IV. EFFECT OF SALTS ON THE CYTOCHROME OXIDASE

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INTRODUCTION

Although it was shown that cytochrome oxidase is widely distributed with cytochromes in cells of aerobic organisms and one of the most important oxidizing enzymes for intracellular respiration[1], we have no accurate knowledge about the nature of this enzyme, because its pure enzyme preparation has not yet been obtained.

In a previous paper[2], I examined the preparing method of the crude enzyme preparation and some conditions for the measuring of its activity. And it was found that this oxidase is extracted not by acidic buffer solution but by neutral or basic side of pH, and the optimum pH for the oxidase activity of this preparation in phosphate buffer solution is at pH 7.17.

In this paper, I propose to examine the effect of several salts on the cytochrome oxidase activity. I have not yet found a report descriptive of this problem in literature. I found that the presence of a neutral salt activated the oxygen consumption when hydroquinone is oxidized by this cytochrome oxidase preparation.

EXPERIMENTAL

1) *Preparation of enzyme and measuring method of its activity*

Throughout this study, I have made use of the following procedure. Heart muscle of ox cleaned from fat and ligaments is minced and pressed out blood as much as possible, and then washed five or six times in water with stirring it at the same

time. 50 g. of this pulp is mixed with 15 c.c. M/15 phosphate buffer solution of pH 6.81 and a little sand, and ground in a mortar. The thick paste thus obtained is mixed with 35 c.c. of the same buffer and centrifuged hard. Red-brown cloudy fluid is obtained. This fluid consists of a very finely divided suspension of muscle tissue and contains not only cytochrome oxidase but cytochrome c. Therefore, at the measurement of oxidase activity of this enzyme preparation, the addition of cytochrome c is unnecessary.

The oxidase activity of this preparation is determined by measuring the amount of oxygen used up when hydroquinone is oxidized in Warburg apparatus.

2) *Effect of* NaCl

As the used enzyme preparation is not pure, its activity is not constant according to the condition of extraction. Therefore, at the experiment the control test is carried out at the same time. The effect of NaCl on this enzyme system is shown in Table 1.

Table 1. Effect of NaCl on the cytochrome oxidase. (Enzyme preparation 1 cc., 0.27 M hydroquinone 0.2 c.c. and NaCl solution 1 c.c., at 37°C.)

Concentration of NaCl	0	M/2.4	0	M/7.3	M/11	0	M/22	M/44
O_2 uptake in 20 min. (c. mm.)	147.0	67.6	158.0	180.8	199.0	164.6	218.0	205.6
Relative activity	100.0	46.0	100.0	114.3	126.0	100.0	132.4	124.6

Concentration of NaCl	0	M/66	M/88	0	M/94	M/480
O_2 uptake in 20 min. (c. mm.)	206.0	235.5	233.0	218.0	231.0	220.0
Relative activity	100.0	114.0	112.6	100.0	106.0	101.0

From the results shown in Table 1, it is found that NaCl accelerates the oxidation of this enzyme system, and when the concentration of NaCl is M/22, the activity is maximum.

3) *Effect of* KCl

The properties of KCl resemble that of NaCl chemically. Therefore, I examined the effect of KCl next time, and found its effect also resembles that of NaCl as shown in Table 2.

Table 2. Effect of KCl on the cytochrome oxidase. (Enzyme preparation 1 c.c., 0.27 M. hydroquinone 0.2 c.c. and KCl solution 1 c.c., at 37°C.)

Concentration of KCl	0	M/22	M/44
O_2 uptake in 20 min. (c. mm.)	154.9	212.0	195.6
Relative activity	100.0	136.9	126.0

These data of Table 1 and Table 2 are results of experiments with the fresh enzyme preparation. The activation effect of these salts is the strongest when the oxidase preparation is fresh. If the oxidase preparation is stored in an ice chest, the activation ratio decreases with a decrease of oxidase activity. That is found from the result shown in Table 3 in contrast with Table 1.

Table 3. Effect of NaCl and KCl on the 5 days stored enzyme preparation. (Enzyme preparation 1 c.c., 0.27 M. hydroquinone 0.2 c.c. and salt solution 1 c.c., at 37°C.)

	NaCl			KCl		
Concentration of salt	0	M/22	M/44	0	M/22	M/44
O_2 uptake in 20 min. (c. mm.)	160.0	186.0	168.3	159.2	187.0	178.8
Relative activity	100.0	116.2	105.1	100.0	117.0	112.0

The activity given in Table 3 shows that of the same oxidase preparation with that in Table 1, after 5 days storage in an ice chest.

4) *Effect of* $MgCl_2$

Next time, the experiment on the effect of $MgCl_2$ which has the same anion with NaCl and KCl was carried out. The results are given in Table 4.

Table 4. Effect of $MgCl_2$ on the cytochrome oxidase. (Enzyme preparation 1 c.c., 0.27 M. hydroquinone 0.2 c.c. and salt solution 1 c.c., at 37°C.)

Concentration of $MgCl_2$	0	M/22	M/44	M/440	0	M/240	M/800
O_2 uptake in 20 min. (c. mm.)	167.2	28.5	71.2	189.5	200.0	209.0	210.0
Relative activity	100.0	17.0	42.6	113.0	100.0	104.5	105.0

The activation effect of $MgCl_2$ is different from that of NaCl or KCl, and has a maximum point at the concentration of about M/440 as shown in this table. At the concentration of M/22, at which NaCl or KCl has the strongest activation effect, $MgCl_2$ inhibits considerably the oxidase activity.

From these results, it is found that the activation effect of salts on the cytochrome oxidase depends upon not its anion but its cation.

Summary

1. The effect of NaCl, KCl and $MgCl_2$ on the crude cytochrome oxidase preparation was examined.

2. The presence of these salts activated the activity of the cytochrome oxidase system.

3. The activation effect is maximum at the concentration of M/22 for NaCl and KCl, while it is at the concentration of M/440 for $MgCl_2$.

4. This activation effect of salt depends upon not its anion but its cation.

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