THE SITES OF COBRA VENOM PHOSPHOLIPASE ACTION ON THE RESPIRATORY SYSTEM OF SUBMITOCHONDRIAL PARTICLES

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1. Introduction

The mechanism of phospholipase action on the respiratory system has been studied by a number of authors [1–8]. Among numerous other tasks connected with solution of this problem it seemed of fundamental importance to ascertain the sites of a multienzyme system sensitive to phospholipases. Nygaard [2] suggested that the succinate oxidase system loses its activity owing to lecitinase A splitting a certain factor situated between cytochromes *b* and *c*. Later Minakami et al. [6] showed that there were two labile sites in the NADH oxidase system, one of which directly precedes coenzyme Q, and the other is localized between cytochromes *b* and c_1.

The results of the present study are evidence that treatment of submitochondrial particles with cobra venom results primarily in disturbance of electron transfer at the cytochrome *c* level. The other respiratory chain sites mentioned above probably break down more slowly.

2. Methods

In this study we used ultrasonic submitochondrial particles isolated from the mitochondria of beef hearts after Beyer [9] *. The enzymic properties of the particles are described in our previous paper [11].

The source of phospholipase A was the venom of a Middle Asian cobra ** (Naja Oxiana Eich.) As was shown earlier by Edwards and Ball, cobra venom destroys the respiratory system exclusively as a result of the action of the phospholipase A it contains [3]. Indeed, the other enzymes contained in cobra venom can hardly cause inactivation of the respiratory system [12].

3. Results and discussion

Fig. 1 presents the results of a steady-state analysis of the succinate oxidase system of intact submitochondrial particles and of particles pretreated with cobra venom (55 percent inactivation). Comparison of these results shows that the action of phospholipase A substantially lowers the steady-state level of reduced cytochrome aa_3 and raises the steady-state levels of reduced cytochromes *b* and c_1 (+*c*). After oxygen was exhausted all the cytochromes were completely reduced by succinate. It was established earlier that the succinate dehydrogenase and cytochrome oxidase activities decrease insignificantly when phospholipase A acts on the respiratory system, whereas the succinate:cytochrome *c* reductase and succinate oxidase activities disappear completely [2, 3]. The above results give only very approximate information on phopholipase-sensitive succinate oxidase sites. Minakami et al. [6] discovered such a site between the cytochromes *b* and c_1. If this were the only site the action of phospholipase should have infallibly

* Alkaline particles isolated by the Crane method [10] were also used in the study.

** The cobra venom was obtained from the Institute of Zoology and parasitology of the Academy of Sciences of the UzSSR (Tashkent).

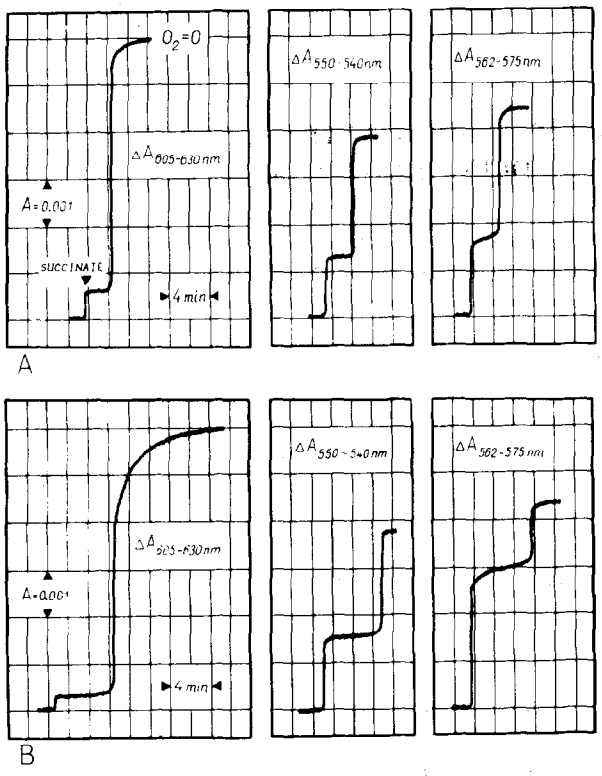


Fig. 1. Changes in steady-state levels of reduced cytochromes as a result of treating submitochondrial particles with cobra venom. The particles (0.6 mg protein per ml) were incubated at 20° with cobra venom (1.8 mg/ml) for 9 min in 80 mM potassium phosphate (pH 7.4). The action of the venom was interrupted by introducing EDTA (1 mM). The mixture was transferred to the cuvettes of a Hitachi 356 spectrometer, succinate (20 mM) was added and the reduction one of the cytochromes was recorded at 22° (A). As a result of treating the particles with cobra venom the succinate oxidase activity decreased 55 per cent. For comparison the data obtained for intact particles are also given (B).

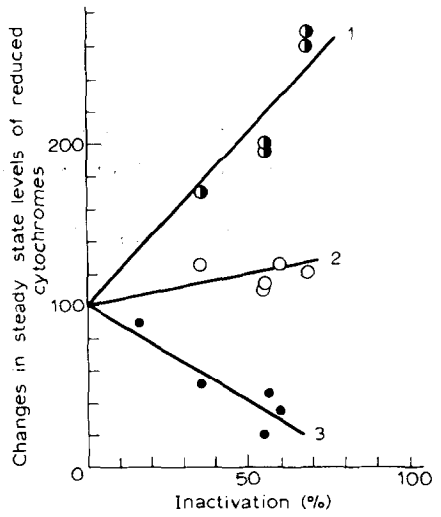


Fig. 2. Steady-state analysis of submitochondrial particles treated with cobra venom for various periods of time. Conditions as in fig. 1. The steady-state levels of reduced cytochromes in intact particles are taken as 100 percent. (1) cytochrome *b*; (2) cytochrome c_1 (+*c*); (3) cytochrome aa_3.

been accompanied by a decrease in the steady-state level of reduced cytochrome c_1 (+*c*). However, this was never observed in our experiments (fig. 2). The results obtained for different times of treatment of the particles with cobra venom form unambiguous evidence that electron transfer is disturbed primarily at the cytochrome *c* level, because only in this case the content of the reduced form of cytochrome c_1 (+*c*) increases or remains unchanged when the steady state level of reduced cytochrome aa_3 decreases sharply*. It follows from a comparison of the data for cytochromes *b* and c_1 (+*c*) that the interaction between them also becomes worse. It is possible, alternatively, that under the conditions indicated phospholipase disturbs electron transfer only between cytochromes c_1 and *c*, as a result of which cytochrome c_1 is reduced, while cytochrome *c* is oxidized. In this case the net steady-state level of their reduction may remain approximately constant if the submitochondrial particles contain equimolecular amounts of cytochromes c_1 and *c* [13]. However, in the light of Minakam's data this point of view seems rather speculative. To ascertain the site of phospholipase action more exactly it is necessary to record separately the steady-state levels of reduced cytochromes c_1 and *c*, which is difficult to accomplish experimentally.

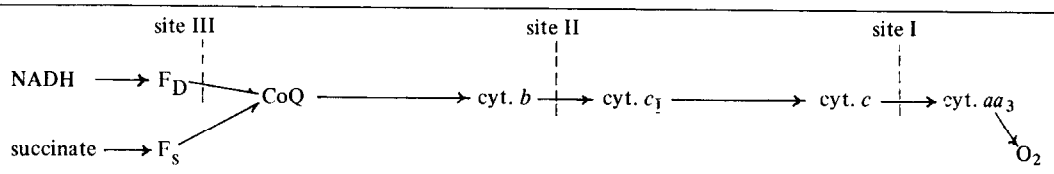
Summing up the results of previous investigations and of the present study it may be concluded that there are three sites of the respiratory system that are highly sensitive to cobra venom phospholipase (see scheme 1).

* The differences between our results and the data of Minakami et al. are probably due to the fact that those of the latter were obtained under conditions where the submitochondrial particles were subject to more drastic destruction.

Table 1
Effect of succinate on inactivation of succinate oxidase system in ultrasonic submitochondrial particles by cobra venom.

Conditions of incubation of particles	Succinate oxidase activity (atoms O/min mg protein)	Steady-state levels of reduced cytochromes (% of completely reduced by substrate)		
		b	$c_1(+c)$	aa_3
Without cobra venom	1.4	29 ± 1	27 ± 2	15 ± 1
With cobra venom	0.9	49 ± 3	34 ± 3	7 ± 1
In the presence of cobra venom and succinate	1.4	30 ± 1	27 ± 2	15 ± 2

Particles (1.5 mg protein per ml) were incubated with cobra venom (4.5 mg per ml) for 1.5 min at 20° in 80 mM potassium phosphate (pH 7.4). When necessary 20 mM of succinate was also added to the medium. After 1.5 min the action of the phospholipase was interrupted by adding 10 mM EDTA. The suspension was centrifuged for 15 min at 40,000 rpm (Spinco L-50, Ti50 rotor). The sediments were suspended in 80 mM potassium phosphate (pH 7.4) containing 10 mM EDTA. The suspension was diluted to a concentration of 0.5 mg protein per ml and the cytochromes' reduction at 22° in the presence of 20 mM succinate was recorded. Simultaneously the succinate oxidase activity of the particles was measured at 38°.



Scheme 1

The quickest to succumb to the action of phospholipase is the site between cytochromes c_1 and aa_3 (site I). The site preceding cytochrome *b* (site III) is comparatively stable. Indeed, it follows from the data of Minakami et al. that cytochrome *b* is reduced readily under conditions where reduction of all the other cytochromes is completely disturbed [6].

Several years ago we showed that succinate and NADH increased the stability of the respiratory chain (reconstituted or contained in submitochondrial particles) to cobra venom phospholipase in the presence of oxygen [14, 15]. This result suggested that the conformation of the respiratory chain changes under conditions favouring electron transfer. The present study gives some idea of the nature of these conformational changes. It follows from this study, in particular, that the action of phospholiphase on all three labile sites of the respiratory system is difficult under the conditions indicated. Below we give results confirming this point of view. It can be seen from the table that incubation of particles with cobra venom results in a decrease of succinate oxidase activity and simultaneously in a change in the steady state characteristics of the system. No such changes were observed when the particles were incubated aerobically in the presence of succinate. It is interesting that under the conditions indicated access of phospholipase to site I is difficult though it is not in the direct vicinity of the site of succinate binding. Taken in conjunction with other facts [14–16] this fact may mean that stabilization of the succinate oxidase system is connected with electron transfer.

Acknowledgements

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