THE SWELLING OF RAT LIVER MITOCHONDRIA INDUCED BY A THERMOSTABILE FACTOR IN CRUDE STAPHYLOCOCCAL α-TOXIN PREPARATIONS

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1. Introduction

Staphylococcal α-toxin is known for its potent hemolytic properties as well as for its general lethal effect. During recent years attention has been paid to the effect of crude and partially purified α-toxin on natural and artificial membranes [1–4]. It has been concluded that α-toxin may react with phospholipids by means of an interaction between a hydrophobic portion of the toxin molecule and the constituent membrane lipids [4, 5]. In the present communication, the swelling of rat liver mitochondria was studied using a crude α-toxin. The purpose of this study was to further elucidate the effect of α-toxin upon natural membranes. Doubts about the composition and properties of purified α-toxin still exist [6–8]. Thus, the results obtained with crude α-toxin may not apply in full to partially purified α-toxin.

2. Materials and methods

A crude batch of lyophylized Staphylococcal α-toxin (Institute of Sera and Vaccine, Prague) rich in α-toxin was used. The toxin was kept at −20°C in evacuated ampoules, each ampoule containing 4,500 hemolytic units (H.U.) of toxin. For immediate use, the content of ampoules was dissolved in 1 ml of distilled water and further diluted to 25 ml with saline. The LD_{50} of the product in rats (i.v.) was 80 H.U.

The heavy mitochondrial fraction of rat liver homogenate was isolated by the methods of de Duve et al. [9]. The mitochondria were used immediately after preparation. Swelling experiments were carried out in 1 X 3 cm tubes and optical density (OD) was measured at 520 nm. The incubation medium consisted of 137 mM KCl; 17 mM tris-HCl buffer pH 7.2 and 105 ± 9 μg of mitochondrial protein per ml. Protein was estimated according to the method of Lowry at al. [10].

3. Results and discussion

As reviewed by Lehninger [11], most of the swelling processes require respiration and are therefore denoted as "active". In addition, liver mitochondria undergo a slow but extensive swelling when incubated under aerobic conditions in isotonic saline media. This is designated "low energy" or irreversible swelling [12, 13]. It has been proposed that this swelling is caused by relaxation of a contractile mechanism regulating the mitochondrial size [14, 15]. Fig. 1 shows that the "low energy" type of mitochondrial swelling was greatly accelerated by addition of crude staphylococcal α-toxin to a suspension of mitochondria incubated at 37°C. The toxin at low concentartion induced swelling almost immediately and the extent depended on the toxin concentration.

The toxin induced swelling even in the presence of respiratory chain inhibitor KCN (fig. 2). Therefore swelling requiring respiration was not affected, at least, under our conditions of analysis. Both the rate and extent of swelling was considerably increased by Ca^{2+} while Mg^{2+} caused inhibition. The binding of Ca^{2+} ions onto the surface of the mitochondrial membrane may alter its electrical characteristics and thus infuence

Table
Effect of heat-denaturated and dialysed crude Staphylococcal α-toxin and plasma addition on the swelling of rat liver mitochondria.

System	Plasma (% ml)	OD_{520}
1. Mitochondria + native ST	–	0.145
2. Mitochondria + heated ST (60°C, 30 min)	–	0.153
3. Mitochondria + boiled ST (100°C, 5 min)	–	0.143
4. Mitochondria + native ST + plasma	5	0.058
5. Mitochondria + native ST + plasma extracted with chlorophorm-methanol (2:1)	5	0.132
6. Mitochondria + dialysed ST	–	0.142

The mitochondria were incubated at 20°C under conditions described in legend to fig. 1. The data were obtained as a difference in optical density at 520 nm between control and crude Staphylococcal α-toxin (ST) treated samples. 4.5 H.U. was used. ST was dialysed at 0°C for 6 hr against redistilled water.

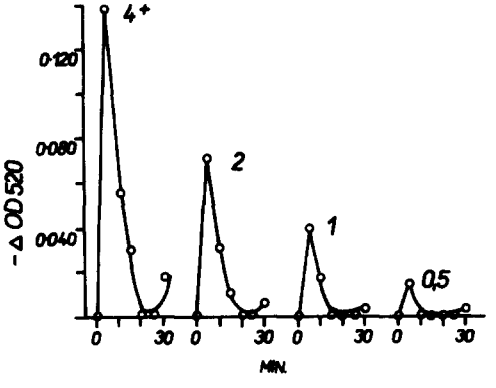

Fig. 1. Effect of various concentrations of crude Staphylococcal α-toxin on the mitochondrial swelling. Mitochondria (107 μg/ml) were added to a medium containing 134 mM KCl, 17 mM tris-HCl buffer pH 7.2 and Staphylococcal α-toxin. Tubes incubated at 37°C were read every 5 min at 520 nm. Each of the points represented differences in OD_{520} reading between control and Staphylococcal α-toxin treated samples assayed under the same conditions. (+ hemolytic units of Staphylococcal α-toxin per ml).

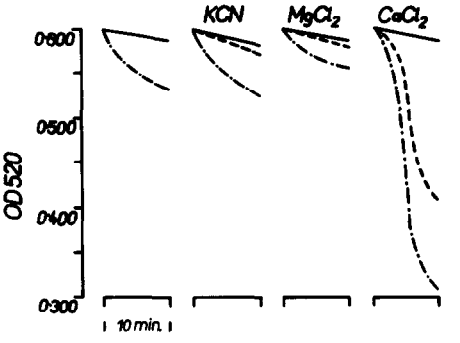

Fig. 2. Effect of KCN, Mg^{2+} and Ca^{2+} on the mitochondrial swelling induced by Staphylocccal α-toxin. Tubes incubated at 20°C were read every 30 sec for 10 min. KCN 2 mM, $MgCl_2$ 1 mM, CaCl 1 mM, Staphylococcal α-toxin 9.5. H.U. Symbols: —— control, - - - - ions, -.-.-.- crude Staphylococcal α-toxin. The more detailed assay conditions are described in legend to fig. 1.

the action of staphylotoxin. It is of interest that isolated mitochondria which have a negative charge similar to that of the cell [16] are very sensitive to basic proteins [17, 18]. Heating crude staphylococcal α-toxin resulted in a complete loss of toxicity (measured as LD_{50}) and hemolytic activity [19]. However, heated, boiled or dialysed toxin still induced swelling in mitochondria (table). The effect of crude staphylococcal α-toxin on mitochondrial swelling thus appears to be associated with a thermostabile factor as yet unknown. The fact that most of the biological properties of α-toxin are thermolabile supports the view that the toxin is an enzyme [20]. Since the effect of crude staphylococcal α-toxin on mitochondrial swelling did not show enzymatic kinetics, the mechanism remains obscure. The above evidence suggests, at least in part, that the swelling factor may exert a charge effect on the membrane surface. Thus the basic nature of the membrane may play an important role with respect to the biological properties of crude staphylcoccal α-toxin. At present, however, the nature of its effect is not clear and the mechanism of action has not been established. Further studies showed that addition of plasma reduced the swelling effect of native crude staphylococcal α-toxin. However, plasma extracted with chloroform-methanol (2:1) was without effect. The reducing effect of natural plasma is probably due to an unknown swelling factor binding to the plasma lipopro-

teins of mitochondrial membranes which could be the receptors for the toxin swelling factor. It is known at present that partially purified α-toxin has amphophilic properties [4]. On the other hand, phospholipids of mitochondrial membranes may be important in the control of mitochondrial swelling [21]. Recent results showed [22–24] that the complement thermostabile factor of crude staphylococcal α-toxin simultaneously accelerated respiration and swittched the flux of "useful energy" from respiration to phosphorylation. In addition, it has biphasic effect upon mitochondrial ATPase activity. These results will be published elsewhere. Further work with the isolated mitochondria will be required before the detailed mechanism of these results is fully understood.

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