TITRATION OF MITOCHONDRIAL ADENOSINETRIPHOSPHATASE WITH DESASPIDIN

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The uncoupling effect on oxidative phosphorylation of desaspidin and some related phlorobutyrophenone derivatives has been demonstrated earlier (Runeberg 1962). This report deals with the effect of these substances on mitochondrial adenosinetriphosphatase (ATPase).

Experimental: Mitochondria and solutions of the substances tested were prepared as described earlier (Runeberg 1962). When indicated, the mitochondria were "aged" by preincubation for 30 minutes at 37° (Potter et al. 1953) or by treatment with 0.1 per cent desoxycholate (Siekewitz et al. 1958). ATPase was assayed at 30° in 2 ml of media containing 50 µmoles tris buffer, pH 7.5, 500 µmoles sucrose, ATP and $MgCl_2$ as indicated. Inorganic phosphate (P_i) was determined according to the method of Ernster et al. (1953).

Results: Desaspidin stimulates the latent mitochondrial ATPase and gives maximal activity when present in amounts of about 0.7 µmoles per g mitochondrial protein. Higher concentrations are inhibitory to the reaction. The optimal concentration of desaspidin is directly proportional to the amount of mitochondria present in the reaction mixture (Fig. 1).

ATPase activation curves of similar shape were obtained with different concentrations of the following phlorobutyro-

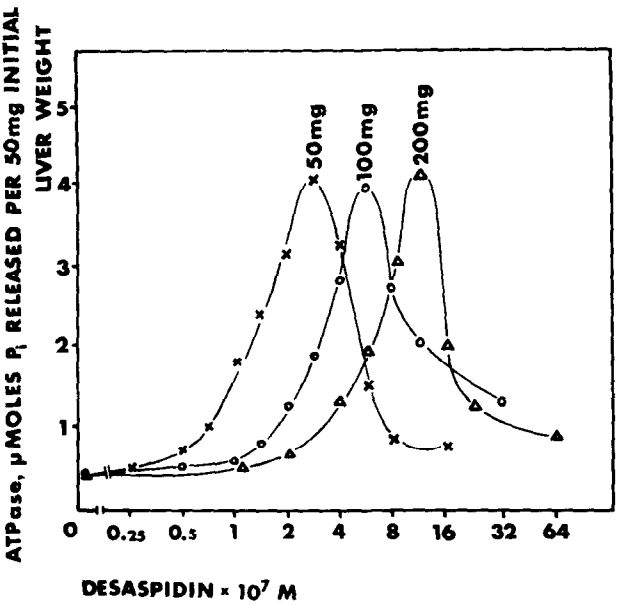

Fig. 1. TITRATION OF MITOCHONDRIA WITH DESASPIDIN

Mitochondria from 50, 100 or 200 mg rat liver were incubated in the medium described, containing 40 μmoles ATP. The ordinate gives the ATPase activity as μmoles P_i released per 50 mg liver in 15 minutes.

phenone derivatives, listed in order of decreasing activity: ortho-desaspidin, aspidin, para-aspidin, iso-aspidin, flavaspidic acid, nor-flavaspidic acid and desaspidinol (phlorobutyrophenone-4-methyl ether). Flavaspidic acid is active at concentrations about 25 times higher than desaspidin. Phloroglucinol lacks effect in concentrations up to 10^{-3} M.

With the initially maximally stimulating concentration of desaspidin the activity closely approaches that obtained with 2,4,-dinitrophenol (DNP). With this concentration of desaspidin, the activity slows down during the latter part of the 15-minute reaction time usually employed (Table 1).

Addition of Mg^{++} to the medium does not affect the desaspidin-stimulated ATPase, nor does desaspidin cause mitochondrial swelling. The effects of desaspidin observed are thus not associated with structural damage to the mitochondria.

Table 1. TIME COURSE OF DESASPIDIN- AND DNP-STIMULATED ATPases

Mitochondria from 50 mg liver were used in the medium described, containing 10 µmoles ATP.

ATPase-stimulating agent:	µmoles P_i released after 6 min.:	µmoles P_i released after 15 min.:
none	0.1	0.3
2×10^{-7} M desaspidin	1.90	4.60
2.8×10^{-7} M desaspidin	2.25	4.30
10^{-4} M DNP	2.30	5.60

The effect of desaspidin closely resembles that of DNP, and like DNP-stimulated ATPase (Siekewitz et al. 1958), desaspidin-stimulated ATPase is also inhibited by methylene blue and arsenate. Atebrin exerts the same biphasic effect on both ATPases (Löw 1958), causing further stimulation of the maximal activity at concentrations of 0.25 to 1.0 mM and inhibition at higher concentrations.

It was further interesting to note that at concentrations higher than those stimulating latent ATPase, desaspidin strongly inhibits DNP-stimulated ATPase, while the Mg^{++}-stimulated ATPase of aged mitochondria is not affected (Fig. 2).

When tested together with suboptimal concentrations of DNP desaspidin in suboptimal concentrations acts synergistically with this substance. Optimal concentrations of both yields an ATPase activity equal to that obtained with desaspidin alone, i.e. about 80 per cent of the maximal DNP-stimulated activity when a reaction time of 15 minutes is employed (Fig. 3).

It was not possible to reverse the inhibitory effect of desaspidin on DNP-stimulated ATPase by repeated centrifugations and resuspensions of mitochondria in fresh media.

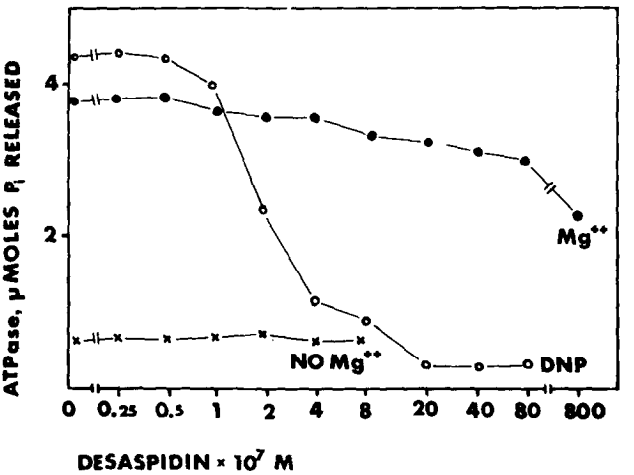

Fig. 2. THE EFFECT OF DESASPIDIN ON DNP- AND Mg^{++}-STIMULATED ATPases

Experimental conditions as in Table 1. ATPase was stimulated in fresh mitochondria with 10^{-4} M DNP and in aged mitochondria with 4 x 10^{-3} M $MgCl_2$. Aged mitochondria were also incubated with no addition of $MgCl_2$.

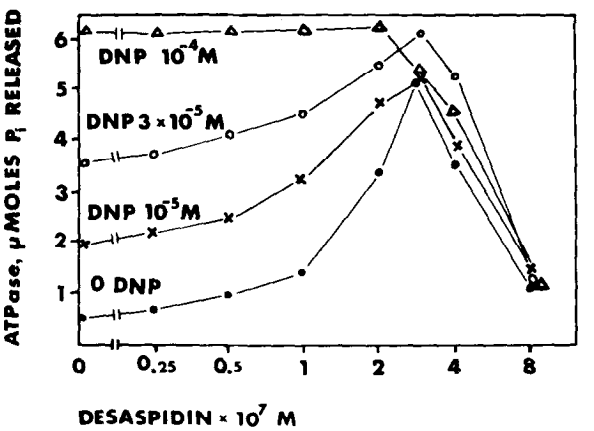

Fig. 3. THE EFFECT OF DESASPIDIN COMBINED WITH DNP

Experimental conditions as in Fig. 2.

Discussion: The fact that the ATPase of aged mitochondria is not affected by desaspidin in concentrations inhibitory to DNP-stimulated ATPase suggests that the inhibitory effect is

exerted at the same, or at least at a closely related point as the stimulating effect. The fact that all the phlorobutyrophenone derivatives tested show ATPase activation curves of similar shape although the concentrations needed vary over a fairly wide range also speaks for a single point of action. It seems as if desaspidin at lower concentrations causes aberrant hydrolysis of a non-phosphate high-energy intermediate of oxidative phosphorylation, while it at higher concentrations and/or with prolonged treatment may be visualized to combine with the enzyme in the same way as has been suggested for DNP (Chance et al. 1963), causing stabilization of the intermediate involved. In relation to its uncoupling effect desaspidin seems to have a stronger tendency to form such a complex than DNP. This also provides an explanation for the respiration-inhibiting effect of desaspidin (Runeberg 1962).

Like some other recently reported inhibitors of mitochondrial enzymes as oligomycin (Huijing and Slater 1961, Ernster et al. 1963), antimycin A and rotenone (Ernster et al. 1963), desaspidin shows a titration effect. The sites of action of these substances are different and if it is assumed that the substances are mainly bound to their respective sites of action it can be calculated that the desaspidin-sensitive site is present in an amount which is about 3 times greater than the oligomycin sensitive site at which P_i enters into the reaction chain.

More detailed experimental results will be published elsewhere.

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