THE SITE OF OLIGOMYCIN ACTION IN CORN MITOCHONDRIA[1]

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Oligomycin is widely believed to inhibit oxidative phosphorylation and associated respiration by blocking the reaction of phosphate with the non-phosphorylated intermediate (Slater, 1963; Wadkins and Lehninger, 1963; Bruni et al, 1964; Racker, 1965). Lardy et al (1964), however, indicate that oligomycin prevents phosphoryl transfer to acceptor; that is, a reaction subsequent to formation of X~P preventing the reversible formation of ATP.

Experiments with corn mitochondria in our laboratory strongly suggest that Lardy's view is correct. Substrate-powered contraction is strongly inhibited by phosphate, and the inhibition is unaffected by oligomycin or Mg^{++} (Stoner and Hanson, 1966). The phosphate inhibition can be partially relieved with ADP + hexokinase trap (Stoner and Hanson, 1966) or completely relieved by 1 mM Ca^{++} (Truelove and Hanson, 1966). The Ca relief is associated with Ca + Pi uptake and increased respiration. Our interpretation is that contraction is associated with formation of I~X. Phosphate depletes I~X in production of X~P, lowering contraction. ADP and Ca discharge ~P in ATP formation and Ca + Pi uptake, respectively, recycling X for further respiration and associated contraction. Since, as with other mitochondria, oligomycin blocks ATP formation and utilization (Stoner and Hanson, 1966) but not Ca + Pi transport (Hodges and Hanson, 1965), we concluded that the inhibition lies between X~P and ATP, and does not affect the Ca-activated discharge of ~P in transport.

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Sanadi (1965) points out that the critical evidence for the view that oligomycin blocks formation of the phosphorylated intermediate consists of observations that arsenate does not relieve oligomycin-inhibited respiration and that oligomycin inhibits arsenate-stimulated ATPase (Estabrook, 1961; Huijing and Slater, 1961).

In recent work we have used arsenate and oligomycin in studying the respiration of corn mitochondria. Typical results with the oxygen electrode are given in Figure 1. The following points seem important:

1 - Corn mitochondria exhibit reasonable respiratory control but have high State 4 rates indicating that they are "loose coupled". We believe the loose coupling is related to the rapid spontaneous swelling; that is, spontaneous hy-

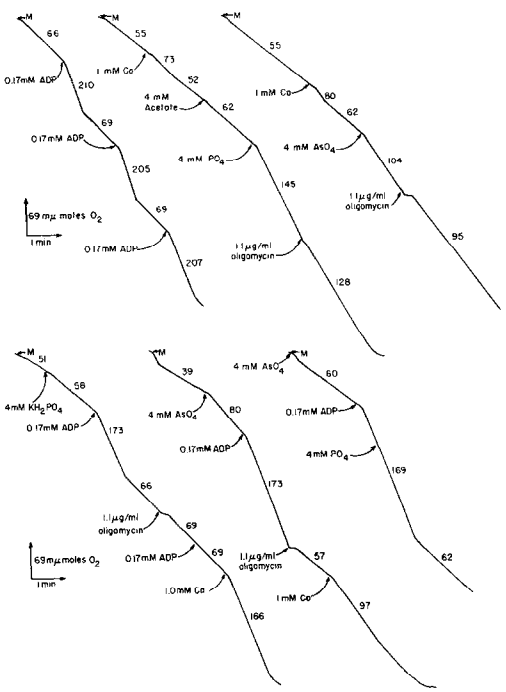

Figure 1. Oxygen consumption by isolated corn mitochondria as affected by oligomycin and arsenate. Tracings of recorder chart made with the Clark oxygen electrode. Mitochondria were isolated from 3-day etiolated corn shoots essentially as previously described (Truelove and Hanson, 1966). Final reaction volume was 2.6 ml containing 0.25 M sucrose, 20 mM Tris (pH 7.2), 1 mg/ml bovine serum albumin, 10 mM succinate, 10 mM pyruvate, 0.23 mM NAD, 0.17 mM TPP, 0.04 mM CoA, and 0.5 mM $MgSO_4$. Reaction was initiated by adding mitochondria at M (0.18 mg N). Values for rates are mμ moles O_2/min. Additions were in 10 μl volumes from stock solutions adjusted to pH 7.2 with Tris.

drolysis of a non-phosphorylated intermediate (Stoner and Hanson, 1966).

2 - Calcium added to an acceptorless system produces a small and transient rise in respiration. (With 0.1 to 0.3 mM Ca a rise in respiration cannot be reliably detected). Addition of acetate has no significant effect. Upon addition of Pi, however, there is a marked rise in respiration. Associated with this respiratory increase is rapid Ca + Pi accumulation (Kenefick and Hanson, 1966). Oligomycin does not stop either the respiration (Figure 1) or the Ca + Pi transport (Hodges and Hanson, 1965; Truelove and Hanson, 1966).

3 - Addition of arsenate to a system containing Ca also increases the respiration rate, although the increase is not so dramatic as with phosphate. Again oligomycin has little effect. Despite the respiratory increase, arsenate does not potentiate Ca uptake (Hodges and Hanson, 1965; Truelove and Hanson, 1966). In recent experiments we were able to detect a small Ca^{45} uptake in the presence of arsenate and oligomycin (Kenefick and Hanson, 1966), but in many previous experiments we failed. With the conditions used here the Ca + arsenate stimulated respiration must be viewed as largely "uncoupled" from transport in contrast to the "coupled" mechanism with Ca + Pi.

4 - The addition of Pi or arsenate to an acceptorless system slightly stimulates respiration, arsenate being more effective. As with other mitochondria, subsequent addition of ADP yields a burst of respiration which terminates in State 4 with phosphate, but which does not terminate with arsenate. If Pi is added to an arsenate containing medium respiratory control is restored, a reflection of the competitive effect of Pi (Ter Welle and Slater, 1964). Addition of oligomycin blocks the arsenate uncoupling as reported by Estabrook (1961) and by Huijing and Slater (1961). However, subsequent addition of Ca accelerates respiration to State 3 rates with phosphate, less so with arsenate.

It is evident that there is a parallel here between the action of Ca and of ADP. In a sense both are components of an "acceptor" system, with ADP taking ~P to ATP formation and Ca diverting ~P to Ca + Pi accumulation, releasing respiration The action of arsenate can be accounted for by assuming with Lardy et al. (1964)

that ~As is unstable, probably on leaving the hydrophobic membrane phase for addition to ADP, or on attempted transport to the aqueous matrix. Both ATP formation and transport are thus uncoupled due to instability of ~As in an aqueous phase.

If this view is correct (see Stoner et al. 1964, for schematic representation) then oligomycin cannot act by blocking X~P formation for it should then block Ca + Pi transport and the phosphate (or arsenate) inhibition of contraction. As pointed out previously (Hanson et al. 1965), there is no compelling evidence with corn mitochondria that Ca is transported at the expense of X~I, accompanied by a passively permeating anion. It is more likely that transport results from a reaction of Ca with X~P $\overline{/}$ Ca + MgX~P $\longrightarrow$ Mg + CaX~P $\longrightarrow$ X + (Ca + Pi) $\overline{/}$. It is assumed here that Chance's (1965) basic concept of X~ binding cations is correct, and that in corn mitochondria where the endogenous Mg is high (Hodges and Hanson, 1965) that Mg will ordinarily dominate. Oligomycin would not interfere strongly with this Ca-activated reaction but would with ATP formation (MgX~P + ADP $\rightleftharpoons$ Mg + X + ATP).

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