THE ACTION OF OLIGOMYCIN AND OF PARA-HYDROXYMERCURIBENZOATE ON MITOCHONDRIAL RESPIRATION STIMULATED BY ADP, ARSENATE AND CALCIUM

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Received May 31, 1966

Thiols are involved in some substrate-level oxidative phosphorylation mechanisms. It is also possible that SH groups might play a role in electron transport phosphorylations. Recently Boyer (1965) proposed an acyl-S compound as a possible intermediate. Several compounds which react preferentially with SH groups were found to interfere with mitochondrial oxidative phosphorylation or its partial reactions. Para-hydroxymercuribenzoate (PMB) which is considered to be a relatively specific SH reagent, uncouples oxidative phosphorylation (Lehninger, 1951; Cooper and Lehninger, 1956), abolishes $^{32}P_i$ - ATP exchange (Cooper and Lehninger, 1957), inhibits the dinitrophenol-stimulated ATPase, activates the latent ATPase of mitochondria (Kielley, 1963) and decreases the exchange of oxygen between water and inorganic phosphate (Boyer, 1965). All these facts indicate that PMB-reactive groups are involved in the trapping of energy during oxidation.

The present study was undertaken in order to localize the site of action of the mercurial in the energy transfer sequence and find the step where thiols might be involved.

Experimental

Rat liver mitochondria isolated according to the procedure of Weinbach (1961) were used. Respiration was measured by the vibrating

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platinum electrode (Gilson Oxygraph) at 24°. The reaction medium contained 100 mM sucrose, 21 mM KCl, 5 mM $MgCl_2$ and was buffered by either 5 mM K-phosphate or 25 mM tris-Cl or by both: the pH was 7.40. Respiratory substrates were 5 mM L-glutamate with or without 5 mM L-malate, 5 mM succinate with or without 5 µM rotenone or 10 mM DL-beta-hydroxybutyrate. The mitochondrial concentration during the experiment was 1.3 to 2.8 mg protein/ml (determined by the biuret method).

Results

PMB, in a narrow concentration range, inhibited the respiratory response of mitochondria to ADP (Fig. 1) or AMP. The effect was similar whether the mitochondria were preincubated in the reaction medium with PMB before the addition of ADP or whether the PMB was added after a prior ADP addition. In the presence of

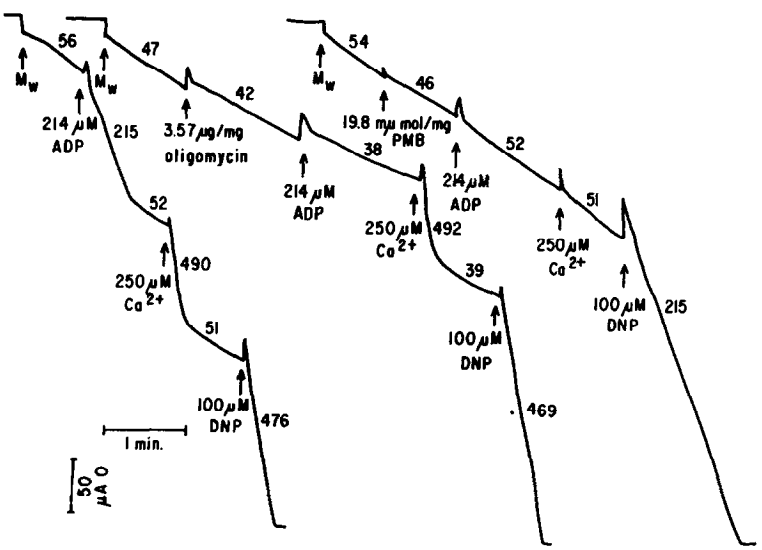

Figure 1

Effect of oligomycin and of PMB on respiration stimulated by ADP and by Calcium.

Succinate (5 mM) as substrate in the presence of rotenone. Mitochondrial protein: 1.45 mg/ml. Respiratory rates (above the tracings) are expressed as m µg atom oxygen/ml/min.

high concentration of ADP (about 1 mM) when the State 3 respiratory rate was sustained until the dissolved oxygen was expended, the time course of the PMB inhibition could be observed (Fig. 2). In the first few seconds after PMB addition the respiration was still maintained at a high level and it decreased only after a lag period of 20 to 30 seconds. In PMB treated mitochondria the respiration was activated again by 100 μM 2,4-dinitrophenol or by 25 μM dicoumarol. It should be mentioned that the uncoupled respiratory rate was also affected by PMB, due to the known effects of the mercurial on individual respiratory enzymes.

The effective concentration of PMB varied somewhat in different mitochondrial preparations. Usually under 8 m μmol/mg protein PMB exerted very slight effect; 15 to 33 m μmol/mg prevented the ADP effect on respiration almost completely while the uncoupled respiration was inhibited about 30 to 50 per cent.

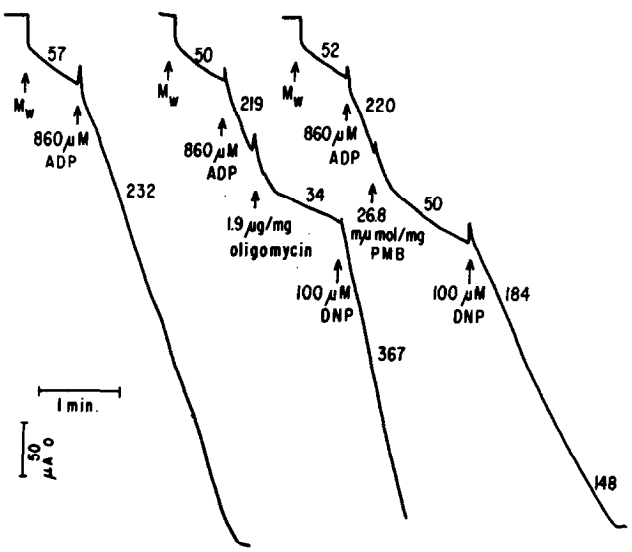

Figure 2

Effect of oligomycin and of PMB added during the course of ADP stimulated respiration

Conditions as in Figure 1. Mitochondrial protein 1.86 mg/ml.

The effect of PMB was similar when glutamate with or without malate, succinate in the presence or in the absence of rotenone or beta-hydroxybutyrate served as substrates for respiration.

From these experiments it was not yet clear whether PMB inhibited the reaction of mitochondria with ADP or interfered at an earlier level with the energy transfer reactions. For this reason the effect of PMB on arsenate stimulated respiration was studied. Arsenate probably uncouples oxidative phosphorylation by substituting for phosphate and forming an unstable arsenylated intermediate (Crane and Lipman, 1953). This is supported by the fact that the arsenate stimulation of respiration is pronounced only in phosphate-free media. As has been reported by Estabrook (1961), it was necessary to pretreat the mitochondria with a small amount of ADP (70 to 210 μM) in order to get a maximal arsenate stimulation. Under these circumstances 1 mM arsenate increased respiration 3 to 5 fold: the rate was the same or slightly less than the ADP stimulated one. PMB prevented the arsenate stimulation almost completely (Fig. 3) and inhibited it after a lag phase when applied during the arsenate stimulation. Arsenate alone, without ADP caused approximately 2 fold increase in respiration: this increase was also inhibited by PMB. As in the ADP experiments reported above, dinitrophenol was again effective in increasing the respiratory rate.

Ca^{2+} in small amounts (100 to 300 M) causes a rapid respiration by "draining" the high-energy intermediates prior to the site of the oligomycin effect (Chance, 1965). This is based on the fact that oligomycin blocks completely the ADP or the arsenate stimulation of respiration but has no effect on the Ca^{2+} "burst". PMB also prevents Ca^{2+}-stimulated respiration (Fig.1).

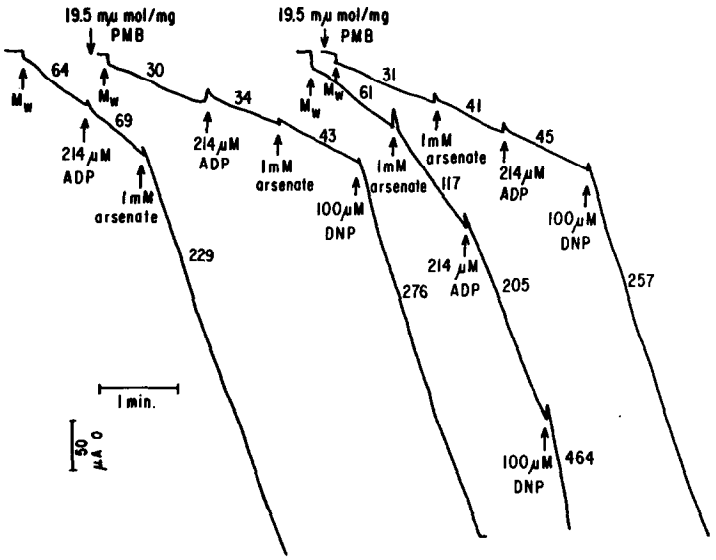

Figure 3

Effect of PMB on respiration stimulated by arsenate.

Succinate as substrate in the presence of rotenone. Phosphate-free medium. Mitochondrial protein: 2.56 mg/ml.

Discussion

PMB resembles, in some respects, the energy transfer inhibitors like oligomycin, aurovertin, guanidine and some guanidine derivatives. It inhibits the respiratory response to ADP or arsenate and the respiratory inhibition can be partially overcome by uncouplers like dinitrophenol or dicoumarol. There is one important difference between the action of PMB and of oligomycin: the latter does not inhibit the Ca^{2+}-induced respiratory jump, whereas PMB does. It seems therefore likely that the site of PMB interaction with the energy coupling reactions is between the dinitrophenol (and/or dicoumarol) and the oligomycin sensitive sites. In agreement with Lardy et al (1964), we have to postulate at least one reaction between the dinitrophenol sensitive step and the entry of phosphate (or arsenate) and it is this reaction which is probably inhibited by PMB. At this step there may be a thiol intermediate.

The experiments reported are consistent with the idea that a thiol intermediate is formed during electron-transport phosphorylation but do not prove it. As Boyer and Schulz (1959) have pointed out, sensitivity of a particular reaction to mercurials, may indicate thiol involvement in the catalytic mechanism, but there might be another interpretation. Mercurial sensitivity can be the result of change in protein structure or of simple steric hindrance of the reaction. Nevertheless, the mercurial sensitivity of one particular reaction in oxidative phosphorylation direct the search for the thiol intermediate toward this site.

The devoted technical assistance of Mrs. Laura Watkins is gratefully acknowledged.

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