PHOSPHATE TRANSPORT IN MITOCHONDRIA ACTION OF MERSALYL ON THE BINDING AND TRANSPORT OF INORGANIC PHOSPHATE

S. PAPA, D. KANDUC and N.E. LOFRUMENTO
Istituto di Chimica Biologica, Università di Bari e Centro di Studio sui Mitocondri e Metabolismo Energetico, Bari, Italy

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1. Introduction

Inorganic phosphate moves across the inner mitochondrial membrane either by exchange-diffusion with hydroxide ions [1, 2] this is equivalent to a phosphoric acid uniport [3] – or by exchange-diffusion with dicarboxylates [1, 2]. Both reactions are inhibited by mersalyl [4–7]. It is unknown whether the two reactions are mediated by two separate systems or if different functional groups of the same system are involved cf. ref. [7].

It is shown in this paper that mersalyl, in the same concentration range at which it inhibits P_i transport, stimulates P_i uptake by rat-liver mitochondria when added after net P_i transport is completed. This finding suggests that mersalyl inhibits the transport of P_i in mitochondria by fixing the anion on binding site(s) in the membrane.

2. Methods

2.1. *Titration of the action of mersalyl on the $^{32}P_i/P_i$ exchange*

After 30 sec preincubation at 10°C in 150 mM sucrose, 20 mM Tris–HCl (pH 7.4), 1 mM $MgCl_2$, 0.5 mM EDTA and 3 μg/ml rotenone, 0.5 μg/ml antimycin A and 15 μg/ml oligomycin to prevent esterification of P_i, mitochondria were incubated 2 min with 1mM $^{32}P_i$. $^{32}P_i$-loaded mitochondria (5 mg protein/ml) were rapidly centrifuged, as described by Pfaff [8; see also ref. 5], through a reaction medium containing unlabelled P_i.

2.2. *$^{32}P_i$ uptake by P_i-depleted mitochondria*

Mitochondria were depleted of endogenous P_i by aerobic preincubation for 10 min at 27°C in 200 mM sucrose, 50 mM Tris–HCl (pH 7.4), 15 mM KCl, 0.5 mM EDTA, 20 mM glucose, 5 mM β-hydroxybutyrate, 0.2 mM ADP and 3 units hexokinase (Sigma, Type C-300). Mitochondria were then washed twice with 250 mM sucrose. P_i was assayed according to Wahler and Wollenberger [9]. P_i-depleted mitochondria (1 mg protein/ml) were incubated with $^{32}P_i$ at 10°C in 150 mM KCl, 20 mM Tris–HCl (pH 7.4), 1 μg/ml rotenone, 0.2 μg/ml antimycin and 5 μg/ml oligomycin and separated from this medium by rapid centrifugation at 4°C. $^{32}P_i$ uptake was calculated by correcting the amount in the mitochondrial extract with that in the sucrose space [see ref. 5].

3. Results

Fig. 1 shows a titration of the action of mersalyl on the exchange-diffusion between mitochondrial $^{32}P_i$ and external unlabelled P_i. This was followed by exposing for 15 sec $^{32}P_i$-loaded mitochondria to a second incubation medium containing unlabelled P_i. Mersalyl was added to $^{32}P_i$-loaded mitochondria at micromolar concentrations 2 min before the exchange started. Up to 7.5 nmoles/mg protein, mersalyl had practically no effect on phosphate translocation [cf. ref. 10]. Above this level mersalyl inhibited the $^{32}P_i/P_i$ exchange; maximal inhibition was reached at about 17 nmoles/mg protein [cf. ref. 7].

The experiments of fig. 2 refer to P_i-depleted mito-

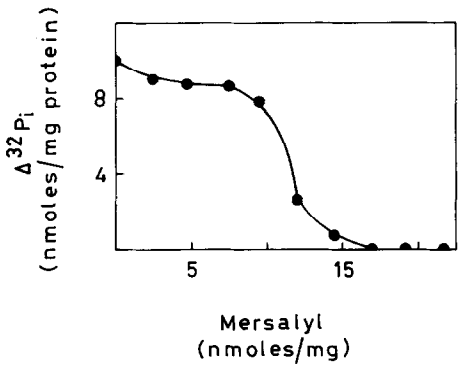

Fig. 1. Titration of the effect of mersalyl on the $^{32}P_i/P_i$-exchange. Mitochondria loaded with $^{32}P_i$ (20 nmoles $^{32}P_i$/mg protein) were centrifuged through a second incubation layer containing the same components of the preincubation mixture and 2 mM unlabelled P_i.

chondria. The depletion reduced the content of P_i from 20–25 to 2–5 nmoles/mg protein. In expt. a (fig. 2) P_i depleted mitochondria were incubated 5 min in the reaction mixture, mersalyl was then added, followed 2 min later by 500 μM $^{32}P_i$. Up to a level of 8 nmoles/mg protein mersalyl had practically no effect on the uptake of $^{32}P_i$ by mitochondria; above this level the mercurial inhibited P_i uptake, maximal inhibition being reached at 16 nmoles/mg protein. In expt. b (fig. 2) P_i-depleted mitochondria were incubated with 500 μM $^{32}P_i$. Separate controls showed that the uptake of $^{32}P_i$ was already completed in 2 min; chemical analysis showed that after this interval there was no further change in the intra- and extramitochondrial content of P_i. After 5 min incubation of mitochondria with $^{32}P_i$, mersalyl was added 2 min before separation of the mitochondria from the medium. Under these conditions mersalyl, in the same concentration range at which inhibited P_i translocation, caused a marked stimulation of $^{32}P_i$ uptake. This stimulation reached a maximum of about 3 nmoles/mg protein at a level of 17 nmoles mersalyl/mg protein (in various experiments this level ranged between 13 and 19 nmoles/mg protein) and partially disappeared as the concentration of mersalyl was further increased. It sould be noted that this stimulatory effect of mersalyl observed in a KCl medium, was much less evident in a sucrose medium.

The experiment of fig. 3 shows that the stimulation of $^{32}P_i$ uptake caused by mersalyl, was practically completed in 2 min. The addition of *n*-butylmalonate,

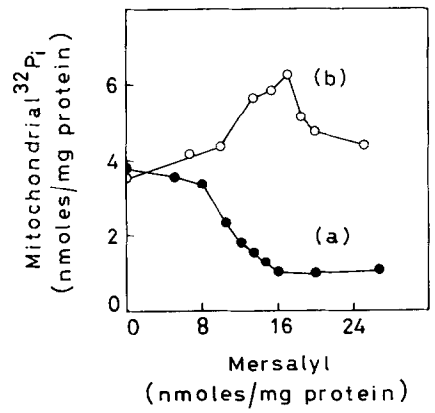

Fig. 2. Effect of mersalyl on $^{32}P_i$ uptake by P_i-depleted mitochondria. Expt. a) P_i-depleted mitochondria were preincubated 5 min in the reaction medium; mersalyl was then added, followed 2 min later by 500 μM $^{32}P_i$. After 5 min, mitochondria were centrifuged from the medium; Expt. b) P_i-depleted mitochondria were incubated 5 min in the presence of 500 μM $^{32}P_i$, then mersalyl was added and allowed to react 2 min before centrifugation of mitochondria.

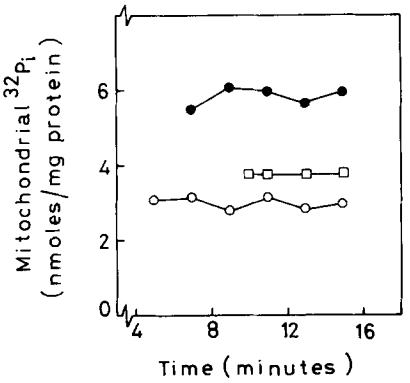

Fig. 3. Time course of the effect of mersalyl and *n*-butylmalonate on $^{32}P_i$ uptake by P_i-depleted mitochondria. (○–○–○) P_i-depleted mitochondria incubated 5–15 min in the presence of 500 μM $^{32}P_i$; (●–●–●) after 5 min incubation with $^{32}P_i$, 17.7 nmoles mersalyl/mg protein were added and allowed to react 2–10 min; (□–□–□) after 5 min incubation with $^{32}P_i$ and 4 min with 17.7 nmoles mersalyl/mg protein, 2 mM *n*-butylmalonate was added and allowed to react for 1–7 min.

4 min after mersalyl, caused in 1 min an almost complete release of the extra $^{32}P_i$ taken up by mitochondria under the influence of mersalyl.

4. Discussion

Mersalyl inhibits P_i transport either if added before or after P_i (figs. 1 and 2a) [cf. ref. 11]. On the other hand when mersalyl is added to P_i-depleted mitochondria after $^{32}P_i$, under conditions at which $^{32}P_i/P_i$ exchange and any net transport of P_i are completed, the mercurial induces an extra uptake of $^{32}P_i$ by mitochondria. This stimulation of $^{32}P_i$ uptake cannot simply be due to a selectivity of the inhibition by mersalyl of the P_i/OH^- and P_i/P_i exchange, in fact the stimulation is maximal at a level of mersalyl which gives complete inhibition of the $^{32}P_i/P_i$ exchange-diffusion. This and the finding that the extra $^{32}P_i$ taken up by mitochondria under the influence of mersalyl is almost completely released upon the addition of *n*-butylmalonate suggest that what is promoted by mersalyl is the binding of P_i to the mitochondrial membrane [see also refs. 12 and 13].

The titer of the stimulatory effect of mersalyl on the uptake of $^{32}P_i$ suggests that it is directly involved in the inhibition of P_i transport by the mercurial. This could be explained on the basis of a mobile P_i carrier with functional groups moving from one side to the other of the membrane depending upon the relative concentration of P_i in the two aqueous phases and the direction of the flux [11]. When mersalyl is added to mitochondria suspended in a P_i-free medium, the inhibitor reacts with the carrier orientated towards the inner side of the membrane, when added to mitochondria equilibrated with added P_i mersalyl finds the functional groups of the carrier distributed between the inner and the outer position. It is proposed that blockage of SH groups by mersalyl produces a conformational change of the carrier with two consequences: immobilization and enhanced binding capacity of the carrier. The transport of P_i will be inhibited regardless of the position in which the carrier is immobilized, but only when the carrier is orientated towards the outer side and in equilibrium with $^{32}P_i$ will the enhanced binding capacity become apparent.

The effect of mersalyl described could, alternatively, be explained by supposing that mersalyl stimulates the binding of P_i to unspecific anion binding sites. These sites will compete with the specific carrier-sites for P_i-binding with consequent inhibition of P_i-transport. The fact that the stimulatory effect of mersalyl on the uptake of P_i is in large part reversed by concentrations of *n*-butylmalonate which selectively inhibit the P_i–dicarboxylate exchange, would, however, favour the possibility that the effect of the mercurial takes place principally at the level of the phosphate translocator. The specificity as well as the biphasicity (see fig. 2b) of the effect of mersalyl are under further investigation.

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