PHOSPHATE CARRIER OF LIVER MITOCHONDRIA: TWO EQUIVALENT SH-GROUPS IN THE CARRIER UNIT

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SUMMARY Phosphate carrier activity of mitochondria that had become swollen during the aerobic accumulation of calcium acetate was measured indirectly by monitoring the turbidity changes. The exchange between extramitochondrial phosphate and intramitochondrial acetate was inhibited by SH-group binding reagents. Part of the SH-groups of the carrier could be blocked by mersalyl without loss of activity whereas liberation of the same groups restored carrier activity when all the other SH-groups were irreversibly blocked. A symmetrical structure of the carrier is proposed with two equivalent SH-groups; each of them is able in itself to maintain carrier function.

Most of the information available on the mitochondrial phosphate carrier is due to its sensitivity to various SH-group reagents. Organic mercurial compounds inhibit the carrier at those low levels that do not affect other mitochondrial functions (1-5) and inhibition is complete on addition of 8-10 nmoles mersalyl per mg mitochondrial protein (4-6, cf. however 7). It was found earlier that no inhibition of the carrier occured until a considerable fraction of the inhibitory amount of the mercurial was added (5-7). This behaviour may reflect an intrinsic, fundamental property of the carrier. Similar result could however be obtained if some mitochondrial component with a high affinity for SH-group reagents had bound the mercurial before the carrier reacted with the latter; another explanation would be that in the assay systems employed carrier activity was in excess to the actual phosphate transport, i.e. the system was kinetically not competent (see Ref. 8). It was necessary therefore to find a suitable system: in the present paper the exchange of phosphate for acetate in calcium-acetate loaded mitochondria was employed and the reaction of the carrier with low, non-inhibitory amounts of mersalyl was investigated.

EXPERIMENTAL Rat liver mitochondria were isolated according to Johnson and Lardy (9). Shrinkage following the addition of phosphate to mitochondria that

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Abbreviation: NEM, N-ethylmaleimide

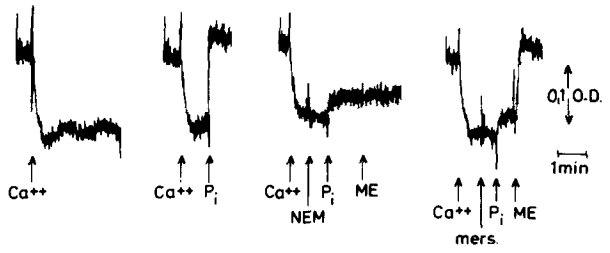


Fig. 1. The effect of SH-reagents on the phosphate-acetate exchange through the mitochondrial membrane. The reaction medium contained 1.50 mg mitochondrial protein/ml. The addition were: Ca^{2+} : 113/uM $CaCl_2$; P_i: 2 mM tris-phosphate; NEM: 100 /uM; ME: 200/uM 2-mercaptoethanol; mers: 13.3/uM mersalyl = 8.9 nmoles per mg protein.

had become swollen during calcium acetate accumulation was the indicator of the phosphate carrier activity. Volume changes of the mitochondria were monitored at room temperature (24-26 ^{o}C) by recording changes in the apparent optical density of the suspension at 520 nm using a Beckman DK 2 spektrophotometer. The reaction medium contained 125 mM sucrose, 20 mM KCl, 25 mM tris-Cl, 5 mM $MgCl_2$, 4 mM tris--succinate, 8.3 mM tris-acetate and 1/uM rotenone: the final pH was 7.4. Other additions were as indicated in the figures.

RESULTS AND DISCUSSION The aerobic uptake of calcium acetate into mitochondria in the absence of phosphate is accompanied by swelling which is proportional to the amount of calcium acetate accumulated (10-12, 2). Phosphate added to these swollen mitochondria exchanges rapidly with the intramitochondrial acetate (10-11). As the intramitochondrial calcium phosphate thus formed is only sparingly soluble, the swelling is reversed, the rate of shrinkage being more rapid than the swelling (Fig. 1). The rate of shrinkage was used to measure phosphate transport.

The movement of phosphate causing the shrinkage is mediated by the phosphate carrier as known inhibitors of the latter interfered with the shrinkage (Fig. 1). The inhibitory effect of NEM was not reversed by 2-mercaptoethanol, while mersalyl abolished the shrinkage in a reversible manner. There is some increase in the turbidity in the inhibited state on the addition of phosphate. The nature of this increase is under investigation: it does not occur when succinate is replaced by ascorbate plus tetramethyl-paraphenylenediamine.

In this phosphate-acetate exchange system the rate of shrinkage could depend either on the carrier mediated inward transport of phosphate or on the diffusion limited outflow of acetate (or, according to Ref. 13 and 14, the diffusion of acetic acid). According to the kinetic analyses of the system the rate of shrinkage depends

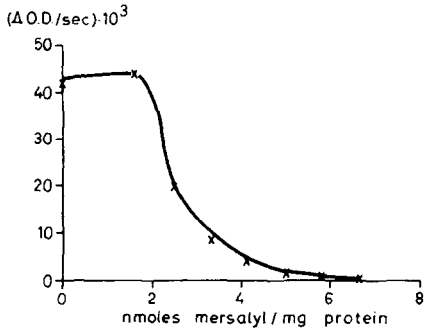
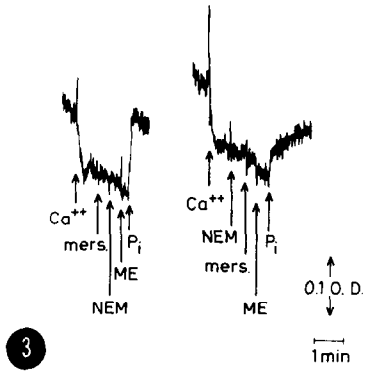


Fig. 2 The relation of the phosphate transport to the amount of mersalyl. 1.59 mg mitochondrial protein per ml was added to the medium, followed by 113 μM $CaCl_2$ and the indicated amount of mersalyl. One minute later 2 mM tris-phosphate was added and the rate of the resulting optical density change was plotted on the ordinate.

on the phosphate carrier activity (Fonyó, Palmieri and Quagliariello, unpublished data). This conclusion is also supported by the fact that under a variety of conditions the movement of acetate through the mitochondrial membrane is faster than that of phosphate (8, 11).

The mersalyl sensitivity of the phosphate carrier could be evaluated from the effect of the mercurial on the rate of shrinkage. In the experiment shown in Fig. 2 no decrease in the rate occured when 1.6 nmoles mersalyl/mg protein was added whereas addition of 6.6 nmoles/mg protein resulted in an almost complete inhibition. This raised the problem whether one-fourth of the inhibitory amount of mersalyl did react with the carrier or, alternatively other mitochondrial proteins with even higher activity for SH-group reagents bound the mercurial before it could react with the carrier. This latter possibility was considered in the case of NEM by Johnson and Chappell (8). The reaction of low, non-inhibitory amounts of mersalyl with the carrier was therefore investigated.

If an inhibitory amount of mersalyl was added to mitochondria it formed a mercaptide and the carrier was not more able to react with NEM. As a consequence of this, under these conditions 2-mercaptoethanol restored carrier activity even in the presence of NEM (Fig. 3). The mercurial "protected" the carrier from the irreversible inhibition by NEM. No reactivation of the carrier took place if NEM was added first and mersalyl later. A "protecting" effect of low, non-inhibitory amounts of mersalyl could also be demonstrated (Fig. 4). A non-inhibitory amount of mersalyl was added first to the mitochondria, followed by an excess of NEM sufficient to give complete inhibition. The added phosphate did not reverse the swelling. The addition of a two-



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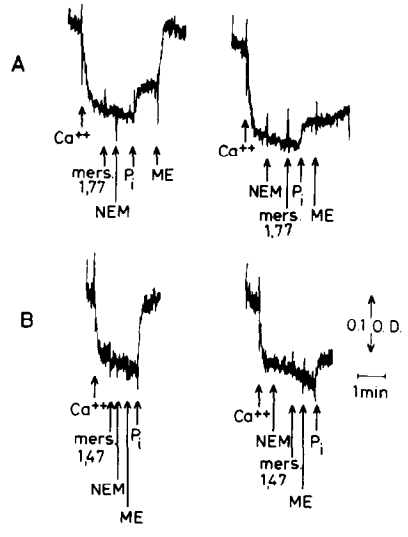

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Fig. 3 Reversible and irreversible inhibition of the phosphate carrier depending on the sequence of inhibitor additions. 1.81 mg mitochondrial protein per ml. Addition as in Fig 1 except that mersalyl added was 10.7 μM = 5.9 nmoles per mg protein.

Fig 4 The protection of the thiol groups of the phosphate carrier by non-inhibitory amounts of mersalyl. The mitochondrial protein content of the incubation medium was 1.50 mg/ml in "A" and 1.81 mg/ml in "B". The amount of mersalyl added is given on the figure as nmoles/mg protein; this amount of the mercurial did not change the rate of shrinkage following phosphate addition. Other additions as in Fig. 1.

fold excess of 2-mercaptoethanol caused rapid shrinkage of the mitochondria (Fig. 4A). If the sequence of additions was changed and the addition of the non-inhibitory amount of mersalyl was followed by NEM and excess 2-mercaptoethanol then shrinkage occured immediately on addition of phosphate (Fig. 4B). In this latter experiment the rate of shrinkage was equal to the restored carrier activity and indicated the proportion of the thiol groups of the carrier that reacted with mersalyl. In the experiment shown in Fig. 4B the recovery of the SH-groups bound by the non-inhibitory amount of mersalyl restored 39 per cent of the original carrier activity. No reversal of the carrier activity by 2-mercaptoethanol occured if NEM was added first and mersalyl later (Fig. 4A and B).

No inhibition of the phosphate carrier occurs when one part of its SH-groups is bound. Moreover the same SH-groups which could be blocked by mersalyl without any loss of the carrier activity were sufficient to support transport when they were liberated and the rest of the SH-groups irreversibly blocked. Consequently the carrier

has more than one SH-group per unit and they are functionally equivalent: if either one of them is free the carrier maintains its function.

The equivalence of the two SH-groups in the carrier function suggests a symmetrical structure of the carrier with 2 free thiols per unit. This would not be a unique structure as a symmetrical unit has also been postulated for the Na-K-carrier system of the membrane ATPase by Stein et al /15/. Thus the symmetricity of the membrane carrier systems may have general significance.

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