INHIBITION OF MALATE TRANSPORT AND ACTIVATION OF PHOSPHATE TRANSPORT IN MITOCHONDRIA BY ETHYLMERCURITHIOSALICYLATE

Helmut FREITAG and Bernhard KADENBACH
Biochemie, Fb Chemie d. Philipps-Universität Hans-Meerwein-str., 3550 Marburg, FRG

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1. Introduction

Different phosphate-transport systems have been described in mitochondria [1]. Since so far no phosphate carrier protein could be isolated, the identification of phosphate-transport systems rely only on indirect studies using SH-inhibitors. The reactivity of SH-inhibitors, however, depends strongly on the conditions applied [2]. The phosphate/proton-symporter of mitochondria is sensitive against SH-inhibitors like mersalyl or MalNEt [1,3]. The effect of thiomersal on phosphate transport in rat liver mitochondria has been studied [4]. The MalNEt- or PMS-inhibited phosphate transport could be reactivated by thiomersal. Here we show that the activation of phosphate transport by thiomersal paralleles an inhibition of malate transport. The data suggest that in the presence of thiomersal the phosphate/dicarboxylate antiporter behaves like a phosphate/proton symporter.

2. Materials and methods

MalNEt, thiomersal and mersalyl were obtained from Serva (Heidelberg), rotenone from Sigma (München). [^{14}C]Malate (30 mCi/mmol) and [^{3}H]-sucrose (3 Ci/mmol) were from Amersham Buchler (Braunschweig). All other chemicals were of analytical grade.

Rat liver mitochondria were isolated by standard procedures [5]. Swelling of mitochondria was done as in [5] either in 100 mM ammonium phosphate (pH 7.4), 2 mM EDTA, 1 μM rotenone, or in 90 mM ammonium malate, 2 mM EDTA, 1 μM rotenone and 10 mM Tris–HCl (pH 7.4). The uptake of [^{14}C]malate was measured at 20°C under energized conditions in a medium containing 140 mM KCl, 10 mM Tris–HCl (pH 7.3), 2 mM ascorbate, 0.2 mM *N,N,N',N'*-tetramethyl-*p*-phenylenediamine, 2.4 mM [^{3}H]sucrose (2 μCi) and 7.9 mM [^{14}C]malate (1 μCi). The reaction was started by addition of mitochondria. Aliquots were taken and centrifuged for 30 s in an Eppendorf centrifuge at the indicated times. The supernatant was removed immediately by suction. The sediment was dissolved in 300 μl 2% SDS and counted in 10 ml scintillation fluid (Rotiscint 22, Roth, Karlsruhe). The uptake of [^{14}C]malate was corrected for the extramatrix space with [^{3}H]sucrose. Protein was measured by the biuret method [6].

Abbreviations: MalNEt, *N*-ethylmaleimide; PMS, *p*-chloromercuri-phenylsulfonate; thiomersal, ethylmercurithiosalicylate; SDS, sodium dodecylsulfate

3. Results and discussion

The uptake of malate into mitochondria as measured by swelling in isotonic ammonium malate, requires the addition of phosphate, which is first taken up by the MalNEt-sensitive phosphate/proton symporter and then is released in exchange with malate (fig.1A). Preincubation of mitochondria with MalNEt therefore results in inhibition of malate uptake by the swelling procedure (fig.1B). In [4] we showed that the MalNEt-inhibited phosphate uptake into mitochondria could be reactivated by thiomersal. We therefore expected also a reactivation of the phosphate-induced malate uptake with MalNEt inhibited mitochondria. However, as shown in fig.1B, thiomersal does not reactivate the swelling of mitochondria in isotonic malate, induced by phosphate and inhibited

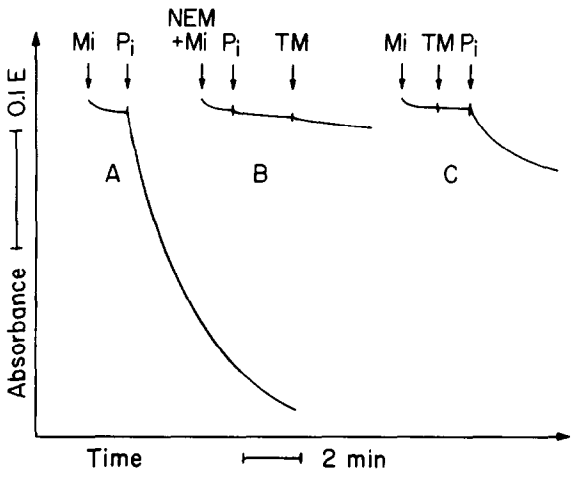

Fig.1. Effect of thiomersal on the swelling of mitochondria in isotonic ammonium malate. The swelling medium contained 0.25 mg mitochondrial protein/ml. Additions: NH_4–P_i, 2 mM: thiomersal(TM), 32 μM. Mitochondria (Mi) were preincubated for 1 min at 0°C with MalNEt (NEM) (26.6 nmol/mg)

by MalNEt. Instead, thiomersal alone inhibits this malate uptake (fig.1C).

To show directly the inhibition of malate uptake by thiomersal, the uptake of [^{14}C]malate was measured under energized conditions (fig.2). Phosphate stimulates the uptake of [^{14}C]malate in analogy to the swelling procedure. Thiomersal inhibits the uptake of [^{14}C]malate in the absence and presence of phosphate. The incomplete inhibition may be due to an insufficient thiomersal concentration (fig.3) or to alternative transport systems (e.g., citrate/malate antiporter [7]). These results show that thiomersal inhibits the phosphate/dicarboxylate antiporter.

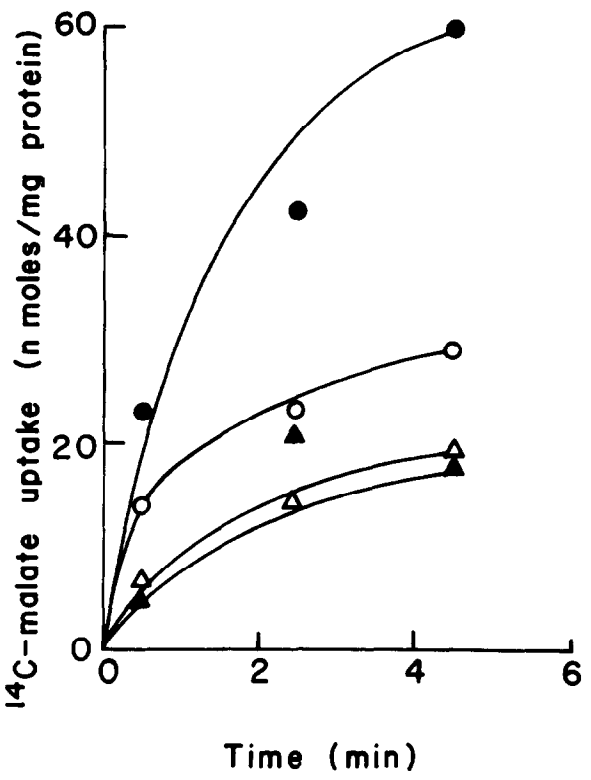

Fig.2. Inhibition of [^{14}C]malate uptake into mitochondria by thiomersal. For details see section 2. The uptake of [^{14}C]-malate was measured in the absence (○—○, △—△) or presence (●—●, ▲—▲) of 1 mM P_i, and in the absence (○—○, ●—●) or presence (△—△, ▲—▲) of 33.2 μM thiomersal. Mitochondrial protein was 1 mg/ml.

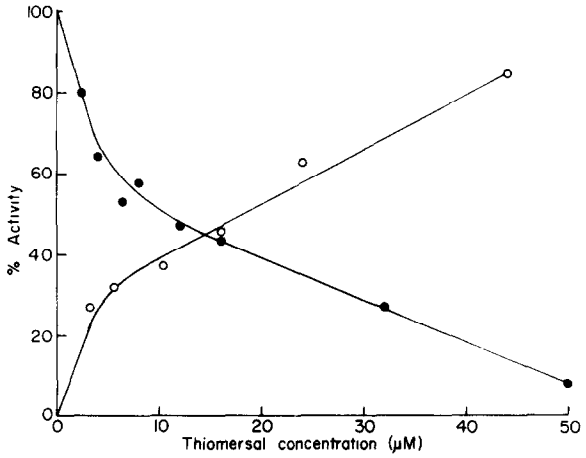

Fig.3. Concentration dependence of the effect of thiomersal on the phosphate and malate uptake in mitochondria. The swelling of mitochondria in isotonic phosphate and malate media was performed as in section 2. (○—○) Mitochondria were preincubated for 1 min at 0°C with mersalyl (18 nmol/mg mitochondrial protein) and added to the phosphate medium. The swelling was started by the addition of the indicated amount of thiomersal. The % activity indicated at the ordinate of the figure is related to the maximally reactivated swelling rate by thiomersal (= 100%), which was 56% of the rate measured in its absence. (●—●) Mitochondria were added to the malate medium followed by the addition of the indicated amount of thiomersal. The reaction was started after 1 min with 2 mM phosphate. For both titrations mitochondrial protein was 0.25 mg/ml.

The concomitant inhibition of malate uptake and reactivation of inhibited phosphate uptake by thiomersal may be explained by two alternative reactions:

(1) Thiomersal reacts independently with the dicarboxylate carrier protein causing inhibition and with the phosphate carrier protein causing reactivation after inhibition with SH-inhibitors.
(2) Thiomersal does not react with the phosphate carrier protein, which remains inhibited by mersalyl or MalNEt in its presence. But it reacts with the dicarboxylate carrier protein, changing its function from an antiporter into a symporter.

Thiomersal could thus lead to inhibition of the dicarboxylate/phosphate antiport with a concomitant induction of a new capacity, a net phosphate transport. If the latter alternative is valid, the concentration dependence of the inhibition of malate uptake and the reactivation of phosphate uptake should show a strong correlation. This correlation was indeed found as shown in fig.3, where the effect of various thiomersal concentrations on the uptake of phosphate and malate was measured by the swelling procedure. This result supports explanation (2), which suggests a conversion of the dicarboxylate antiporter.

A MalNEt- or mersalyl-insensitive phosphate transport can also be found under other experimental conditions (without thiomersal). Such observations lead to the postulation of a Ca^{2+}/P_i-symporter [8], an ADP + P_i/ATP-antiporter [9] or were a matter of discussion [10]. They could more easily be interpreted by the assumption of a functional conversion of the phosphate-transport system. In [11,12] a similar suggestion on a conversion of the phosphate-transport system in mitochondria has been made. Also for the K^+-transport system of mitochondria a conversion of the function was postulated [13]. However, further data are necessary to prove this concept.

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