ETHYLMERCURITHIOSALICYLATE – A NEW REAGENT FOR THE STUDY OF PHOSPHATE TRANSPORT IN MITOCHONDRIA

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1. Introduction

The physiological function of mitochondria requires the transport of P_i across the inner mitochondrial membrane for 3 main metabolic reactions: (1) Uptake of phosphate together with ADP for the synthesis of ATP within the matrix; (2) Exchange of phosphate against dicarboxylates for metabolite fluxes between cytosol and matrix; (3) Uptake and release of phosphate together with Ca^{2+} for calcium homeostasis in the cytoplasm [1]. For these functions 4 separate phosphate transport systems have been described in mitochondria which could only be differentiated by their sensitivity against SH-group inhibitors: (1) The electroneutral phosphate/proton symporter is inhibited by MalNEt and low concentrations of *p*-chloromercuribenzoate and mersalyl [2–4]; (2) The electroneutral dicarboxylate antiporter, exchanging dicarboxylates or phosphate against each other, is inhibited by *p*-chloromercuribenzoate, mersalyl and the dicarboxylate analogon butylmalonate, but not by MalNEt [5–7]; (3) The electrogenic phosphate uniporter is inhibited by mersalyl [8] and with inverted inner membrane vesicles by *p*-chloromercuribenzoate and MalNEt [9]; (4) The electrogenic calcium/phosphate symporter was found insensitive against MalNEt and mersaylyl [10,11]. The latter transport system, however, does not seem to represent a separate transporter, because the insensitivity against MalNEt and *p*-chloromercuribenzoate could not be corroborated [12,13].

The simultaneous occurrence of the electroneutral phosphate/proton symporter (phosphate uptake) and the electrogenic phosphate uniporter (phosphate release) in mitochondria, would result in a futile cycle, driven by the mitochondrial proton pump. Therefore a strong regulation of the phosphate transport system has to be assumed. The existence of only one regulated transport system which functions either as proton/phosphate symporter or as phosphate/dicarboxylate antiporter has also been suggested [14–16]. Here the effect of the antiseptic ethylmercurithiosalicylate (thiomersal), which contains sulfur and mercury in a covalent linkage, on the phosphate uptake of mitochondria is described. The data suggest a regulated sensitivity of the phosphate/proton symport against SH-inhibitors.

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

2. Materials and methods

MalNEt, thiomersal and thiosalicylic acid were purchased from Serva (Heidelberg), PMS and rotenone from Sigma (St Louis). [^{32}P]Phosphate and [^{3}H]-sucrose (3 Ci/mmol) were obtained from Amersham Buchler. All other chemicals were of analytical grade.

Rat liver mitochondria were isolated by standard procedures [17]. Swelling of mitochondria was done as in [16], either in 100 mM ammonium phosphate (pH 7.3), 2 mM EDTA and 1 μM rotenone (A), or in 80 mM potassium phosphate (pH 7.3), 40 mM ammonium chloride, 1 μM rotenone (B). The uptake of [^{32}P]phosphate was measured in aliquots (800 μl) taken from cuvettes containing 2 ml swelling medium (B) and 0.2 μCi [^{32}P]phosphate plus 2 μCi [^{3}H]-sucrose, at the indicated times after addition of mitochondria. After centrifugation for 30 s in an Eppendorf centrifuge the supernatant was immediately removed and the pellet dissolved in 300 μl 2% SDS and counted in 10 ml scintillation fluid. Protein was determined by the biuret method [18].