THE TRANSPORT OF THIOSULPHATE IN RAT LIVER MITOCHONDRIA

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1. Introduction

Thiosulphate administered to animal tissues is converted to sulphate [1], which is formed from the intermediate, sulphite, by sulphite oxidase [2]. The initial conversion of thiosulphate to sulphite is catalysed by two enzymes, thiosulphate sulphurtransferase (rhodanese) and thiosulphate reductase [3], both of which are found in mitochondria [4,5], thiosulphate sulphurtransferase being localised exclusively in the mitochondrial matrix [6]. This raises the question of the permeability of mitochondria to thiosulphate and sulphite. Recent work [7] has indicated that the sulphate and sulphite anions may be transported by the dicarboxylate carrier of rat liver mitochondria and that sulphite may also permeate by an additional mechanism equivalent to an exchange for hydroxyl ions. This paper examines the permeation of thiosulphate and presents evidence that thiosulphate transport is also catalysed by the dicarboxylate carrier.

2. Materials and methods

[^{32}P]Phosphoric acid, [1,5-^{14}C]citrate, [5-^{14}C]-oxoglutarate, [1-^{14}C]malonate, [1,4-^{14}C]succinate, [U-^{14}C]malate, [^{35}S]sulphuric acid and [^{35}S]thiosulphate labelled in the outer sulphur atom were obtained from the Radiochemical Centre, Amersham, U.K.

Rat liver mitochondria were isolated and loaded with various labelled metabolites as described previously [7–9]. The loading procedure leads to an intramitochondrial concentration of metabolites of 8–20 mM. The exchanges between the intramitochondrial labelled anious and externally-added anions were done in 1 ml medium containing 100 mM KCl, 20 mM Tris–HCl, 1 μg antimycin, 1 mM EGTA and metabolite-loaded mitochondria (approx. 2 mg protein), the pH was 7.4 and the temperature was 8°C. After 2 min preincubation the exchange reactions were started by adding the external, unlabelled anions and they were terminated 1 min later by centrifugation for 1 min in an Eppendorf bench centrifuge (model 3200). The mitochondrial pellet was extracted and the radioactivity measured [7]. In some reactions the medium contained the inhibitors *N*-ethylmaleimide, 2-butylmalonate, or mersalyl as indicated. Each exchange was accompanied by a control experiment in which no external anion was added.

The kinetics of thiosulphate uptake were measured by the 'inhibitor stop' method as described before [9]. The reaction medium (1 ml) contained 0.22 M sucrose, 10 mM Tris–HCl, 10 mM KCl, 1 mM $MgCl_2$, 1 μg rotenone 1 mM *N*-ethylmaleimide and phosphate-loaded mitochondria (approx. 2 mg protein), pH 7.0 and temperature 3°C.

The rate of mitochondrial swelling was measured by recording the decrease in E_{623}.

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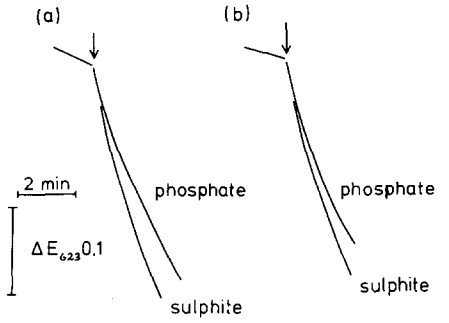

Fig. 1. A comparison between mitochondrial swelling in ammonium thiosulphate (a) and ammonium malonate (b). The incubations contained 100 mM ammonium thiosulphate (a) or 100 mM ammonium malonate (b), 20 mM Tris–HCl, 0.5 mM EDTA, 1 μg rotenone and 2.1 mg mitochondrial protein. At the arrow 5 mM ammonium phosphate or 5 mM ammonium sulphite was added. Final vol. 2.5 ml, pH, 7.4.

3. Results

Fig. 1 shows the swelling of rat liver mitochondria suspended in iso-osmotic ammonium thiosulphate (a) and, as a control, ammonium malonate (b). Little swelling occurs in either medium until 5 mM phosphate or sulphite is added. Phosphate and, as recently shown, sulphite can both permeate by a process equivalent to an exchange for hydroxyl ions [7,10]. In addition both are transported by the dicarboxylate carrier [7,11]. Thus, in the control experiment, ammonium phosphate or sulphite permeates and malonate enters in an exchange for phosphate or sulphite catalysed by the dicarboxylate carrier [7,11]. Since similar results are seen with ammonium thiosulphate it is suggested that thiosulphate also can enter in exchange with phosphate and sulphite.

Table 1 reports the effluxes of intramitochondrial anions elicited by the addition of extramitochondrial thiosulphate. It is reasonable to assume that any efflux occurring does so by exchange with the externally-added anion. Each series of exchanges includes an experiment in which the externally-added anion is the same as the intramitochondrial anion. In this case, the exchange is the maximum obtainable and provides a reference for the other exchanges involving thiosulphate.

External thiosulphate exchanges to about the maximum degree with internal malonate, malate, succinate, phosphate and sulphate, all of which are substrates of the dicarboxylate carrier [7,11]. Furthermore, these exchanges are strongly inhibited by butylmalonate and mersalyl, two inhibitors of the dicarboxylate carrier [12,13], but not by *N*-ethylmaleimide. In contrast, thiosulphate exchanges very poorly with oxoglutarate and citrate, substrates of the oxoglutarate and tricarboxylate carriers respectively [11,14]; in these experiments, an additional control exchange was done using either malonate or malate.

It is important to note that the concentration of mersalyl used (0.1 mM, 50 nmoles/mg mitochondrial protein) was greater than that needed to inhibit the dicarboxylate carrier (25 nmoles/mg, 13) but insufficient to inhibit significantly the oxoglutarate and tricarboxylate carriers [8,15]. Thus, in control experiments, addition of 0.1 mM mersalyl decreased phosphate–malonate exchange from 88–4% (catalysed by the dicarboxylate carrier, [11]), but decreased the malonate–malonate, malate–malate and succinate–succinate exchanges reported in table 1 by less than 7% (catalysed by the dicarboxylate, oxoglutarate and, in the case of malate, the tricarboxylate carriers [11,12,16]).

In fig. 2 the kinetics of thiosulphate influx are presented in double reciprocal form. The plot is linear and displays a K_m value of approx. 1 mM and a V_{max}

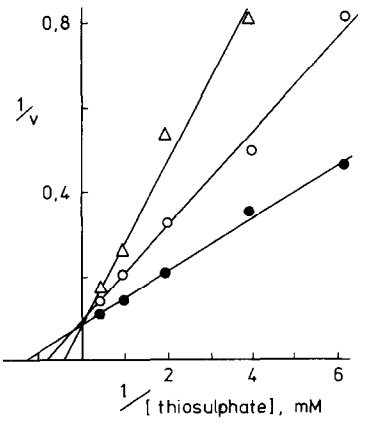

Fig. 2. The dependence of the rate of [^{35}S]thiosulphate uptake on the external thiosulphate concentration at 3° C and pH 7.0. Experimental details are given in the text. Additions: (●) nil; (○) 1 mM phosphate; (△) 0.5 mM malonate. The units of v are nmole thiosulphate influx/mg mitochondrial protein per min.

Table 1
The exchanges between labelled, intramitochondrial anions and extramitochondrial thiosulphate

Intramitochondrial anion	Extramitochondrial anion (2 mM)	Inhibitor	% Exchange after 1 min
Sulphate	Sulphate	–	74
Sulphate	Thiosulphate	–	81
Sulphate	Thiosulphate	*N*-Ethylmaleimide	77
Sulphate	Thiosulphate	Butylmalonate	6
Sulphate	Thiosulphate	Mersalyl	1
Phosphate	Phosphate	–	70
Phosphate	Thiosulphate	–	66
Phosphate	Thiosulphate	*N*-Ethylmaleimide	61
Phosphate	Thiosulphate	Butylmalonate	1
Phosphate	Thiosulphate	Mersalyl	3
Malonate	Malonate	–	95
Malonate	Thiosulphate	–	92
Malonate	Thiosulphate	*N*-Ethylmaleimide	86
Malonate	Thiosulphate	Butylmalonate	0
Malonate	Thiosulphate	Mersalyl	6
Malate	Malate	–	85
Malate	Thiosulphate	–	75
Malate	Thiosulphate	*N*-Ethylmaleimide	69
Malate	Thiosulphate	Butylmalonate	1
Malate	Thiosulphate	Mersalyl	4
Succinate	Succinate	–	82
Succinate	Thiosulphate	–	74
Succinate	Thiosulphate	*N*-Ethylmaleimide	76
Succinate	Thiosulphate	Butylmalonate	0
Succinate	Thiosulphate	Mersalyl	2
Oxoglutarate	Oxoglutarate	–	76
Oxoglutarate	Malonate	–	71
Oxoglutarate	Thiosulphate	–	1
Citrate	Citrate	–	63
Citrate	Malate	–	58
Citrate	Thiosulphate	–	6

The experimental details are explained in the text. The values given are the percentage decreases in the radioactivity content of the mitochondria on the addition of extramitochondrial anions (potassium salts). The inhibitor concentrations used were: 2 mM *N*-ethylmaleimide, 20 mM butylmalonate and 0.1 mM mersalyl.

of 15 nmoles/min per mg protein at 3°C and pH 7.0. Phosphate and malonate decrease the rate of thiosulphate uptake, inpart, at least, by increasing the K_m value for thiosulphate.

4. Discussion

Thiosulphate exchanges in a butylmalonate and mersalyl-sensitive way with recognised substrates [7,11] of the dicarboxylate carrier. In addition, phosphate and malonate inhibit thiosulphate uptake in a largely competitive manner. The most straightforward explanation of these data would seem to be that the dicarboxylate carrier is able to transport thiosulphate.

In contrast, the evidence suggests that thiosulphate is not transported by the phosphate, oxoglutarate or tricarboxylate carriers. Phosphate permeation, catalys-

ed by the dicarboxylate carrier, may be selectively inhibited by butylmalonate [12,17,18], thus permitting phosphate transport by the phosphate carrier alone. Under these conditions, thiosulphate does not exchange significantly with phosphate. Furthermore, specific inhibition of the phosphate carrier by *N*-ethylmaleimide [13] causes little inhibition of any exchange involving thiosulphate. Malate, malonate and succinate are all substrates of the dicarboxylate and oxoglutarate carriers, and, in addition, malate is transported by the tricarboxylate carrier [11,12,14]. The contribution of these systems to the exchanges with thiosulphate may be resolved by the use of mersalyl, which, when used as described, selectively inhibits the dicarboxylate carrier [7,8,15]; it also inhibits the thiosulphate–dicarboxylate exchanges. Direct evidence against the involvement of the oxoglutarate and tricarboxylate carriers in thiosulphate transport is provided by the small degree to which thiosulphate can exchange with oxoglutarate and citrate.

The lack of mitochondrial swelling in ammonium thiosulphate alone confirms the absence of a mechanism of thiosulphate entry in exchange for hydroxyl ions or together with protons. The stimulation of swelling by phosphate and sulphite agrees with the suggested capacity of the dicarboxylate carrier to transport thiosulphate.

References

[1] Pirie, N. W. (1934) Biochem. J. 28, 1063.
[2] Macleod, R. M., Farkas, W., Fridovich, I. and Handler, P. (1961) J. Biol. Chem. 236, 1841.
[3] Sorbo, B. (1964) Acta Chem. Scand. 18, 821.
[4] De Duve, C., Pressman, B. C., Gianetto, R., Wattiaux, R. and Appelmans, F. (1955) Biochem. J. 60, 604.
[5] Koj, A., Frendo, J. and Janik, Z. (1967) Biochem. J. 103, 791.
[6] Greville, G. D. and Chapell, J. B. (1959) Biochim. Biophys. Acta 33, 267.
[7] Crompton, M., Palmieri, F., Capano, M. and Quagliariello, E., Biochem. J., in press.
[8] Palmieri, F., Stipani, I., Quagliariello, E. and Klingenberg, M. (1972) Eur. J. Biochem. 26, 587.
[9] Palmieri, F., Prezioso, G., Quagliariello, E. and Klingenberg, M. (1971) Eur. J. Biochem. 22, 66.
[10] Chappell, J. B. and Crofts, A. R. (1966) in Regulation of Metabolic Processes in Mitochondria (Tager, J. M., Papa, S., Quagliariello, E. and Slater, E. C., eds.), p. 293, Elsevier, Amsterdam.
[11] Chappell, J. B. and Haarhoff, K. M. (1967) in: Biochemistry of Mitochondria (Slater, E. C., Kaniuga, Z. and Wojtczak, I., eds.) p. 75, Academic Press, London and New York.
[12] Robinson, B. H. and Chappell, J. B. (1967) Biochem. Biophys. Res. Commun. 28, 249.
[13] Meijer, A. J., Groot, G. S. P. and Tager, J. M. (1970) FEBS Letters, 8, 41.
[14] Meijer, A. J. and Tager, J. M. (1966) Biochem. J. 100, 79P.
[15] Quagliariello, E. and Palmieri, F. (1971) Biochimica Applicata 18, 191.
[16] Papa, S., Lofrumento, N. E., Kanduc, D., Paradies, G. and Quagliariello, E. (1971) Eur. J. Biochem. 22, 134.
[17] Meijer, A. J. and Tager, J. M. (1969) Biochim. Biophys. Acta 189, 136.
[18] Johnson, R. N. and Chappell, J. B. (1970) Biochem. J. 116, 37P.