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Mechanism of Mitochondrial Transport of Thallous Ions

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Abstract

Rat liver mitochondria were found to swell under nonenergized conditions when suspended in media containing 30–40 mM $TlNO_3$. Respiration on succinate caused a rapid contraction of mitochondria swollen under nonenergized conditions. In the presence of thallous acetate, there was a rapid initial swelling under nonenergized conditions until a plateau was reached; respiration on succinate then caused a further swelling. Trace amounts of ^{204}Tl (less than 100 μM) equilibrated fairly rapidly across the mitochondrial membrane. The influx of Tl^+ was able to promote the decay not only of a valinomycin-induced K^+-diffusion potential but also of respiration-generated fields in the inner membrane in accordance with the electrophoretic nature of Tl^+ movement. Efflux of Tl^+ showed a half-time of about 10 sec at 20°C and was not affected appreciably by the energy state. Efflux was retarded by Mg^{2+} and by lowering the temperature. The data indicate that Tl^+ when present at high concentrations, 30 mM or more, distributes across the mitochondrial inner membrane both in response to electrical fields and to Δ pH. In energized mitochondria the uptake of Tl^+ would occur electrophoretically, while Tl^+/H^+ exchange would constitute a leak. In the presence of NO_3^-, the movements of Tl^+ are determined by that of NO_3^-, indicating short-range coupling of electrical forces. At low concentrations of Tl^+, 5 mM or less, there was no indication of a Tl^+/H^+ exchange, which appears to be induced by high concentrations of Tl^+.

Key Words: Thallium; mitochondria; transport; membrane potential.

Introduction

The monovalent Tl^+ cation resembles K^+ in regard to its size, the ionic radii being 0.147 and 0.133 nm, respectively (Weast and Astle, 1979). Tl^+ is able to substitute for K^+ in activating some enzymes, e.g., ($Na^+ + K^+$)-ATPase (Britten and Blank, 1968) and pyruvate kinase (Kayne, 1971). In contrast to K^+, Tl^+ can readily penetrate membranes that generally have a low permeability toward monovalent cations. This is the case for the erythrocyte plasma

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membrane (Skulskii *et al.*, 1978a), some bacteria (Skulskii *et al.*, 1977; Bakker, 1978), and mitochondria (Melnick *et al.*, 1976; Skulskii, 1977). In mitochondria, Tl^+ appears to competitively inhibit K^+ movements (Barrera and Gomez-Puyou, 1975; Diwan and Lehrer, 1977). Tl^+ may therefore be a suitable probe in the study of the mechanism of K^+ transport in mitochondria.

We have previously shown (Skulskii *et al.*, 1978b) that Tl^+ is able to move electrophoretically in response to induced diffusion potentials in mitochondria, though this does not exclude other transport mechanisms. The in/out ratios of Tl^+ have been used to estimate the magnitude of transmembrane potentials in erythrocytes (Skulskii *et al.*, 1978a) and bacteria (Bakker, 1978). In view of the central role of membrane potentials in the mechanism of energy transduction in mitochondria, it might be worthwhile to further characterize the transport of Tl^+ in mitochondria and thereby explore the possibilities of using it as a nonlipophilic probe of the transmembrane potentials. We therefore systematically studied the swelling of mitochondria in the presence of thallous ions as the nitrate and acetate salts in both nonrespiring and respiring mitochondria. We have found that the swelling behavior of mitochondria indicates the presence of Tl^+/H^+ exchange mechanism, which would complicate the use of Tl^+ as a probe of the transmembrane potential.

Materials and Methods

Rat liver mitochondria were prepared by a conventional method as described elsewhere (Wikström and Saris, 1969). Their content of protein

Table I. Rates of Net Efflux of Tl^+ and K^+ from Mitochondria[a]

Conditions	Temperature (°C)	Halftime	
		Tl^+	K^+
Nonrespiring	20	7–15 sec	25 min
Nonrespiring	10	75 sec	—
Nonrespiring	0	6 min	150 min
Nonrespiring + 10 mM Mg^{2+}	0	45 min	—
Respiring	20	6–24 sec	—

[a] In the case of Tl^+ efflux, mitochondria (2.5 mg/ml) were equilibrated with minute concentrations (less than 100 μM) of ^{204}Tl-labeled Tl^+ in a medium containing 250 mM sucrose, 1 μM rotenone, 10 mM Tris-acetate, 10 mM Tris-succinate, pH 7.4. In nonrespiring mitochondria, respiration was inhibited by 5 mM NaN_3. Then the suspension was diluted 1:10 by nonradioactive medium, and samples removed for the estimation of the mitochondrial Tl content. For the study of K^+ leakage, flame photometry was used.

was estimated by a modification of the Folin–Ciocalteu procedure (Zak and Cohen, 1961) in the presence of sodium taurocholate with bovine serum albumin serving as standard. The mitochondrial content of Tl^+ was measured using ^{204}Tl, specific activity 440 Ci/mol (The Radiochemical Centre, Amersham, U.K.), by transferring aliquots of the incubation suspension to ice-cold 0.25 M sucrose solution containing 10 mM $MgSO_4$ (see Table I), filtering through Millipore^R filters, pore size 0.45 μm, washing the filters with the sucrose–$MgSO_4$ solution, and measuring radioactivity with a β-spectrometer equipped with a Geiger-Müller tube. Swelling was monitored with the Aminco-Chance DW-2 photometer at 520 nm. Membrane potential changes were recorded with the safranine method using the wavelength pair 554–524 nm (Åkerman and Wikström, 1976). FCCP (*p*-trifluoromethoxyphenylhydrazone) was a kind gift of Dr. P. G. Heutler, DuPont, Baltimore, Maryland.

Results and Discussion

Swelling of Nonrespiring Mitochondria in the Presence of Tl^+

There was a substantial swelling of nonrespiring mitochondria in the presence of rather high, 30–40 mM, concentrations of $TlNO_3$ (Fig. 1, broken lines), as was reported previously (Skulskii, 1977). There was no swelling in the presence of KNO_3 (not shown). Since NO_3^- readily penetrates the mitochondrial inner membrane electrogenically (see review by Brierley, 1976), swelling of mitochondria can only be obtained when the cation also

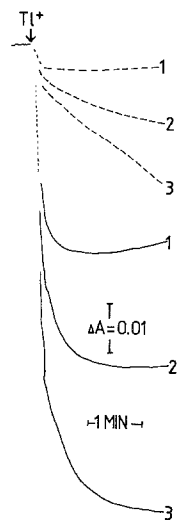

Fig. 1. Swelling of nonrespiring mitochondria in media containing Tl^+. The medium contained 10 mM Hepes, 6 μM rotenone, 2 μg oligomycin/ml, 2 mM KCN, and mitochondrial protein 1.5 mg/ml, pH 7.2. Final concentrations: (1) 20 mM Tl^+ and 140 mM sucrose; (2) 30 mM Tl^+ and 120 mM sucrose; (3) 40 mM Tl^+ and 100 mM sucrose. The Tl^+ was added at the arrow from a 300 mM stock solution as acetate (continuous lines) or as nitrate (broken lines).

moves electrogenically, as K^+ does in the presence of valinomycin. These data thus confirm our previous report of the electrophoretic movement of Tl^+ (Skulskii *et al.*, 1978b). The mechanism of swelling in the presence of $TlNO_3$ seems to occur according to Scheme A in Fig. 2.

In the presence of Tl^+ as the acetate, there was a fast initial swelling of nonrespiring mitochondria (Fig. 1, continuous lines). The rate of swelling was much faster than in the presence of $TlNO_3$. However, swelling was halted when a plateau was reached. Apparently, an osmotic equilibrium was reached which was determined by the relative proportions of the penetrant thallium acetate and the nonpenetrant sucrose which was used to keep the overall osmolarity constant.

It is generally accepted (Brierley, 1976; Mitchell and Moyle, 1969) that the acetates of monovalent cations penetrate resting mitochondria only if there is a cation/H^+ antiporter. Swelling of nonrespiring mitochondria in the presence of thallium acetate would thus occur as shown in Scheme B, Fig. 2. Acetic acid diffuses rapidly into the matrix compartment and forms acetate and H^+. Tl^+ enters in exchange for H^+. The swelling in the presence of thallium acetate is thus strong evidence in favor of the existence of a cation/H^+ antiporter. Mitochondria swell rapidly in the presence of sodium acetate but not in the presence of potassium acetate, which has been interpreted as showing that the electroneutral exchange system shows a high specificity, favoring Na^+ over K^+ (Brierley, 1976; Mitchell and Moyle, 1969). In this system Tl^+ behaves as an analogue of Na^+ rather than of K^+. Tl^+ has been reported to show some competitive inhibition of Na^+ efflux and (Na^+ + K^+)-ATPase in erythrocytes (Brierley, 1974). It is therefore not so surprising that some Na^+-like effects are seen in mitochondria too. On the

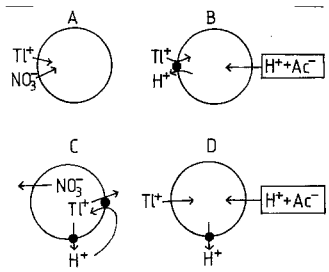

Fig. 2. Schemes of Tl^+ transport in mitochondria. (A) Nonenergized swelling in the presence of nitrate. Tl^+ and NO_3^- enter by diffusion that is overall electroneutral. (B) Nonenergized swelling in the presence of acetate. Acetate enters by diffusion as acetic acid and dissociates, and Tl^+ enters in exchange for H^+. (C) Contraction under energized conditions in the presence of nitrate. NO_3^- is extruded electrophoretically, H^+ by respiratory chain-linked H^+ pumps. H^+ reenters in exchange for Tl^+ by the Tl^+/H^+ antiporter. (D) Energized swelling in the presence of acetate. Tl^+ enters electrophoretically. Acetate enters by diffusion as acetic acid, which dissociates, and protons are extruded by H^+ pumps.

other hand, even if K^+/H^+ exchange does not occur in intact mitochondria, such an exchange may easily be induced by a variety of treatments (Brierley, 1976; Duszynski and Wojtczak, 1977; Azzone *et al.*, 1978a). It is possible that the high concentrations of Tl^+ used in swelling experiments induce a Tl^+/H^+ exchange that does not occur at low Tl^+ concentrations.

Swelling of Respiring Mitochondria in the Presence of Tl^+

Electron flow through the components of the respiratory chain will generate electric fields across or in the inner membrane (see discussions by Mitchell, 1977 and Williams, 1978). In addition a gradient of H^+ is formed either by the operation of loops according to the chemiosmotic schemes of Mitchell (1977) or rather by electrogenic transport of H^+ out of the matrix compartment. The redox reaction of at least one respiratory chain component, cytochrome oxidase, is coupled to a H^+ pump (Wikström, 1977). These phenomena will drastically alter the behavior of mitochondria in the swelling experiments upon energization (Fig. 3). It is seen that there is a strong stimulation of swelling upon addition of a respiratory substrate, succinate, to mitochondria that have reached a plateau in resting conditions in the presence of acetate. In the presence of nitrate, a contraction was induced (Fig. 3, continuous lines) or swelling was inhibited when succinate was present from the beginning of the experiment (not shown).

The stimulation of uptake of thallium acetate in energized conditions is similar to the enhanced uptake of other monovalent cations (Brierley, 1974, 1976). Accumulation of acetate in the matrix would be stimulated by the removal of H^+ that is formed by dissociation of acetic acid diffusing into the matrix. Monovalent cations are thought to move electrophoretically. Indirect evidence has been presented in favor of this model (Brierley *et al.*, 1978),

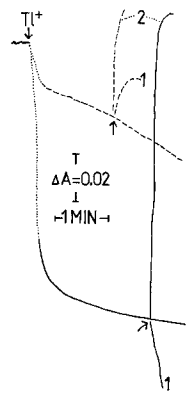

Fig. 3. Volume changes induced by energization in mitochondria swollen under nonenergized conditions. Experimental conditions as in Fig. 1, traces 3, but KCN was omitted for succinate respiration. Additions at arrows: (1) 2 mM Tris-succinate; (2) 360 nM valinomycin. Addition of 1 μM FCCP had an insignificant effect. Continuous lines indicate presence of acetate, broken lines indicate presence of nitrate.

which has been adapted to Tl^+ in Scheme D, Fig. 2. It is, however, possible that there is a short-range coupling of Tl^+ influx to electrogenic H^+ efflux.

The electric fields generated in the inner membrane upon energization would extrude anions and promote uptake of cations that sense the field. In mitochondria, the efflux of nitrate appears to be the dominant feature and the uptake of Tl^+ is prevented, or Tl^+ may even be extruded. Extrusion of NO_3^- will take place at the expense of the membrane potential. Tl^+ would be driven out by the coupled action of the H^+ pump and the cation/H^+ antiporter (Scheme C, Fig. 2). Alternatively, there might be a short-range coupling between the fluxes of Tl^+, NO_3^-, and H^+. Several laboratories have reported respiration-driven contraction of mitochondria swollen under nonenergized conditions in the presence of various nitrate salts (Brierley *et al.*, 1977; Azzone *et al.*, 1978b).

Effect of Valinomycin on Mitochondria in the Presence of Tl^+

Addition of valinomycin to mitochondria that have undergone swelling under resting conditions caused an extensive contraction due to efflux of K^+ in the presence of both acetate and nitrate (Fig. 3). It is evident that under these conditions only negligible amounts of valinomycin–Tl^+ complexes were formed; and the rate of influx of the free Tl^+ was not sufficient to compensate for the efflux of K^+.

Addition of the uncoupling agent FCCP, which increases the permeability of the membrane toward H^+, had no effect on the mitochondrial volume in nonrespiring mitochondria (not shown).

The Rate of Efflux of Tl^+

Mitochondria were suspended in a medium that contained minute concentrations of ^{204}Tl-labeled salt (less than 100 μM). When equilibrium in the distribution of Tl^+ across the mitochondrial membranes had been reached, net efflux of Tl^+ was induced by dilution of the suspension by addition of Tl^+-free medium. Table I shows a relatively fast efflux at 20°C. For comparison, the efflux of K^+ was also measured and found to be several orders of magnitude slower than the rate of Tl^+ efflux. The rate of efflux was not appreciably affected by the energy state of the mitochondria. It is also shown in Table I that the rate of efflux was decreased by lowering the temperature, as could be expected. It is of interest that Mg^{2+} also retarded the efflux of Tl^+. Mg^{2+} has been reported to inhibit the efflux of K^+ in heart mitochondria (Chavez *et al.*, 1977). In heart mitochondria K^+ efflux was stimulated by respiration (Chavez *et al.*, 1977), which was without effect in rat liver mitochondria (Table I).

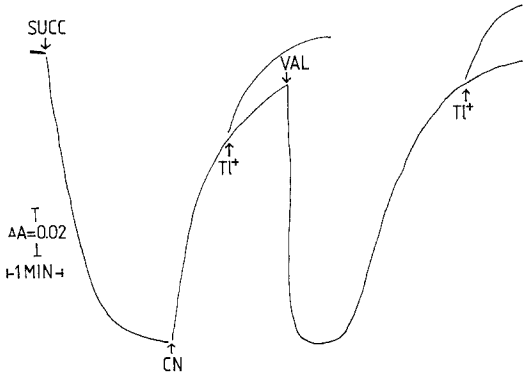

Fig. 4. Stimulation by Tl^+ of the decay of potentials formed by respiration and by valinomycin-induced efflux of K^+ from mitochondria. The medium contained 250 mM sucrose, 10 mM Hepes, pH 7.2, 6 μM rotenone, 2 μg oligomycin/ml, 100 μM EGTA, 18 μM safranine, and mitochondrial protein 1.5 mg/ml, pH 7.2. Additions: 2 mM Tris-succinate, 2 mM NaCN, 1 mM Tl acetate, and 360 nM valinomycin.

Interaction of Tl^+ with Electric Fields and ΔpH in Mitochondria

We have earlier shown that Tl^+ moves in response to valinomycin-induced K^+ diffusion potentials in mitochondria (Skulskii *et al.*, 1978b). Figure 4 shows that small concentrations of Tl^+, 1 mM, were able to increase the rate of decay of the safranine signal, which is formed during respiration on succinate and which decays on inhibition of respiration. This shows that Tl^+ is able to interact with electric fields in the membrane in the absence of valinomycin. Figure 4 also shows the effect of Tl^+ on the decay of the valinomycin-induced diffusion potential.

The interaction of Tl^+ with the fields is too slow to be seen in respiring mitochondria (Skulskii *et al.*, 1978b), since the rate of respiration on succinate is fast enough to compensate for any decay by Tl^+ movements. Figure 4 shows that the safranine signal obtained by respiration and by valinomycin-induced efflux of K^+ was of the same magnitude. The rate of decay brought about by Tl^+ in the presence of valinomycin seems somewhat higher than in its absence, possibly due to changed permeability properties of the membrane in the presence of valinomycin. Electrophoretic influx of Tl^+ due to the electric field and Tl^+ efflux by Tl^+/H^+ exchange would, if occurring simultaneously, bring about cycling of Tl^+. This would constitute an energy drain on mitochondria. In this case the rate of release of Ca^{2+} following inhibition of respiration should be increased. Figure 5 shows that there was no increased rate of release of accumulated Ca^{2+} up to 5 mM Tl^+, while there was a clear stimulation at 10 mM Tl^+. The exchange Tl^+/H^+

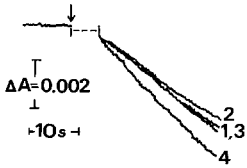

Fig. 5. Effect of Tl^+ on Ca^{2+} efflux from mitochondria. Ca^{2+} (100 μM) had been accumulated by the mitochondria (2.0 mg protein/ml) suspended in a medium containing 200 mM sucrose, 30 mM Hepes, pH 7.2, 2 mM Tris-succinate, 6 μM rotenone, and 80 μM murexide. Tl + K acetate was kept constant as 15 mM. At the arrow, 1 μg antimycin A was added per milligram protein in order to inhibit respiration. Concentration of Tl^+: (1) 0 mM; (2) 1 mM; (3) 5 mM; (4) 10 mM. Absorbance was recorded at 540–507 nm.

would also increase the permeability towards H^+. With low concentrations of Tl^+, we were, however, unable to see any such effects. The rate of pH change due to influx of H^+ into mitochondria after an acid pulse was not changed by 1 mM Tl^+ (not shown).

Conclusion

The mechanism of Tl^+ transport in mitochondria seems to be in accordance with the general schemes proposed for monovalent cation transport in mitochondria. Tl^+, an analogue of K^+, may penetrate the inner membrane electrogenically or by electroneutral Tl^+/H^+ exchange. The electrogenic or electrophoretic movement of Tl^+ explains the ability of Tl^+ to cause decay of signals of the membrane potential probe safranine. It also explains the swelling of mitochondria in media containing $TlNO_3$ under nonenergized conditions and the inhibition or reversal of this under energized conditions. The stimulation of swelling under energized conditions in the presence of Tl^+ as the acetate is also explained in this way. Tl^+/H^+ exchange occurs in the presence of Tl^+ acetate under nonenergized conditions. This mechanism seems to be induced by high concentrations of Tl^+, 10 mM or more, while no evidence was obtained for an exchange at low concentrations of Tl^+. Tl^+ distributions may therefore be used, with caution, to study membrane potentials in mitochondria.

Efflux of trace concentrations of Tl^+ was fairly rapid and could be inhibited by Mg^{2+} and by lowering the temperature.

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