UNCOUPLING OF MITOCHONDRIAL OXIDATIVE PHOSPHORYLATION BY THALLIUM

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SUMMARY: Thallium in the absence of ionophores stimulates succinate oxidation rates in rat liver mitochondria. Mitochondria oxidizing ascorbate undergo large amplitude swelling in the presence of thallium, indicating that thallium is actively accumulated. Similar responses with potassium are possible only if valinomycin is present. Thallium thus appears to act as an uncoupler of energized mitochondria, and mitochondrial membranes can differentiate thallium from potassium.

The effect of the monovalent thallous ion (Tl^+) in biological systems is thought to resemble that of potassium ions (K^+) since both ions have similar ionic charges and similar crystal radii; 1.33 Å for K^+ and 1.47 Å for Tl^+ (1). Thus, it has been observed that Tl^+ can effectively replace K^+ in the activation of K^+ dependent enzymes such as pyruvate kinase (2) and Na^+,K^+-ATPases (3,4), and in depolarization of muscle fiber membranes (5). Membranes appear unable to distinguish Tl^+ from K^+, and it has been noted that the transport of Tl^+ across red blood cell membranes was competitive with K^+ (6).

Spencer et al. (7) observed that mitochondria in the axons of peripheral nerve fibers became enlarged and swollen after exposure to thallium salts. Tl^+ may bind to mitochondrial membranes (8) causing changes in their molecular structure. In our studies it was noted that thallium can be actively transported into isolated rat liver mitochondria without the addition of an ionophore. Active thallium accumulation resulted in swelling of the mitochondria and uncoupling of oxidative phosphorylation.

METHODS AND MATERIALS

Rat liver mitochondria were isolated by the method of Stancliff et al. (9) and submitochondrial particles were prepared by sonication of the mitochondria according to Kielley and Bronk (10). Oxidative phosphorylation was measured polarographically using a Clark oxygen electrode (11). Volume changes in mitochondria due to active Tl^+ or K^+ uptake were followed in a Varian 635 spectrophotometer at 540 nm.

ATPase activity was measured as previously described (12). Segments of the electron transport chain (NADH oxidase, succinoxidase, and ascorbate-TMPD* oxidase) were measured by published methods (13). Protein concentrations were determined by the Biuret method (14) using bovine serum albumin as a standard.

ATP, ADP, NADH, pyruvate kinase, lactate dehydrogenase, phosphoenol pyruvate, valinomycin, and bovine serum albumin were purchased from Sigma Chemical Co. All other chemicals were reagent grade.

RESULTS AND DISCUSSION

The effect of thallium(ous) acetate on succinate oxidation in isolated rat liver mitochondria is shown in Fig. 1. State 4 respiration rates were stimulated by the addition of Tl^+, and at high Tl^+ concentrations (20 mM) the rate approached the state 3 respiration rate obtainable by ADP addition. Half maximal stimulation of succinate oxidation was reached at about 6.5 mM Tl^+. Stimulation of respiration by Tl^+ reduced the stimulation increment resulting from ADP addition. The effect of Tl^+ thus resembles the uncoupling of mitochondrial oxidative phosphorylation by K^+ plus valinomycin (15), however, with Tl^+ no ionophore is necessary. Twenty-five mM K^+ in the absence of valinomycin did not significantly stimulate succinate oxidation rates.

Two possibilities may account for Tl^+ stimulation of mitochondrial respiration; 1. Tl^+ has a damaging effect on the integrity of the inner mitochondrial membrane, or 2. Tl^+ is actively accumulated by respiring mitochondria and thereby deenergizes them. The first possibility was dismissed since we could restore stimulation of succinate oxidation rates by ADP in mitochondria which were treated with 25 mM Tl^+, after

*Abbreviations: TMPD, tetramethylphenylenediamine

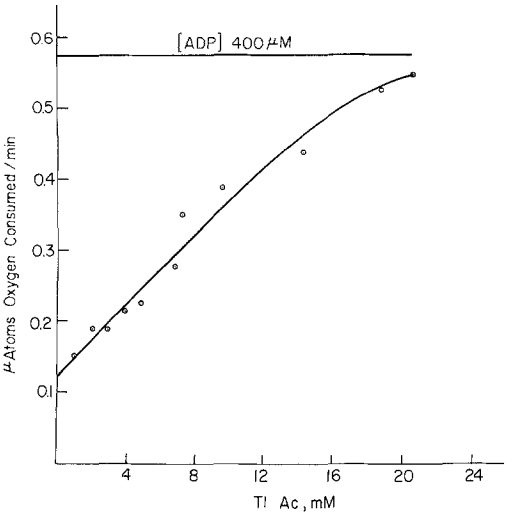

Fig. 1 Stimulation of Succinate Oxidation by Thallium(ous) Acetate in Rat Liver Mitochondria. Oxygen consumption was followed polarographically at 25°C using a Clark oxygen electrode in a 5 ml reaction medium consisting of 250 mM sucrose, 3 mM $MgCl_2$, 3 mM $NaPO_4$, 5 mM sodium succinate, 5 mM Tris-acetate buffer (pH 7.5) and various concentrations of thallium acetate. ADP was added to make a final concentration of 400 uM. Mitochondria were present at 1.2 mg/ml.

separation of the mitochondria from Tl^+ by centrifugation.

The second possibility was tested by comparing ascorbate-TMPD dependent K^+ uptake in whole mitochondria to the same energy dependent process for Tl^+ uptake. Ascorbate oxidation with TMPD is sufficient to energize mitochondria to actively drive K^+ accumulation when valinomycin is present. Such a process can be followed spectrophotometrically since water follows the uptake of K^+, resulting in swelling of the mitochondria and a decrease in optical density. A typical trace, measured at 540 nm, is shown in Fig. 2A. Swelling with K^+ in the absence of valinomycin does not occur (Fig. 2C) since this cation is impermeable to the inner mitochondrial membrane. Tl^+, in the absence of any added ionophore, caused ascorbate-TMPD energized mitochondria to undergo large amplitude swelling associated with active ion accumulation (Fig. 2B). Such a finding indicates that mito-

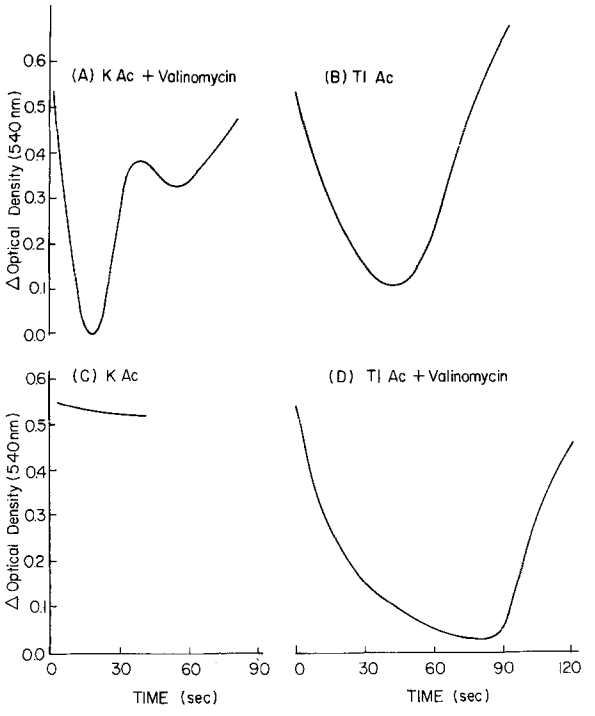

Fig. 2. Volume Changes in Mitochondria due to Tl^+ or K^+ Uptake. The reaction was measured in a 3 ml volume consisting of 100 mM sucrose, 5 mM Tris-acetate buffer (pH 7.5), plus either 25 mM potassium acetate or 25 mM thallium(ous) acetate. The mitochondria were energized by addition of 10 umoles of ascorbate plus 0.6 umoles of tetramethylphenylenediamine. 0.01 ug/ml valinomycin was added where indicated.

chondria are naturally permeable to Tl^+. Swelling with Tl^+ was slower than swelling with K^+ plus valinomycin; this may reflect differences in permeability. Mitochondria also swell with Tl^+ when valinomycin was added (Fig. 2D), however, in this latter case the rate of volume change was slower than when valinomycin was absent. Valinomycin would not be expected to promote mitochondrial permeability to Tl^+ since valinomycin cannot replace the hydration shell of Tl^+ (4) which would be necessary for the formation of a Tl^+-valinomycin complex. Valinomycin, however, may interfere with active Tl^+ transport.

We also examined the effects of Tl^+ on various segments of the electron transport chain and on ATPase activity in submitochondrial

Table 1. Effect of TlAc and KAc on Electron Transport and ATPase Activity in Submitochondrial Particles

Cation addition	NADH oxidase[a]	Succinoxidase[a]	Ascorbate-TMPD oxidase[a]	ATPase[b]
		Specific Activities		
None	0.109	0.076	0.190	1.33
8.3 mM TlAc	0.118	0.066	0.183	0.97
8.3 mM KAc	0.105	0.069	0.198	1.11

[a]specific activity is expressed as uatoms oxygen/min-mg.

[b]specific activity is expressed as uatoms ATP/min-mg.

particles (Table 1). In every case we were unable to find significant inhibition or stimulation of activities by Tl^+ when compared to similar tests with K^+.

The uncoupling of oxidative phosphorylation in rat liver mitochondria by Tl^+ appears to result from an active accumulation of Tl^+ by energized mitochondria. Tl^+ does not irreversibly alter membrane integrity nor inhibit electron transport or ATPase activities. The effect of Tl^+ in the absence of an ionophore is similar to the response of energized mitochondria to K^+ plus valinomycin (15). The finding that the mitochondrial membrane can differentiate Tl^+ from K^+ suggests the possibility of a natural carrier or ionophore in the inner mitochondrial membrane which can selectively bind and transport Tl^+. Thus Tl^+ translocation resembles Ca^{++} transport for which a carrier has been suggested (16).

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