J. Membrane Biol. 44, 187–194 (1978)

Electrophoretic Transport of Tl^+ in Mitochondria

Igor A. Skulskii, Margarita V. Savina, Vadim V. Glasunov, and Nils-Erik L. Saris*

Institute of Evolutionary Physiology and Biochemistry, Academy of Sciences, Leningrad, U.S.S.R., and the Department of Medical Chemistry, University of Helsinki, Helsinki, Finland

Received 10 July 1978

Summary. The distribution of Tl^+ between rat liver mitochondria and the medium was studied; millimolar or smaller concentrations of Tl^+ were labeled with ^{204}Tl. The Tl^+ distribution responded to transient diffusion potentials in a way that indicated electrophoretic movements of Tl^+. The diffusion potentials were induced by efflux of K^+ in response to addition of valinomycin to nonrespiring mitochondria suspended in a medium with low concentrations of K^+ or by efflux of H^+ induced by making the medium more alkaline in the presence of a protonophorous (proton-conducting) uncoupling agent. Changes in membrane potential induced by valinomycin were followed with the aid of safranine. Tl^+ brought about collapse of the diffusion potential. It is concluded that Tl^+ is able to penetrate the mitochondrial membrane electrophoretically.

It has been shown that Tl^+, in contrast to K^+, may readily penetrate the membranes of erythrocytes [10] and bacteria [9]. It has also been found that the cell/medium distribution of Tl^+ in erythrocytes can be used for estimating membrane potential [10]. The interaction of Tl^+ with mitochondria is, however, controversial. The uptake of Tl^+ by mitochondria has either been interpreted as being due to energy-independent binding [3] or to permeation of the inner membrane with osmotic swelling [7, 8]. Tl^+ at comparatively high concentrations, >5 mmol/liter, inhibits mitochondrial K^+ fluxes [3, 4]. The inhibition of influx of K^+ is competitive, and since efflux of K^+ also is inhibited, Tl^+ may compete with K^+ for transport sites on the matrix side, too [4].

In this study we present evidence that Tl^+ indeed is able to permeate the mitochondrial inner membrane. The movement of Tl^+ is shown to be electrophoretic. It may therefore be possible to use Tl^+ as a probe of the

* *To whom reprint request should be addressed:* Department of Medical Chemistry, University of Helsinki, SF-00170, Helsinki 17, Finland.

0022-2631/78/0044-0181 $01.60

membrane potential, which is of central importance in theories on the mechanism of oxidative phosphorylation.

Materials and Methods

Rat liver mitochondria were prepared by conventional methods [11, 12]. The uptake of Tl^+ was measured using ^{204}Tl, sp. act 440 Ci/mol (Amersham, England), diluted with nonradioactive thallium acetate when 150 μmol/liter or higher concentrations were used. The uptake was initiated by the addition of mitochondria to the incubation medium, samples of 0.10 ml were taken and mixed with 2.0 ml ice-cold thallium-free medium, and mitochondria were separated by filtration through Millipore® filters, pore size 0.6 μm. The filters were washed once with 2 ml ice-cold medium, dried, and the radioactivity was measured in a β-spectrometer with a Geiger-Müller tube. The background of the filters was determined by filtering aliquots of the incubation medium. The results of the uptake experiments were expressed as a Tl-distribution ratio "r" calculated from the radioactivity of ^{204}Tl per mg mitochondrial protein, assuming matrix space to correspond to 1 μl/mg protein, and that of 1 μl medium. The K^+ content of mitochondria was determined by flame emission photometry after extraction of K^+ from washed mitochondria into 0.1 mol/liter nitric acid. The amount of mitochondrial protein was measured by the Lowry procedure with bovine serum albumin V as standard [6]. Membrane potential was measured with safranine [1, 2] by using the wavelength pair 524–554 nm in an Aminco-Chance dual wavelength photometer Model DW-2.

CCCP (Carbonyl cyanide *m*-chlorophenyl hydrazone) was purchased from Sigma Chemical Co., St. Louis, Mo.; FCCP (Carbonyl cyanide *p*-trifluoromethoxy phenylhedrazone) was a kind gift of Dr. P. G. Heutler.

Results

1. Tl^+ Distribution Changes Induced by Diffusion Potentials

A diffusion potential – negative inside – can readily be created by increasing the permeability towards K^+ by addition of the ionophore valinomycin to non-respiring mitochondria suspended in a medium containing no K^+ or low concentrations of K^+. Figure 1 shows that in the presence of trace amounts of Tl^+, 100 μmol/liter, induction of a diffusion potential caused a transient uptake of Tl^+. The maximal uptake was obtained in about 30 s, and then the initial steady-state ratio was again approached with a half-time of about 30 s. At lower valinomycin concentrations this relaxation was slower (data not shown).

In the presence of a protonophorous uncoupling agent, no diffusion potential is formed and no transient uptake of Tl^+ was observed, since the outflux of K^+ was compensated for by influx of H^+.

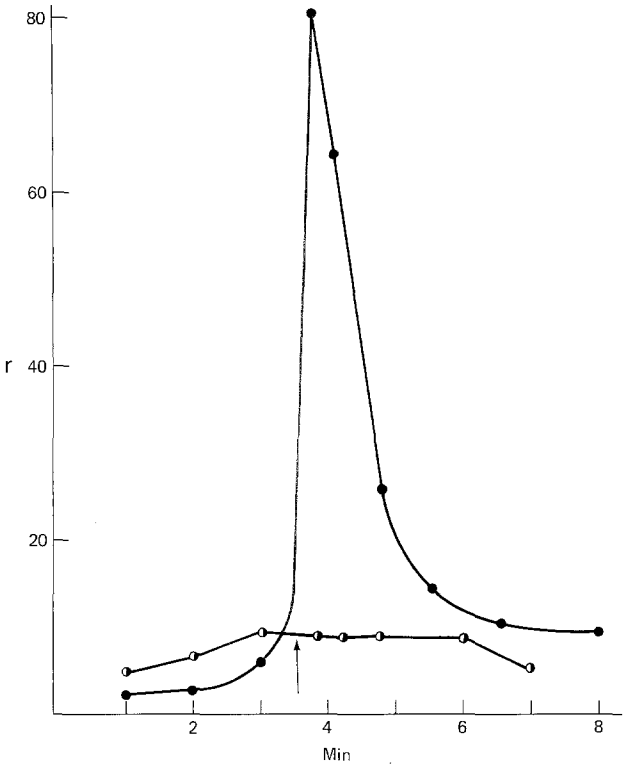


Fig. 1. The uptake of Tl^+ in response to a K^+ diffusion potential. The medium contained 150 mmol/liter sucrose, 50 mmol/liter Tris-acetate, 5 μmol/liter rotenone, 150 μmol/liter Tl^+, pH 7.4, 20–22 °C. Mitochondria contained 120 nmol K^+/mg protein (2 mg mitochondrial protein/ml). r: $\{Tl^+\}_i/\{Tl^+\}_o$; ↑: Addition of valinomycin; ◑—◑: 1 μmol/liter CCCP present

The magnitude of the valinomycin-induced diffusion potential can be varied according to the Nernst equation by varying the concentration of K^+ in the medium. Figure 2 summarizes the results of a series of experiments similar to the one in Fig. 1. It is seen that the magnitude of the Tl^+ uptake was related to the magnitude of the K^+ ratios.

A diffusion potential can also be obtained by decreasing the H^+ concentration in the medium in conditions in which the mitochondria have been rendered permeable towards H^+ ions by addition of a protonophorous uncoupling agent. Figure 3 shows that, indeed, a spike of transient Tl^+ uptake was obtained by increasing the pH of the medium. After this, however, the steady-state distribution was altered, probably because of a change in the Donnan equilibrium. The transient Tl^+ uptake was seen only with very low Tl^+ concentrations, 1 μmol/liter;

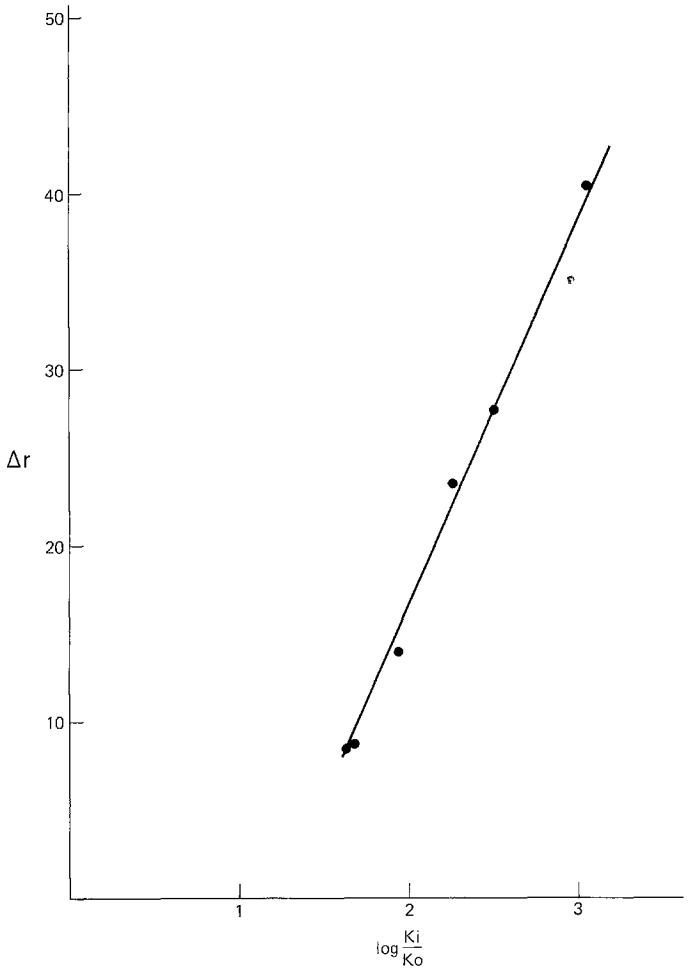
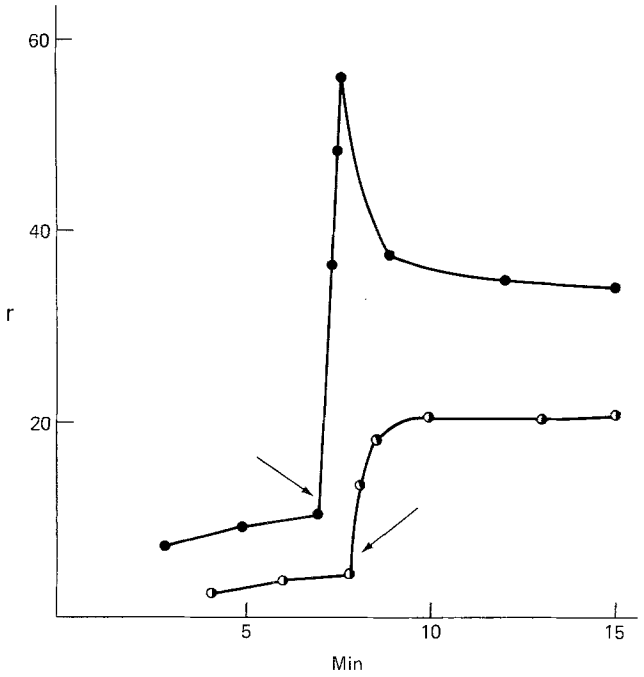


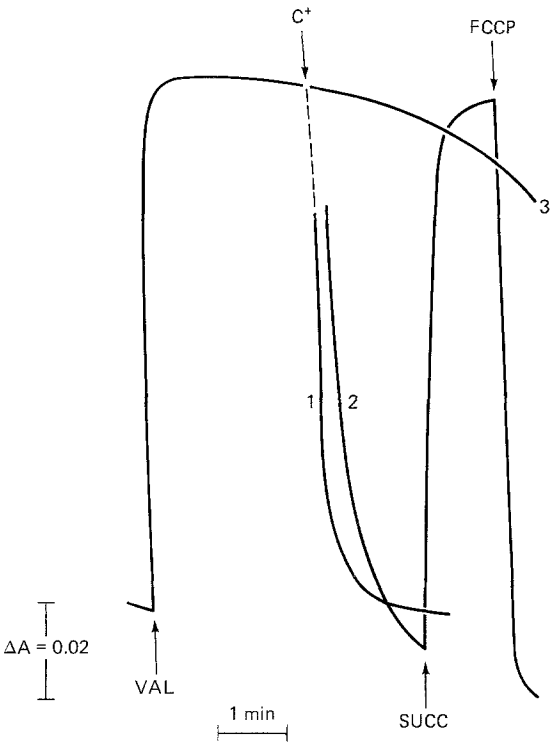
Fig. 2. The relationship between uptake of Tl^+ induced by valinomycin to the initial K^+ gradient. Conditions were as in Fig. 1 but varying concentrations of K^+ were used in the medium. The Tl^+ uptake was measured at 30 s after the additions of valinomycin and expressed as increment in Tl^+ ratios over steady-state ratios

Fig. 3. The uptake of Tl^+ in response to a H^+ diffusion potential. Conditions were as in Fig. 1. Mitochondria were pre-incubated with 1 μmol/liter CCCP for 10 min before the addition of Tris-base to increase the pH (arrows). ●—●: 0.5 μmol/liter Tl^+, pH change from 6.8 to 7.7; ◑—◑: 150 μmol/liter Tl^+, pH change from 6.0 to 7.4

Fig. 4. Collapse of K^+ diffusion potential effected by Tl^+. The medium contained 250 mmol/liter sucrose, 10 mmol/liter Hepes, pH 7.2, 5 μmol/liter rotenone, 20 μmol/liter safranine and 1.5 mg/ml mitochondrial protein. ↑VAL: addition of valinomycin; C^+: addition of cation 10 mmol/liter. Trace 1: cation added was K^+; Trace 2: cation added was Tl^+; Trace 3: cation added was Na^+ or none



3



4

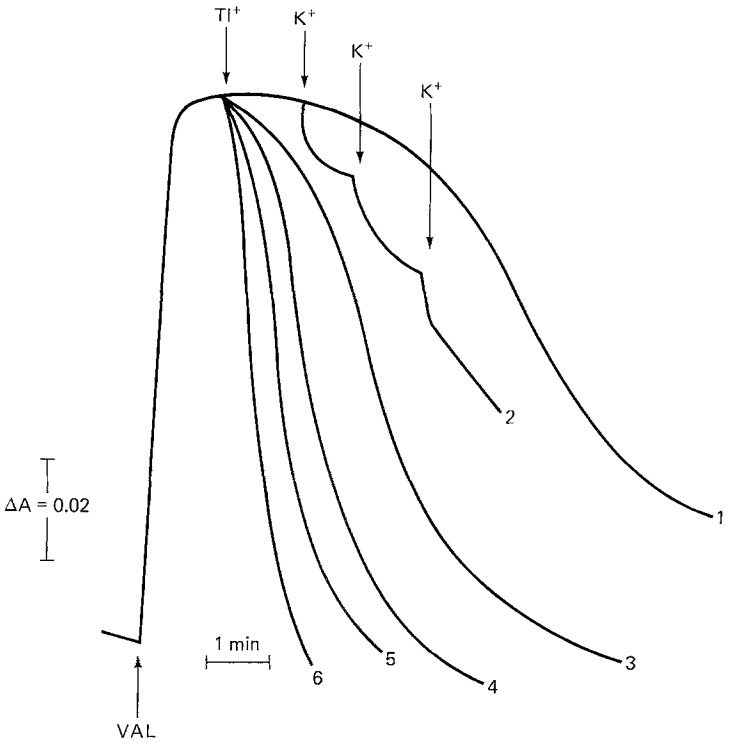


Fig. 5. Collapse of the diffusion potential effected by small amounts of Tl^+. Experimental conditions were as in Fig. 4. Trace 1: Control without addition of cations; Trace 2: Control with successive additions of 0.25 mmol/liter K^+; Trace 3–6: Tl^+ added as nitrate, 0.25, 0.5, 1.0, and 2.0 mmol/liter, respectively

with higher concentrations, only a shift of the steady-state distribution was observed.

2. *Collapse by Tl^+ of Mitochondrial Membrane Potential*

Safranine is a hydrophobic cation that exhibits a spectral shift as a response to a membrane potential [1, 2]. Figure 4 shows the formation of the diffusion potential when valinomycin is added to mitochondria suspended in a K^+-free medium. The potential slowly decays (trace 3). Addition of 10 mmol/liter K^+ abolished the signal almost completely (trace 1) by diminishing the K^+ gradient. Addition of a corresponding amount of Tl^+ also caused the potential to collapse (trace 2). Na^+ was without effect (trace 3).

Figure 4, trace 2, also shows that after the membrane potential has been abolished by this rather high concentration of Tl^+, the mitochon-

dria could still be induced to exhibit the safranine signal by addition of succinate. Respiration thus caused "energization" of the mitochondria. Addition of an uncoupling agent then abolished the safranine signal.

In Figure 5 we studied the effect of low Tl^+ concentrations. Even 0.25 mmol/liter Tl^+ increased the rate of relaxation of the membrane potential, and higher concentrations were progressively more effective.

Discussion

The data presented demonstrate an uptake of Tl^+ in response to diffusion potentials in mitochondria. The uptake is transient, which strongly suggests an electrophoretic movement of Tl^+ or of its complex with an intrinsic carrier. It could be suggested that there is a formation of a complex with valinomycin in the K^+ experiments. However, Tl^+ forms only weak complexes with valinomycin [5] and we have not obtained any indications of complex formation in our experiments. Another interpretation would be that the transient uptake of Tl^+ was due to the formation of binding sites or to changes in the affinity of binding sites when a diffusion potential is induced. but this seems highly unlikely. Certainly, Tl^+ may to some extent be bound to phospholipids and to other binding sites, and we cannot know exactly the activity of Tl, nor of other ions, in the matrix space.

The Tl_i^+/Tl_o^+ ratios obtained (Figs. 1 and 2) were much smaller than the initial K^+ ratios. The response of Tl^+ to changes in the membrane potential were somewhat sluggish. The rates of Tl^+ influx were probably smaller than the rates of K^+ efflux. It seems probable that by the time of peak Tl^+ ratios, the K^+ ratios were already much diminished. Probes of the membrane potential, like safranine, respond fast to changes in membrane potential and are useful in kinetic studies, while Tl^+ may prove useful in steady-state conditions.

The collapse of the diffusion potential brought about by Tl^+ (Figs. 4 and 5) could be due to increased H^+ permeability (uncoupling) or release of endogeneous Ca^{2+}, followed by reuptake (Ca^{2+}-cycling). However, the effect of Tl^+ was unaffected by the presence of EGTA, which by chelating Ca^{2+} prevents Ca^{2+} uptake. We could not observe any signs of uncoupling due to increased H^+ permeability. Thus, the release of accumulated Ca^{2+} from mitochondria, upon inhibition of respiration, which is stimulated even by slight uncoupling, was unaffected by millimolar concentrations of Tl^+ (data not shown). Energization of mitochondria by

succinate respiration was unaffected by the presence of 10 mmol/liter Tl^+ (Fig. 4). It has been reported [7] that mitochondria, which have been incubated in media with high concentrations of Tl^+ (25 mmol/liter), were still able to exhibit ADP-stimulated respiration after separation from the medium by centrifugation. This shows that no membrane damage had occurred. We therefore conclude that the abolition of the diffusion potential by Tl^+ indeed was due to penetration of Tl^+ itself.

These results support our earlier interpretation, based upon rapid equilibrium of mitochondrial Tl^+ with the medium after dilution, that Tl^+ is able to penetrate the mitochondrial membranes [8]. Our results also support the opinion that swelling in nonenergized conditions [8], as well as respiration-driven swelling [7] in media containing rather high concentrations of Tl^+, is due to penetration of Tl^+.

This study was supported by a grant from the Sigrid Jusélius Foundation in Finland.

References

1. Åkerman, K.E., Saris, N.-E.L. 1976. Stacking of safranine in liposomes during valinomycin-induced efflux of potassium ions. *Biochim. Biophys. Acta* **426**:624
2. Åkerman, K.E.O., Wikström, M.K.F. 1976. Safranine as a probe of the mitochondrial membrane potential. *FEBS Lett.* **68**:191
3. Barrera, H., Gomez-Puyou, A. 1975. Characteristics of the movement of K^+ across the mitochondrial membrane and the inhibitory action of Tl^+. *J. Biol. Chem.* **250**:5370
4. Diwan, J.J., Lehrer, P.H. 1977. Inhibition of mitochondrial potassium ion flux by thallous ions. *Biochem. Soc. Trans.* **5**:203
5. Eisenman, G., Szabo, G., Ciani, S., McLaughlin, S., Krasne, S. 1973. Ion binding and ion transport produced by neutral lipid-soluble molecules. *In:* Progress in Surface and Membrane Science. Vol. 6, p. 187. J. F. Danielli, M.D. Rosenberg, and D.A. Cadenhead, editors. Academic Press, New York
6. Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J. 1951. Protein measurement with the Folin reagent. *J. Biol. Chem.* **193**:265
7. Melnick, R.L., Monti, L.G., Motzkin, S.M. 1976. Uncoupling of mitochondrial oxidative phosphorylation by thallium. *Biochem. Biophys. Res. Commun.* **69**:68
8. Skulskii, I.A. 1977. Transport of monovalent thallium ions through mitochondrial membranes. *Dokladi Acad. Sci. U.S.S.R.* **232**:945
9. Skulskii, I.A., Glasunov, V.V., Rjabova, I.D., Gorneva, G.A. 1977. Selective permeability of bacterial membranes for monovalent thallium ions. *Biochimija* **42**:1637
10. Skulskii, I.A., Manninen, V., Järnefelt, J. 1978. Factors affecting the relative magnitudes of the ouabain-sensitive and the ouabain-insensitive fluxes of thallium ion in erythrocytes. *Biochim. Biophys. Acta* **506**:233
11. Stancliff, R.C., Williams, M.A., Utsumi, K., Packer, L. 1969. Essential fatty acid deficiency and mitochondrial function. *Arch. Biochem. Biophys.* **131**:629
12. Wikström, M.K.F., Saris, N.-E.L. 1969. Effect of hydroxylamine on respiration and oxidative phosphorylation. *Eur. J. Biochem.* **9**:160