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THE CHLORIDE TRANSPORT INDUCED BY TRIALKYL-TIN COMPOUND ACROSS ERYTHROCYTE MEMBRANE

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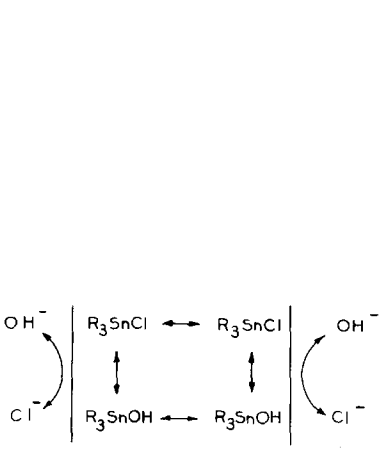
Summary

The effect of tripropyl-tin chloride on anion permeability was studied using red cells previously treated with a covalent binding inhibitor 4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonic acid (SITS) to inhibit completely and irreversibly the natural anion transport system. It was demonstrated that the tin compound can mediate chloride-hydroxide and chloride-chloride exchanges across the "impermeabilised" erythrocyte membrane. In the non hemolytic range, the rate of exchange increased with the concentration of the tin compound in a non linear fashion, and no saturation effect was seen. The temperature profile of the chloride self exchange induced by tripropyl-tin was studied and the apparent activation energy found was 29 Kcal/mol.

The tripropyl-tin chloride cannot mediate a chloride-bicarbonate exchange. Because of this discriminatory effect between hydroxide and bicarbonate, the tin compound can be useful in certain experimental conditions as seen for the study of the anion "carrier" of the red cell membrane (Cousin, J.L., Motais, R. and Sola, F. (1975) J. Physiol. Lond. 253, 385—399).

The trialkyl-tin compounds (R_3SnX) have been shown to have several effects on mitochondria, notably inhibition of oxydative phosphorylation [1,2] and induction of chloride-hydroxide exchange across the membrane [3]. The effect on anion exchange cannot be due to an interaction with the specific anion-transporting systems of mitochondria since it can also be observed with erythrocyte and artificial lipid membrane [4]. It is assumed [4] that the trialkyl-tin exists in the membrane either in the hydroxide or chloride form and, according to the anion concentration gradients in the aqueous solutions it shuttles between the two lipid-water interphases (Fig. 1). Thus the tin compound would act as a carrier for Cl^- and OH^-. It would be an interesting model for the transmembrane exchange of anions when the only driving forces are



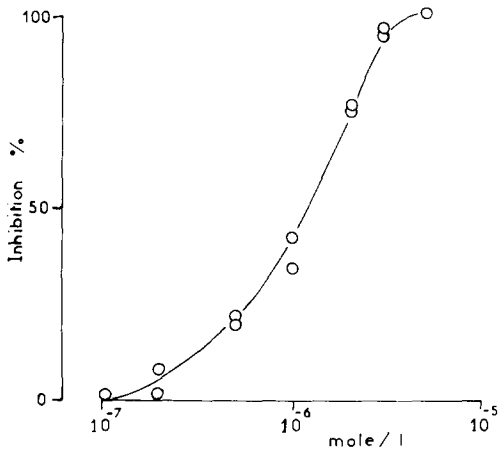


Fig. 1. The assumed mechanism for chloride-hydroxide exchange mediated by a trialkyl-tin compound.

Fig. 2. Inhibition of chloride equilibrium self exchange by covalently bound SITS at different concentrations. The cells were exposed to the various concentrations of the agent at 5°C for 2 h in darkness (hematocrit 5%). Subsequently the excess agent was removed by washing the cells five times (twice with 0.5% albumin in the ringer solution). The cells were resuspended in a medium without SITS at a hematocrit of 0.5% for measuring ^{36}Cl efflux at 0°C.

concentration gradients across the membrane, a situation found in the red blood cell for anions such as Cl^-, OH^- and HCO_3^-.

In an attempt to define some of the permeability properties of these compounds, therefore, experiments were carried out to answer the following two questions.

(1) Can the tin-compounds mediate not only a chloride-hydroxide exchange but also a chloride-bicarbonate exchange? (2) Can the tin compounds mediate an exchange between internal and external chloride (chloride self exchange) as would be expected if the assumed model (Fig. 1) is valid?

Experiments were performed using the tripropyl-tin chloride ($(CH_3CH_2CH_2)_3SnCl$) as prototypical of trialkyl-tin compounds and ox erythrocyte as the biological membrane. The chloride permeability was measured by determining the rate of tracer efflux from radioactively labelled red cells either under steady-state conditions of anion concentrations for demonstrating the Cl/Cl exchanges (technique in ref. 9), or after suspension in a Na^+ gluconate solution, for studying Cl^-/OH^- and Cl/HCO_3^- exchanges (technique in ref. 8). However, in order to permit a clear understanding of the permeability properties of the tin compound, the "natural" anionic permeability of erythrocytes was previously abolished. This was accomplished by fully inhibiting the transporting system with a specific inhibitor, 4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonic acid (SITS), which can be covalently bound at the transporting site [5]. Indeed, as illustrated in Fig. 2, after incubation of ox red cells in a Ringer solution containing SITS (B.D.H.) at 10^{-5} M (hematocrit 5%, 5°C, pH 7.4) followed by extensive washing with albumin, the inhibition of chloride self exchange is practically total and irreversible. Impermeabilisation of the red cell membrane to organic anions can also be obtained with the same treatment [6].

Thus, the effect of tripropyl-tin chloride on the permeability properties of red cell membranes was studied after such an impermeabilising treatment of the membrane by SITS 10^{-5} M. The tripropyl-tin chloride (B.D.H.) was added to the external medium as an alcoholic solution (100 µl/50 ml). At this concentration ethanol has no effect on the anion transport.

(a) Chloride-hydroxide exchange

To demonstrate that tripropyl-tin chloride produces a chloride-hydroxide exchange across the erythrocyte membrane, the following experimental procedure was used. Red cells were labeled with radioactive chloride and then the membrane was made impermeable to anions by binding covalently SITS as described above. After extensive washing, packed cells were injected into an isotonic unbuffered and HCO_3^- free sodium gluconate medium (165 mM). As expected, since the membrane has been made impermeable to anions, chloride did not leak out of the cell (Fig. 3a) and no pH modification of the unbuffered external solution was observed (Fig. 4a). In the same experimental conditions, but with tripropyl-tin chloride present in the medium at 10^{-4} M, a leak of internal chloride was observed immediately after suspension of the red cells (Fig. 3b). Such an increase of the chloride permeability induced by the tin compound could be explained either by a co-diffusion of NaCl (chloride being carried by the tin, and internal sodium by the "natural" transporting system for Na^+) or by an exchange diffusion between internal chloride and an available external anion, as postulated by the model. The first assumption is untenable since the red cell membrane has a well known low permeability for cations. The exchange diffusion between internal chloride and external gluconate is unexpected because the translocation by tripropyl-tin of gluconate, a big and very polar molecule, seems unlikely. Thus, in our experimental conditions the only external anion available for exchange with internal chloride is OH^-; such an exchange OH^-_{ext}/Cl^-_{int} must induce an acidification of the unbuffered external medium, and this is indeed observed as seen in Fig.

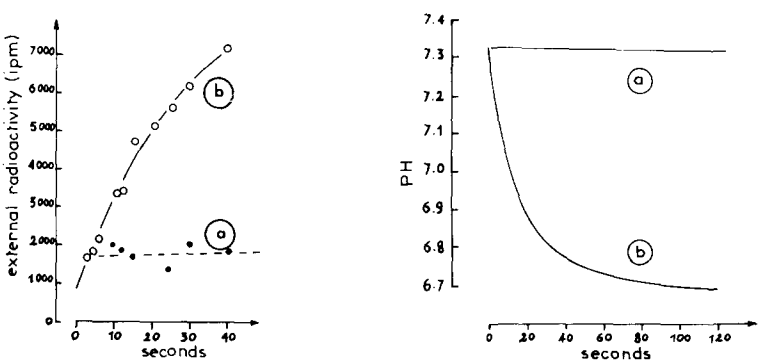


Fig. 3. Effect of tripropyl-tin chloride on the chloride efflux when "impermeabilised" red cells are suspended in an unbuffered gluconate medium. (a) In absence of tin compound; (b) in presence of tin compound (10^{-4} M).

Fig. 4. Effect of tripropyl-tin chloride on the external pH when impermeabilised red cells are suspended in an unbuffered gluconate medium. (a) Without tin compound; (b) with tin compound (10^{-4} M).

4b. These results confirm the previously published data, using the osmotic swelling technique [4,7], indicating that the tin compounds allow an exchange between chloride and hydroxide across the erythrocyte membrane.

(b) Chloride-bicarbonate exchange

In order to test the possibility that tripropyl-tin chloride could similarly mediate a chloride-bicarbonate exchange, the following experiments, illustrated in Fig. 5, were performed. "Impermeabilised" red cells, labelled with radioactive chloride were suspended in an isotonic solution of sodium gluconate, buffered with 20 mM HEPES (pH 7.4), and containing 10^{-4} M tripropyl-tin; the increase of radioactivity in the external medium (control curve a) represents the efflux of ^{36}Cl due to the exchange between internal chloride and external OH^-. No pH modification occurs since the solution is buffered with HEPES. If the tripropyl-tin can mediate a Cl^-/HCO_3^- exchange across the membrane it would be expected that in the presence of HCO_3^- in the external medium the chloride efflux would be enhanced. It can be seen (curve b) however, that the pattern of chloride efflux is unchanged by the addition, at time zero,

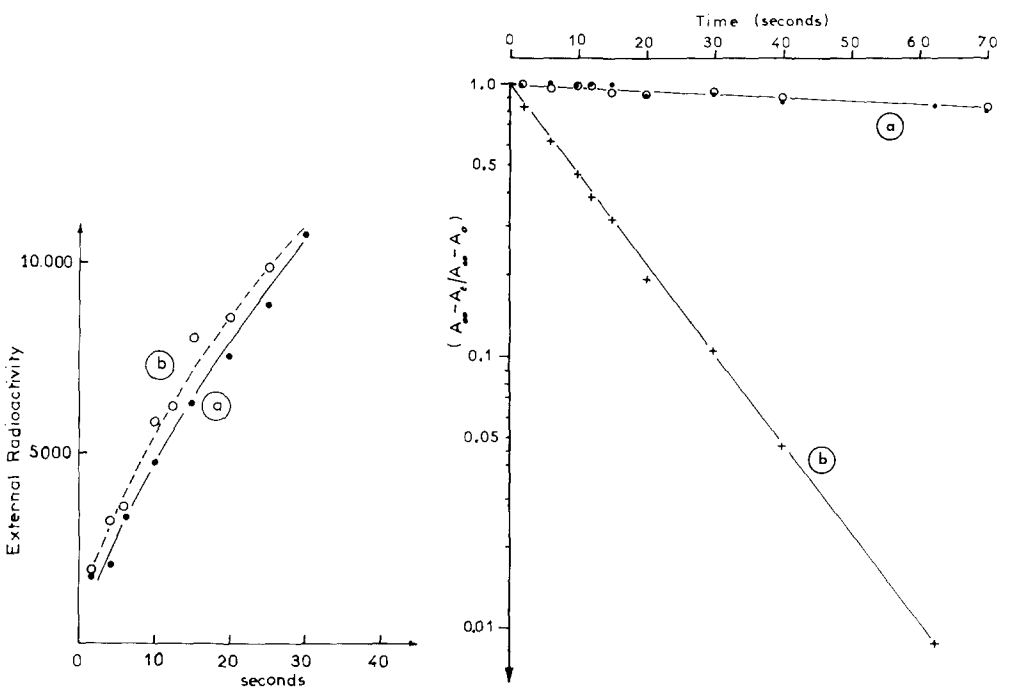


Fig. 5. Effect of addition of HCO_3^- to the external medium on chloride efflux mediated by tripropyl-tin chloride in impermeabilised red cells. (a) In absence of HCO_3; (b) in presence of 3 mM HCO_3. (The gluconate medium is strongly buffered so that addition of bicarbonate does not induce pH modification.)

Fig. 6. The rate of steady-state chloride self exchange at 13°C, pH 7.3 between impermeabilised red cells and the external medium with (X) and without (●,○) tripropyl-tin chloride. Ordinate: ln $(A_\infty - A_t)/(A_\infty - A_0)$, where A_∞, A_t and A_0 are the concentration of isotope in the external medium at equilibrium, time t, and 0 respectively.

of 3 mM HCO_3^- to the medium, i.e. a concentration of bicarbonate 10^4 times higher than the concentration of available hydroxide (because the solution is strongly buffered, addition of HCO_3 does not induce any pH modification).

The fact that tripropyl-tin chloride sharply discriminates between HCO_3^- and OH^- enables it to be used as a tool for studying the permeability characteristics of certain membranes. Thus, using this discriminatory property, we have recently been able to demonstrate that the physiological transporting system for anions in the cell highly mediates the bicarbonate transfer, but very poorly the hydroxide transfer [8].

(c) Chloride-chloride exchanges

The possibility that the tripropyl-tin chloride can induce a chloride self exchange, as expected from the assumed model (Fig. 1), has been tested in experiments illustrated in Fig. 6.

"Impermeabilised" red cells, labelled with radioactive chloride were suspended in a Ringer solution 150 mM NaCl, 10 mM KCl; 20 mM Tris pH 7.4). Under such steady-state conditions of anion concentration, the rate of tracer efflux from the cell was measured, as described previously [9] with and without tripropyl-tin chloride in the Ringer solution.

It can be observed that without the tin compound in the external medium (curve a) the radioactive chloride efflux is very small. This efflux might be due either to a residual self exchange of chloride through the incompletely inhibited transporting system or to a leak of Cl through the pathway of non-mediated anion transfer, operationally defined as "conductance pathway" [10]. In the presence of tripropyl-tin however, the chloride efflux is greatly increased (curve b) and since the experiments were run under steady conditions of anion concentration this result shows that, as expected, tripropyl-tin induces an exchange between external chloride and internal chloride.

The influence of tripropyl-tin concentration on the rate of chloride self exchange is illustrated in Fig. 7 (curve a). The given concentrations are those in the external medium with an 0.5% hematocrit and absorption on to the glass surface which was not taken into account. It can be seen that the rate of chloride self exchange increased abruptly from $3 \cdot 10^{-5}$ M to $3 \cdot 10^{-4}$ M in a non linear fashion. No saturation of the tripropyl-tin effect was seen in the non hemolytic range. With the amount of tripropyl-tin chloride in the membrane being unknown it is not possible to calculate the turnover number for Cl transport by tripropyl-tin chloride. The same effects can be observed for the Cl^-/OH^- exchange measured in isotonic gluconate medium (curve b).

The rate of chloride self exchange mediated by tripropyl-tin was studied over a temperature range 0—22° C. It is linearly related to the reciprocal of absolute temperature (Fig. 8) and from these data the apparent activation energy for the Cl efflux was estimated to be 29 Kcal/mol. It should be noted that this value is similar to the apparent activation energy measured on the same biological material (within the range 0—35° C) for the physiological self exchanges of organic (oxalate 26 Kcal/mol, ref. 9) and inorganic (Cl 25.9 Kcal/mol, ref. 11) anions.

The value can be compared with that found for valinomycin-induced K^+ permeability in the human red cell in the range 18—28° C, which was found

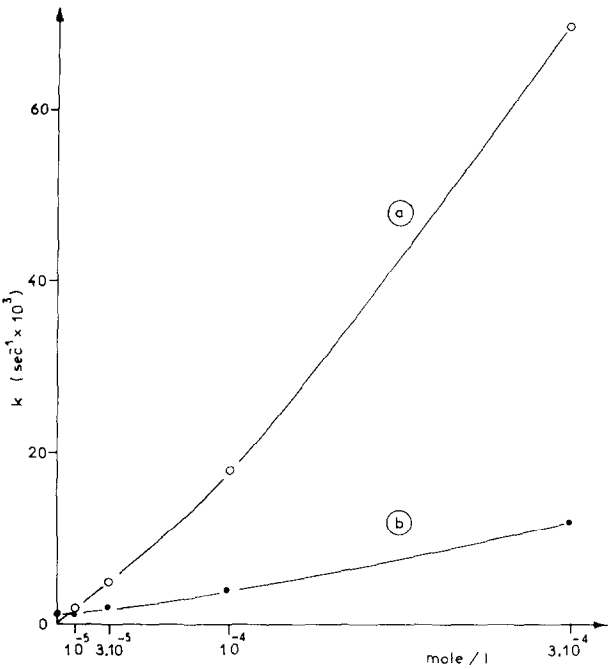


Fig. 7. Influence of the tripropyl-tin concentration on the rate of chloride efflux in impermeabilized red cells. (a) In a ringer solution; (b) in a sodium gluconate solution. Ordinate: the rate constant of (chloride efflux with tin compound—chloride efflux without tin compound). Abscissa: the concentration of tripropyl-tin chloride in the external medium, hematocrit 0.5%.

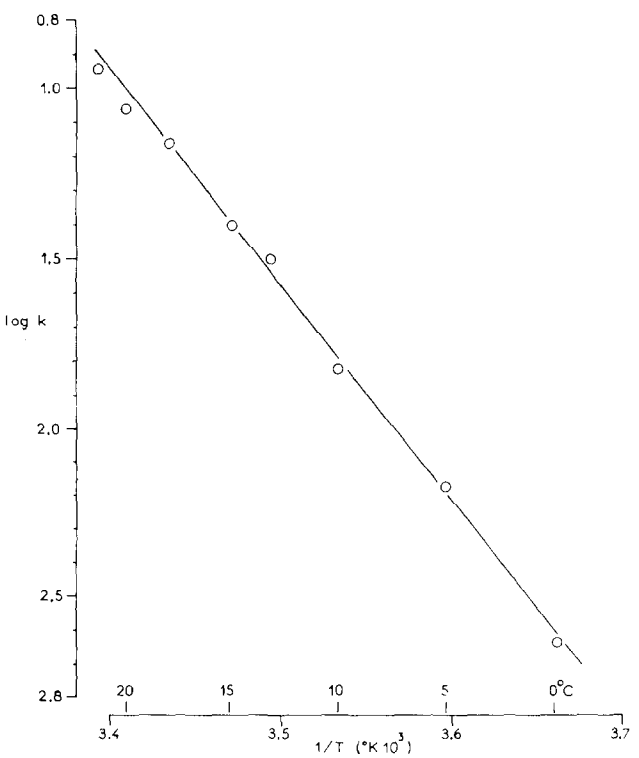


Fig. 8. Temperature dependence of chloride self exchanges mediated by tripropyl-tin chloride (10^{-4} M) in impermeabilised red cells.

to be 21 Kcal/mol [12] or 17.4 Kcal/mol when correction was made for the influence of temperature on valinomycin absorption by the membrane. Such an effect of temperature on the partitioning of tripropyl-tin between the bulk and the membrane has not been measured.

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