Biochimica et Biophysica Acta, 735 (1983) 185–188
Elsevier

BBA Report

BBA 70127

CADMIUM AND THALLOUS ION PERMEABILITIES THROUGH LIPID BILAYER MEMBRANES

JOHN GUTKNECHT

*Department of Physiology, Duke University Medical Center and * Duke University Marine Laboratory, Beaufort, NC 28516 (U.S.A.)*

(Received April 21st, 1983)
(Revised manuscript received August 9th, 1983)

Key words: Cadmium; Thallium; Tl^+ permeability; Cl^- permeability; Lipid bilayer

Cadmium (Cd^{2+}) and thallous ion (Tl^+) permeabilities were measured in planar (Mueller-Rudin) lipid bilayer membranes made from diphytanoylphosphatidylcholine in decane. Permeabilities of the electroneutral Cl^- complexes, measured with tracers (^{109}Cd and ^{204}Tl), were about 10^{-8} cm · s^{-1} for $CdCl_2$ and 10^{-6} cm · s^{-1} for TlCl. Electrical conductance measurements showed that permeabilities to Cd^{2+} and Tl^+ were approx. 10^{-11} cm · s^{-1}, similar to the Na^+ permeability. The low permeabilities to both Cd^{2+} and $CdCl_2$ are consistent with biological studies which suggest that Cd transport and toxicity are protein mediated and correlated with Cd^{2+}, not $CdCl_2$, concentration. However, the low bilayer permeability to Tl^+ raises questions about recent reports that Tl^+ is a lipid permeable cation in biological membranes and liposomes. An alternative explanation for the lipid permeable behavior of Tl^+ is presented, based on the diffusion of TlCl and other complexes of Tl^+ with inorganic and organic anions.

Heavy metal transport is important in physiology and toxicology, but the mechanisms of heavy metal transport are largely unknown. Recently I found that inorganic mercury (Hg^{2+}) diffusion through lipid bilayer membranes is 'facilitated' by Cl^- due to the very high permeability of $HgCl_2$ [1]. Cadmium (Cd^{2+}) and thallous ion (Tl^+) also form neutral complexes with Cl^- and other anions under physiological conditions [2]. Cadmium is important in environmental health and toxicology [3,4], and thallous ion is being used as a potassium analogue in biological systems [5,6] and as a probe for estimating transmembrane voltage [7,8]. Thus it is important to find out whether Cl^- complexes of these metals can diffuse through lipid bilayer and biological membranes.

Lipid bilayer (optically black) membranes were formed by the brush technique of Mueller and Rudin [9]. Membranes were formed from a mixture of diphytanoylphosphatidylcholine in *n*-decane (20 mg/ml). Membranes were formed on a 1.8 mm^2 hole in a polyethylene partition which separated two magnetically stirred solutions of 1.1 ml each. Aqueous solutions contained Cl^- or NO_3^- salts of Na^+, Cd^{2+} or Tl^+. The ionic strength was 0.15 and the pH was 5.8–6.1. Concentrations of metal complexes with Cl^- or NO_3^- were calculated from stability constants tabulated by Smith and Martell [2]. Temperature was 24 ± 2°C.

After a stable membrane was formed, 20–50 μCi of ^{109}Cd or ^{204}Tl was injected into the rear compartment. The rate of appearance of radioactivity in the front compartment was measured by continuous perfusion (1–2 ml · min^{-1}) and collection of samples at 3-min intervals. The samples were collected by aspiration into a vacuum trap. During the flux experiment the rear compartment was sampled with a microsyringe. Radioactivity was measured in a liquid scintillation counter.

* Mailing address for correspondence and reprint requests.

The one-way flux of solute was calculated by the equation:

$$J = \frac{{}^{*}C^{F}}{t\,A\,sa^{R}} \quad (3)$$

where J is the flux ($mol \cdot cm^{-2} \cdot s^{-1}$), ${}^{*}C^{F}$ is the total amount of tracer (cpm) entering the front compartment during the time interval t (s), A is the surface area of the membrane (cm^2) and sa^R is the specific activity of tracer in the rear compartment ($cpm \cdot mol^{-1}$).

Permeability coefficients of electroneutral complexes were calculated from tracer fluxes using the relation $P = J/c$, where c is the concentration of $CdCl_2$ or TlCl. Unstirred layer corrections were not necessary because the observed P was always less than 1% of the unstirred layer permeability, which is about 10^{-3} $cm \cdot s^{-1}$ [1].

The membrane resistance was measured at 3-min intervals by applying a known voltage pulse across the membrane in series with a known resistance. The membrane potential was recorded as the potential difference between two calomel-KCl electrodes which made contact with the front and rear solutions.

Metal ion permeabilities were calculated from the relation, $P_i = RTG_i/Z_i^2F^2c_i$ [10], where G_i and c_i are the ionic conductance and the free (noncomplexed) ion concentration, respectively, and R, T, Z and F have their usual meanings. Ionic conductances were calculated from the total membrane conductance (G_m) and the transference number deduced from ionic diffusion potentials [11].

Diphytanoylphosphatidylcholine was obtained from Avanti (Birmingham, AL). $^{109}CdCl_2$ and $^{204}TlNO_3$ were obtained from New England Nuclear (Boston, MA) and ICN (Irvine, CA), respectively.

Table I shows the permeability coefficients of Cd^{2+} and Tl^+ and their neutral Cl^- complexes, $CdCl_2$ or TlCl. For comparison, Hg^{2+} and Na^+ permeabilities are shown also. The metal ion permeabilities are uniformly low, approx. 10^{-11} $cm \cdot s^{-1}$. These permeabilities correspond to conductances in the range of 3–9 $nS \cdot cm^{-2}$, which is the normal range for diphytanoylphosphatidylcholine/decane bilayers. The permeabilities to $CdCl_2$ and TlCl (10^{-8} to 10^{-6} $cm \cdot s^{-1}$) were much higher than the ionic permeabilities but much lower than the $HgCl_2$ permeability (10^{-2} $cm \cdot s^{-1}$) observed previously [1] (Table I). These relatively low permeabilities of $CdCl_2$ and TlCl are due to the greater polarity and ionic character of $CdCl_2$ and TlCl, compared to the nonpolar, covalent character of $HgCl_2$ [12]. From the data shown in Table I I estimate that > 99% of the Cd and Tl fluxes through bilayers occur by nonionic diffusion of $CdCl_2$ and TlCl.

TABLE I

PERMEABILITY COEFFICIENTS OF METAL IONS AND THEIR NEUTRAL CHLORIDE COMPLEXES THROUGH LIPID BILAYER MEMBRANES

Metal	Permeability coefficient ($cm \cdot s^{-1}$)		[a]Relative concn. of neutral Cl complex (percent) of total metal) [c]
	Metal ion	Neutral complex [b]	
Cd^{2+}	$< 1.1 \cdot 10^{-11}$	$4.1 \cdot 10^{-8}$	24
Tl^+	$1.8 \cdot 10^{-11}$	$1.1 \cdot 10^{-6}$	23
Hg^{2+}	$< 3.8 \cdot 10^{-11}$	$1.3 \cdot 10^{-2}$ [d]	18
Na^+	$1.0 \cdot 10^{-11}$	–	< 1

[a] Maximum standard deviations were ±60%, including estimated uncertainties in the association constants, which are very sensitive to ionic strength [2].

[b] Tracer fluxes were measured in NaCl (150 mM), plus Cd^{2+} or Tl^+ (0.1 mM). $CdCl_2$ and TlCl permeabilities were calculated as the one-way flux divided by the concentration of $CdCl_2$ or TlCl (calculated from data in column 4). Tracer permeabilities measured in 100 mM $NaNO_3$ were below the limit of detection ($< 10^{-8}$ $cm \cdot s^{-1}$ for Cd and $< 5 \cdot 10^{-8}$ $cm \cdot s^{-1}$ for Tl).

[c] Calculated for physiological conditions (150 mM Cl^-), using association constants tabulated by Smith and Martell [2].

[d] $HgCl_2$ permeability is from previous study [1].

Cd^{2+} also forms electroneutral complexes with organic anions, e.g., monocarboxylic acids [24]. In one experiment I measured the Cd permeability in the presence of sodium propanoate (100 mM, pH 7). Under these conditions the fraction of cadmium dipropanoate was about 10% of the total Cd. The total Cd permeability coefficient was below the limit of detection, i.e., $< 10^{-8}$ $cm \cdot s^{-1}$; therefore the cadmium dipropanoate permeability was $< 10^{-7}$ $cm \cdot s^{-1}$.

Previous studies on cadmium transport and toxicity suggest that Cd transport is carrier media-

ted [13] and that Cd toxicity is related to the free Cd^{2+} concentration rather than the concentration of $CdCl_2$ [3,14]. My results are consistent with these biological studies, because both Cd^{2+} and $CdCl_2$ apparently have lower permeabilities through lipid bilayers than through biological membranes. For example, from the rate of Cd^{2+} uptake by Chinese hamster ovary cells [13] I estimate a Cd^{2+} permeability of about 10^{-6} cm · s^{-1} at 37°C, several orders of magnitude higher than the values shown in Table I. Therefore it seems likely that cadmium ions traverse biological membranes by protein mediated pathways which are not present in unmodified lipid bilayers.

Several recent studies suggest that Tl^+ is a 'lipid permeable cation' in biological membranes and liposomes because Tl^+ equilibrates faster than K^+ and distributes approximately as predicted by the Nernst equation and the transmembrane voltage (V_m) [5,8]. However, the low Tl^+ permeability shown in Table I raises doubts about the ability of Tl^+ to act as a lipid permeable cation. For example, lipid permeable cations such as tetraphenylphosphonium and tetraphenylarsonium have bilayer permeabilities 10^{-9} to 10^{-8} cm · s^{-1} [15,16], substantially higher than the Tl^+ permeability of $1.8 \cdot 10^{-11}$ cm · s^{-1} (Table I).

An alternative explanation for the lipid permeable behavior of Tl^+ is that Tl^+ crosses the membrane primarily as complexes with anions or organic molecules [2,17]. For example, one study of Tl^+ uptake by liposomes utilized high concentrations of tris buffer at alkaline pH [5]. Tris forms a variety of highly stable complexes with Cu^{2+}, Ag^+ and other metals [18,24], and Tl^+ might show similar complexation behavior. Permeability coefficients of neutral complexes in the range of 10^{-8}–10^{-7} cm · s^{-1} could probably explain the Tl^+ equilibration rates observed in small liposomes [5].

In biological membranes Tl^+ permeabilities are usually 10–100-times higher than K^+ or Rb^+ [5–7,19], which may reflect a significant lipid permeability [5,8] and/or a high selectivity of Tl^+ for K^+ transport pathways [20,21]. In human red blood cells the Tl permeability is about $2 \cdot 10^{-8}$ cm · s^{-1} at 20°C, which I calculated from the efflux rate constant of 1.2 h^{-1} [6] and the volume/surface area ratio of $6.1 \cdot 10^{-5}$ cm [23]. The permeation mechanism has not been established, and Tl^+ complexation with anions may be important. In red blood cells, the activation energy for Tl^+ efflux is 11 kcal · mol^{-1} in NO_3^- saline and 16 kcal · mol^{-1} in SO_4^{2+} saline [6]. Since $TlSO_4^-$ is the predominant Tl species in SO_4^{2-} saline [2], transport of $TlSO_4^-$ via the red-cell anion carrier should be considered, analogous to the carrier mediated transport of $LiCO_3^-$ and $NaCO_3^-$ [22].

Several investigators have used Tl^+ as a probe for measuring the transmembrane voltage in cells and liposomes [7,8]. The application of this method requires that Tl^+ movement through membranes be 'electrophoretic', i.e., driven only by the electrochemical gradient of Tl^+. Thus, active transport of Tl^+ via the K^+ transport pathway must be either intrinsically slow or specifically inhibited [7,21]. Another disadvantage of this method is caused by complexation of Tl^+ with Cl^- and other anions. This complexation causes the equilibrium ratio of total Tl, i.e., $[Tl_T]^i/[Tl_T]^o$, to differ from the $[Tl^+]^i/[Tl^+]^o$ predicted by the Nernst equation.

This problem is illustrated in Fig. 1, which shows the theoretical distribution of Tl in a red blood cell at 25°C. External concentrations of Cl^- and Tl^+ are arbitrarily set at 150 mM and 1.0 mM, respectively, and V_m is assumed to be −10 mV.

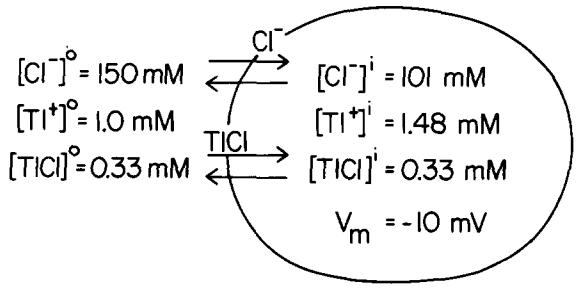

Fig. 1. Theoretical distribution of Tl^+ and TlCl across a red-cell membrane, assuming that only TlCl and Cl^- cross the membrane, $V_m = -10$ mV and $T = 25$°C. External Cl^- and Tl^+ concentrations were arbitrarily set at 150 mM and 1.0 mM, and $[Cl^-]^i$ was calculated from the Nernst equation, i.e., $[Cl^-]^o/[Cl^-]^i = \exp(ZFV_m/RT)$. TlCl concentrations were calculated from the association constant, i.e., $[TlCl]/[Tl^+][Cl^-] = 2.2$. In this example, the total Tl ratio, $[Tl_T]^i/[Tl_T]^o$, is 1.35. However, the same ratio is obtained by assuming that Tl^+ rather than TlCl is the permeant species. Note that the total Tl ratio (1.35) differs significantly from the inverse Cl^- ratio (1.48), which causes an error if the total Tl ratio is used to calculate V_m from the Nernst equation.

TlCl concentrations are calculated from the association constant of approx. 2.2 at $I = 0.15$. Both TlCl and Cl^- are assumed to easily cross the membrane. Thus, $[Cl^-]^o/[Cl^-]^i = \exp(ZFV_m/RT)$, and $[TlCl]^i = [TlCl]^o$. Tl^+ is in equilibrium with TlCl and Cl^-, which results in an equilibrium ratio of total Tl of 1.35. However, an identical Tl ratio is obtained if one assumes that Tl^+ equilibrates with V_m and TlCl cannot cross the membrane. Thus, tracer measurements of Tl equilibria do not provide unequivocal evidence for Tl^+ diffusion. Furthermore, the existence of TlCl, $TlNO_3$, or any other Tl complex causes an error when total Tl distribution is used to estimate V_m. For example, in Fig. 1 the V_m calculated from the Cl^- ratio is -10.1 mV, whereas the V_m calculated from the total Tl ratio is -7.9 mV.

This work was supported by USPHS grants ES 02289 and GM 28844. I thank Drs. W.S. Sunda and S.A. Simon for helpful advice and discussions.

References

1 Gutknecht, J. (1981) J. Membrane Biol. 61, 61–66
2 Smith, R.M. and Martell, A.E. (1976) Critical Stability Constants, Vol. 4. Plenum Press, New York
3 Engel, D.W., Sunda, W.G. and Fowler, B.A. (1981) in Biological Monitoring of Marine Pollutants (Vernberg, F.J., Calabrese, A., Thurberg, F.P. and Vernberg, W., eds.), pp. 127–144, Academic Press, New York
4 Kopp, S.J., Glonek, T., Perry, H.M., Erlanger, M. and Perry, E.F. (1982) Science 217, 837–839
5 Rabon, E.C. and Sachs, G. (1981) J. Membrane Biol. 62, 19–27
6 Skulskii, I.A., Manninen, V. and Jarnefelt, J. (1978) Biochim. Biophys. Acta 506, 233–241
7 Skulskii, I.A. and Manninen, V. (1981) Acta Physiol. Scand. 111, 343–348
8 Bakker, E.P. (1978) Biochemistry 17, 2899–2904
9 Mueller, P., Rudin, D.O., Tien, H.T. and Westcott, W.C. (1962) Circulation 26, 1167–1170
10 Hodgkin, A.L. (1951) Biol. Rev. Camb. Phil. Soc. 26, 339–365
11 Andreoli, T.E., Bangham, J.A. and Tosteson, D.C. (1967) J. Gen. Physiol. 50, 1729–1749
12 Cotton, F.A. and Wilkinson, G. (1972) Advanced Inorganic Chemistry, 3rd Edn., John Wiley Interscience, New York
13 Corrigan, A.J. and Huang, P.C. (1981) Biol. Trace Element Res. 3, 197–216
14 Sunda, W.G., Engel, D.W. and Thuott, R.M. (1978) Environ. Sci. Technol. 12, 410–413
15 Tosteson, M.T. and Wieth, J.O. (1979) J. Gen. Physiol. 73, 789–800
16 Cafiso, D.S. and Hubbell, W.L. (1982) Biophys. J. 39, 263–272
17 Martell, A.E. and Smith, R.M. (1977) Critical Stability Constants, Vol. 3, Plenum Press, New York
18 Smith, R.M. and Martell, A.E. (1975) Critical Stability Constants, Vol. 2, Plenum Press, New York
19 Skulskii, I.A., Manninen, V. and Jarnefelt, J. (1973) Biochim. Biophys. Acta 298, 702–709
20 Neher, E. (1975) Biochim. Biophys. Acta 401, 540–544
21 Damper, P.D., Epstein, W. Rosen, B.P. and Sorensen, E.N. (1979) Biochemistry 18, 4165–4169
22 Becker, B.F. and Duhm, J. (1978) J. Physiol. 282, 149–168
23 Funder, J. and Wieth, J.O. (1976) J. Physiol. 262, 679–698
24 Perrin, D.D. (1979) Stability Constants of Metal-Ion Complexes, Part B, Organic Ligands, Pergamon Press, New York