Ion Transport Through Membranes

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Ion Transport Through Membranes

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Preface

Concentration gradients of ions between cell compartments are signals which provoke successive reactions for cell functions such as energy transduction, muscle contraction, and nerve excitation. Such a gradient can only be brought about by ion transport through the biomembranes separating cell compartments. Accordingly, ion transport is an essential process for many normal physiological functions. Understanding the importance of this problem, we decided to organize an international symposium, which we entitled "Ion Transport through Membranes."

The symposium, sponsored by the Japanese Society of Biochemistry, International Union of Biochemistry, Suzuken Memorial Foundation, and Yagi Memorial Foundation, was held from November 3 to 5, 1985, at the Nagoya Kanko Hotel in Nagoya, Japan. Leading specialists in this field from around the world were invited.

On that occasion, each speaker was asked to present a mini-review, including his or her own findings, of a given area of this broad topic. These manuscripts were then edited to provide a monograph which surveys the most up-to-date knowledge on this exciting topic.

The editors wish to express thanks to the above-mentioned organizations for their financial support, to the authors for their cooperation, and to Madame Alberte Pullman for her help during the planning of the symposium.

Kunio Yagi
Bernard Pullman

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DESIGN OF IONOPHORES FOR A CARRIER-INDUCED ION TRANSPORT THROUGH BULK MEMBRANES

W. Simon
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I. INTRODUCTION

Ion carriers (a class of ionophores) are lipophilic complexing agents having the capability to bind ions reversibly and to transport them across organic membranes by carrier translocation (1,2). Ideally, selective ion carriers render a membrane permeable for one given sort of ion I only. If such ionophore-based membranes are used in cell assemblies of the type

External reference electrode	Sample solution (solution 1)	Membrane	Internal filling solution (solution 2)	Internal reference electrode

and if an electric potential gradient is applied between solutions 1 and 2, an exclusive transport of the ions I across the membrane should result, the transport number

This work was partly supported by the Swiss National Science Foundation, Orion Research Inc. and Corning Ltd.

being then $t_I = 1$. For the same membrane electrode cell the electric potential difference at zero current (e.m.f.) between the external and the internal reference electrodes depends then on the ratio of the activities of the ion I in the solutions 1 and 2. If a constant composition of solution 2 is used, the activities of the selected ion in solution 1 may therefore be measured potentiometrically according to the Nernst equation (3). In particular, neutral carriers, defined as ionophores that carry no charge when not complexed by the transported ion, have led to such cell assemblies (ion-selective electrodes) with a wide range of different selectivities (2). Figure 1 shows a selection of ion carriers which we designed in view of an analytically relevant application in bulk membranes or which we found to exhibit the required properties for such a use. Ionophores 1 - 19 in Fig.1 are neutral carriers for cations, 21 (4) is a charged carrier for anions and 20 (5,6) is a neutral carrier for anions. Some of them have found widespread application in cation sensors for clinical use (for a review see (2)). The ionophores 2 (7), 3 (7), 4 (8), 7 (9), 11 (10), 12 (10), 14 (11), 15 (12) and 18 (13) have been described more recently. Among the different bulk membranes, solvent polymeric membranes based on poly(vinyl chloride) (14) have been studied in especially great detail. Membranes with ∿32 wt.-% poly(vinyl chloride), ∿65 wt.-% plasticizer and ∿1 - 3 wt.-% ionophore exhibit diffusion coefficients of free carriers and carrier/ion complexes of about 10^{-7} to 10^{-8} cm^2 sec^{-1} (15) comparable to values for carriers in black lipid membranes (16).

II. REQUIREMENTS FOR THE DESIGN OF IONOPHORES

Ionophores for an analytically relevant application in solvent polymeric membranes have to meet at least the following three requirements (see also (17)):

A. Ion-Permeability Selectivity

The ionophore has to induce ion-permeability selectivity in membranes. As the ion selectivity of a membrane is related to the free energies of transfer of the ions from the aqueous phase (sample) to the membrane phase, the selectivity of neutral carrier-based membranes obviously depends on various factors. Such factors are mainly (2): (a) the selectivity behaviour of the carrier used, which can be completely characterized by complex stability constants, (b) the extraction properties of the membrane solvent (plasticizer), (c) the

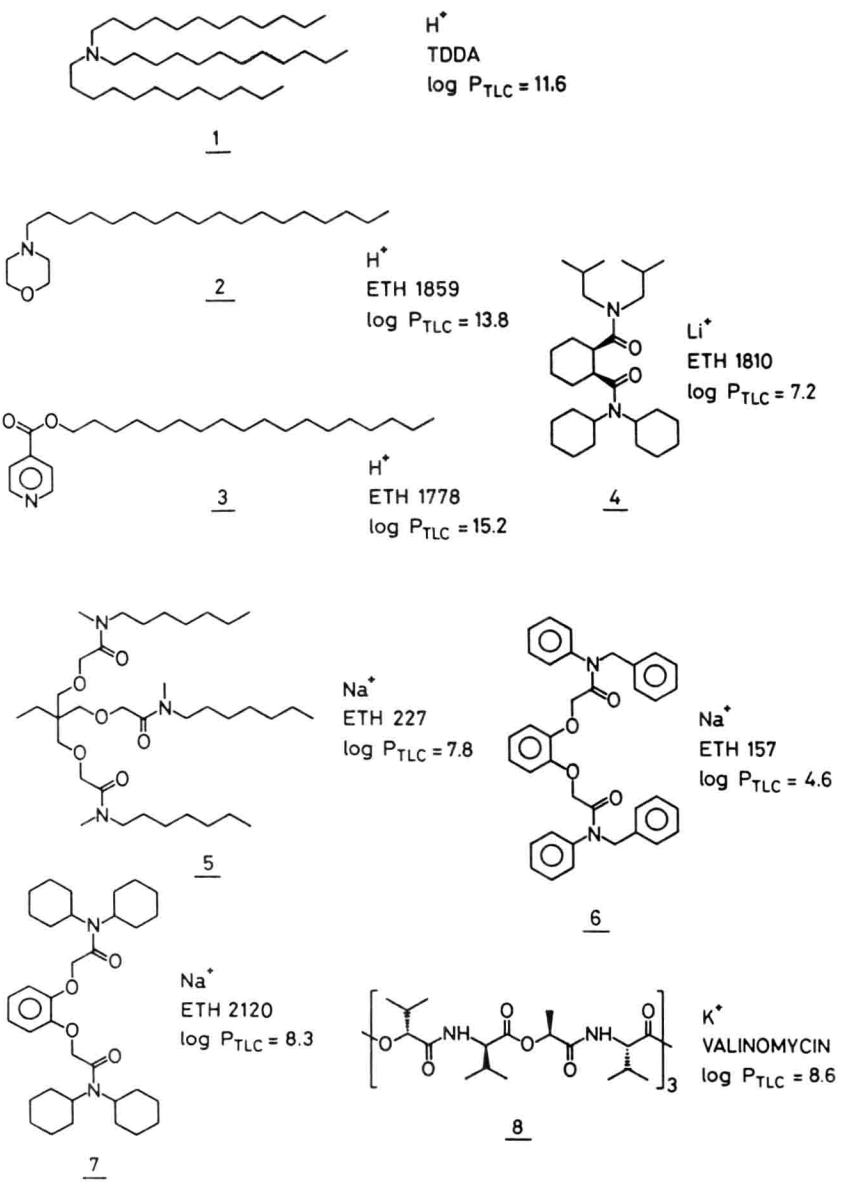
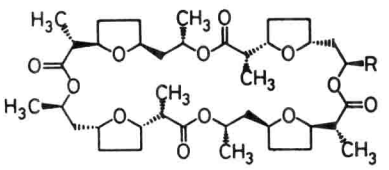


FIGURE 1. Constitutions of the ionophores discussed.



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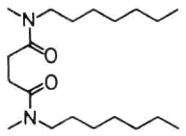
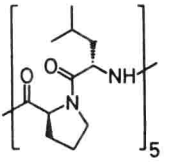
NH_4^+
R = CH_3 : NONACTIN
log P_{TLC} = 5.8
R = C_2H_5 : MONACTIN
log P_{TLC} = 6.5



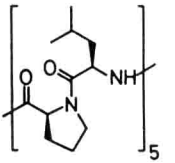
10

Mg^{2+}
ETH 1117
log P_{TLC} = 5.8



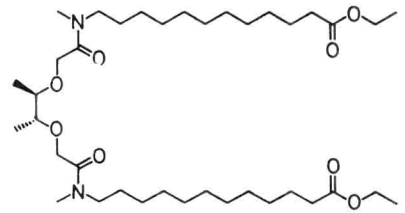
11

Mg^{2+}
CYCLO(L-PRO-L-LEU)$_5$
log P_{TLC} = 3.6



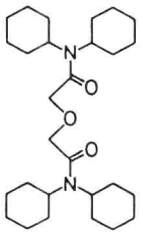
12

Mg^{2+}
CYCLO(L-PRO-D-LEU)$_5$



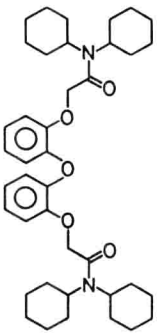
13

Ca^{2+}
ETH 1001
log P_{TLC} = 7.5



14

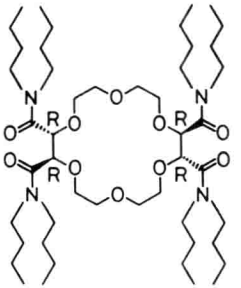
Ca^{2+}
ETH 129
log P_{TLC} = 7.2



15

Ba^{2+}
V 163
log P_{TLC} = 8.2

Figure 1. (Continued).



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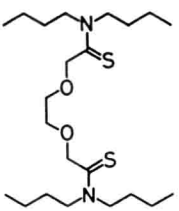
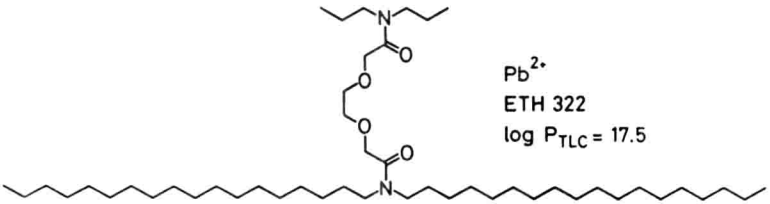
R(+)-PEA$^+$



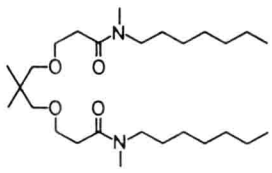
17

Cd^{2+}
ETH 1062
log P_{TLC} = 6.5



18

Pb^{2+}
ETH 322
log P_{TLC} = 17.5



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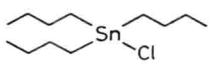
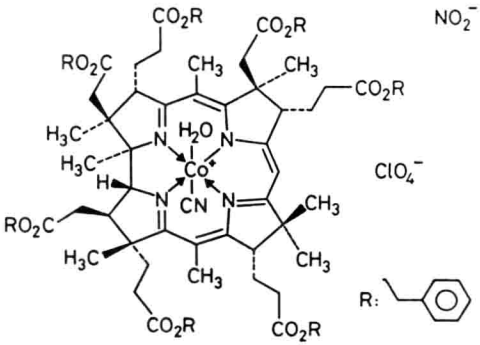
UO_2^{2+}
ETH 295
log P_{TLC} = 6.2



20

Cl^-
TBTCl



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NO_2^-
ClO_4^-
R:

FIGURE 1. (continued).

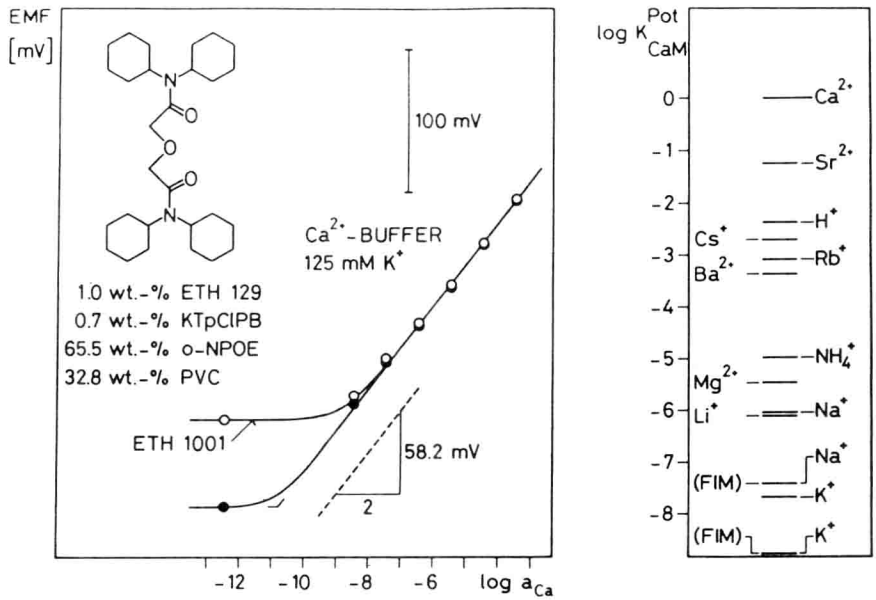

FIGURE 2. Electrode function (left, dots) and potentiometric selectivities (right) of a PVC membrane containing ionophore 14 (ETH 129). The electrode function is determined in calcium-buffered solutions containing a constant background of 125 mM K^+. For comparison the electrode function of a membrane containing ionophore 13 (ETH 1001) is given (circles). The selectivity coefficients (log K^{Pot}_{CaM}) are determined by the separate solution method (SSM) (20). For Na^+ and K^+ they are also given as determined in calcium-buffered solutions by the fixed interference method (FIM) (20).

concentration of the free ligand in the membrane phase and (d) the concentration of ionic sites in the membrane. The effects of (b), (c) and (d) can be described to a large extent by model calculations and may be controlled by adequate membrane technology (2,18,19). Figure 2 corroborates that neutral carriers can induce outstanding selectivities in membranes if adequate membrane technology is applied (11). Reciprocal selectivities $1/K^{Pot}_{CaK}$ (preference of Ca^{2+} over K^+ by the membrane as measured potentiometrically (20)) of up to 10^9 have been obtained (11).

As expected theoretically, the selectivity of complex formation of charged ion carriers in membranes containing predominantly neutral associates (ion / charged ionophore complexes) is only partially exhibited in potentiometric

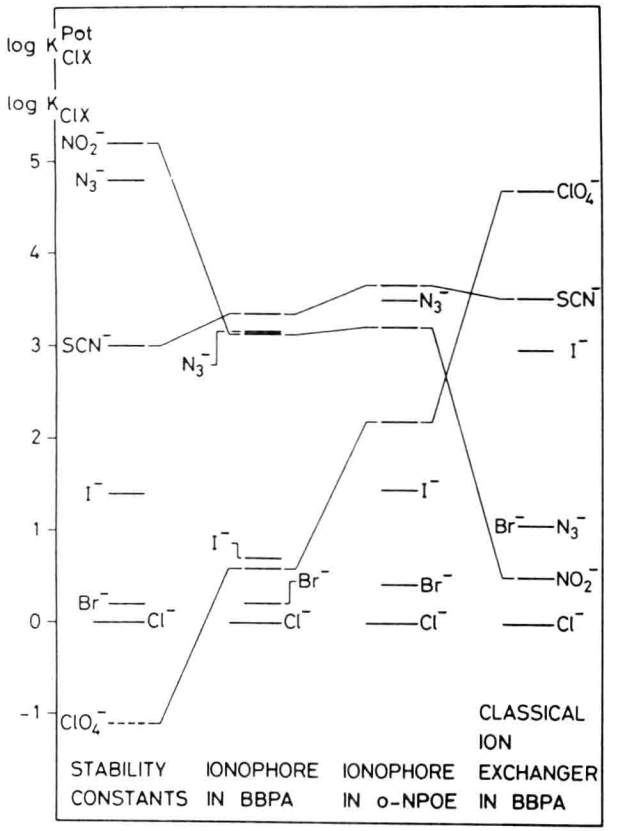

FIGURE 3. Comparison of the relative stability constants (log K_{ClX}) of anion complexes of vitamin B_{12} (column 1) with potentiometric selectivities (log K^{Pot}_{ClX}) of PVC membranes with an analog of ionophore 21 (a,b,d,e,f,g-hexamethyl-c-octadecyl Co-aquo-Co-cyanocobyrinate perchlorate) (columns 2 and 3) and of a PVC membrane based on a classical anion exchanger (BBPA: bis(1-butylpentyl) adipate, o-NPOE: o-nitrophenyl octyl ether).

studies on cell assemblies of the type described above (19). Thus the potentiometric selectivity is found to lie within the limits given by the ratio of the complex formation constants and the selectivity of liquid membranes based on dissociated ion exchangers (21,22). An example is given in Fig. 3 for an anion carrier of the type 21 (for details see (23)).

The design of ionophores with a given selectivity is more problematic (a). Although an overwhelming activity in de-

signing host molecules (ionophores) for selected guest species (ions) is in evidence (24-31), only modest use has been made of model calculations describing the host-guest interactions (25,32-34).

Classical electrostatic models are useful for calculating the ion-molecule interactions near the energy minima for Group IA/IIA cations (35). However, they require a knowledge of molecular parameters normally not available. It has been shown (35) that semi-empirical quantum chemical treatments of ion-ligand interactions often lead to unrealistic results. In contrast, ab initio computations give reliable results even if small but well balanced basis sets are chosen (36-39). The application to realistic ionophores is usually prohibited by the extent of the computation even if small basis sets are used. Through ab initio calculations on model complexes and a representation of the interaction energies by pair potentials (40-44), such large systems are easily made amenable to analysis. Another approach for the description of large systems on the basis of ab initio calculations on small systems is described in (39). For a discussion of the stability of the complexes the sum of the interaction energies and the conformational energy relative to the most stable conformation of the free ligand is relevant. Unfortunately, the computation of such conformational energy changes is still too uncertain (45) (see also (46)). Recently a model based on ab initio calculations has been proposed for the evaluation of conformational energies (47).

B. Lipophilicity of Ionophores

The ionophore must exhibit a high lipophilicity to be confined to the membrane phase for an appropriate period of time. To guarantee a continuous use lifetime of at least one month of a typical solvent polymeric membrane sensor in contact with whole blood or undiluted blood serum, a lipophilicity P (partition coefficient between water and octan-1-ol) of the carrier larger than $10^{8.4}$ is necessary (49). Incorporating adequate structural elements (e.g. alkyl groups) into ionophores, this required lipophilicity may easily be obtained. A reliable estimate for P may be obtained by thin-layer chromatography (17). Such estimated values log P_{TLC} are given in Figure 1 for the neutral ionophores studied. Obviously, most of the ionophores have been designed to exhibit a sufficient lipophilicity for such a desired membrane lifetime.

C. Ligand Exchange Kinetics

The ionophores should form relatively stable complexes with the primary ion I (high selectivity) but, on the other hand, the exchange reaction of the selected ions at the membrane / solution interface must be sufficiently reversible. Therefore, the free energy of activation of the ligand exchange reaction

$$IS + S' \rightleftharpoons IS' + S$$

where S and S' are ionophores, has to be relatively low.

For Zn^{2+} or Cd^{2+} complexes of ligand 17 (Fig. 1), free energies of activation of the ligand exchange reaction of < 45 kJ mol^{-1} (in acetonitrile) have been measured (17,50). Cation permselectivity is indeed observed with $CdCl_2$ in the sample solution (17). As $CdCl^+$ is probably the permeating species, a slope of the electrode response of approximately 60 mV (25°C) is obtained (17). However, in systems with a free energy of activation of the ligand exchange reaction of > 65 kJ mol^{-1} (in acetonitrile) the cationic complexes of the ionophore act as anion exchangers (e.g., complexes with Pt^{2+} or Pd^{2+} (50)). An electrode containing the $PdCl_2$ complex of ligand 17 in the membrane phase therefore responds to the chloride anions in a sample solution of $CdCl_2$ (17). Theoretically related is the requirement that a sufficiently high and constant concentration of ionophore should be present in the membrane phase in the unloaded form (19). If a cation ionophore is predominantly present within the membrane phase in the loaded form, anion-permselectivity is induced (see Fig. 4) (19,48). These findings are in agreement with the experimental evidence that the transport rate of ionophores passes a maximum when increasing the stability constant of the ionophore / ion interaction (51).

In order to keep the free energy of activation of the ligand exchange reaction sufficiently small, the design of ionophores has been focused on non-macrocyclic structures (see Fig. 1).

Using model calculations (section A), CPK model building and adequate membrane technology, it has been possible to design neutral carrier-based membranes that show analytically relevant ion selectivities for a wide range of ions (Fig. 1). Although the synthetic ionophores shown in Fig. 1 are predominantly non-macrocyclic (see section C), there are several reports on the successful application of macrocyclic ionophores (e.g., crown compounds) in ion-selective electrodes (52-54) (for a review, see (2)).