Transport in Biomembranes: Model Systems and Reconstitution

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Preface

The aim of this volume, whose authors are drawn from several disciplines, is to give an up-to-date insight and a deeper understanding of the phenomena related to transport through biomembranes. This collection of papers was originally presented at the Second Course of the School on "Fundamental Aspects of Membrane Phenomena," coordinated by the Italian Group of Membrane Science and Technology.

The topics are organized in a hierarchical fashion, beginning with the transport properties of model systems and moving into topics of increasing complexity such as reconstitution of membrane transport functions.

The rapid expansion of this area can be attributed to a number of technical advances both in forming well-characterized model systems (such as planar black membranes and liposomes) and in isolating and purifying membrane compounds. The chapters in this volume combine the biophysically oriented approach, intended to underline the physical basis of membrane processes, with the biochemically oriented approach, stressing the crucial role played in reconstitution experiments by isolation and purification of membrane proteins, enzymes, and receptors.

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Kinetic Properties of Ion Carriers, Channels, and Pumps

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Permeation of ions through cellular membranes involves special mechanisms different from simple diffusion through the lipid bilayer. In the discussion of possible passive transport pathways, two alternatives are usually considered: carrier and channel mechanisms. A carrier (in its simplest form) may be defined as a transport system with a binding side that is exposed alternately to the left and to the right side (but not to both sides simultaneously). A channel, on the other hand, consists of one or several binding sites arranged in a transmembrane sequence and is accessible from both sides at the same time.

Clear-cut examples of carrier and channel mechanisms in ion transport have been obtained from the study of certain small or medium-sized peptides and depsipeptides. Cyclodepsipeptides, such as valinomycin, have been shown to act by a translatory carrier mechanism which involves a movement of the whole carrier molecule with respect to the lipid matrix of the membrane (1). A well-characterized ion channel is the channel formed by the linear pentadecapeptide, gramicidin A (2). In these cases the distinction between a channel which is more or less fixed within the membrane and a carrier moving within the lipid matrix is unambiguous. The discrimination between carrier and channel mechanisms becomes less obvious, however, in the case of large membrane proteins spanning the lipid bilayer, which are thought to be responsible for ion transport across the cell membrane. Such a protein is unlikely to move as a whole within the membrane. It still can act as carrier (according to the definition given above), however, if a conformational change within the protein switches the binding site from a left-exposed to a right-exposed state. A channel, on the other hand, does not necessa-

rily have a fixed, time-independent structure. Proteins may assume many conformational substates and move from one state to the other. Accordingly, in a channel conformational transitions may occur between states differing in the height of the energy barriers that restrict the movement of the ion. It will be shown below that such a channel with multiple conformational states may approach the kinetic behavior of a carrier. Channel and carrier models should therefore not be regarded as mutually exclusive possibilities, but rather as limiting cases of a more general mechanism.

KINETIC ANALYSIS OF TRANSLATORY ION CARRIERS

Incorporation of certain macrocyclic antibiotics, such as valinomycin, enniatin B, and the macrotetrolides into artificial lipid bilayer membranes result in a strong increase of potassium permeability of the membrane (3). Ion transport mediated by carriers of the valinomycin type occurs in four distinct steps (Fig.1): Association of ion M^+ and carrier S in the interface, (ii) translocation of the complex MS^+, (iii) dissociation of MS^+, (iv) back transport of free carriers S. The kinetics of the carrier may be analyzed by studying the electrical conductance of planar bilayer membranes. In particular, electrical relaxation techniques

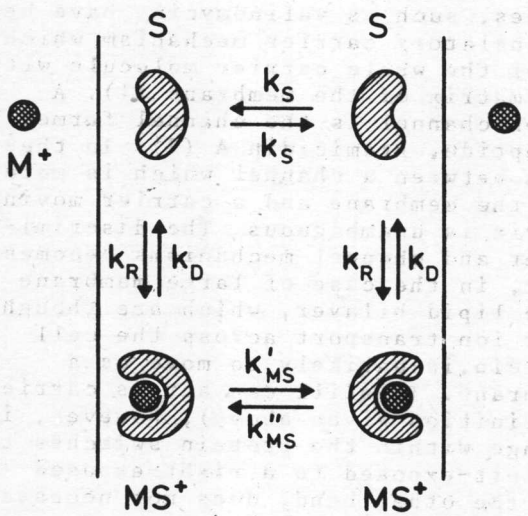

Fig.1.Transport of ion M^+ mediated by a translatory carrier S.

such as the voltage-jump (9) or charge-pulse method (2) may be used to evaluate the individual rate constants. As a specific example, we consider the results obtained for valinomycin/Rb^+ in a monoolein/n-decane membrane (25°C, 1 M RbCl)(2):

$$k_R \simeq 3\times10^5 M^{-1}s^{-1}$$
$$k_D \simeq 2\times10^5 s^{-1}$$
$$k_{MS} \simeq 3\times10^5 s^{-1}$$
$$k_S \simeq 4\times10^4 s^{-1}$$

At one-molar concentration of the transported ion (C_M=1 M), the rate constants of association ($C_M k_R$), dissociation (k_D) and translocation of the loaded carrier (k_{MS}) are approximately equal ($2\text{-}3\times10^5 s^{-1}$). The rate-determining step in this system is the back transport of the free carrier ($k_S \simeq 4\times10^4 s^{-1}$). $k_{MS} \simeq 3\times10^5 s^{-1}$ is the frequency by which the ion-carrier complex crosses the central barrier; the reciprocal value $1/k_{MS}$ 3μsec is the average time required for translocation. This time may be compared with the diffusion time $\tau = d^2/2D$ of a spherical particle of the size of the carrier across the same distance (membrane thickness $d \simeq 5$ nm) in water (diffusion coefficient $D \simeq 3\times10^{-6}$ $cm^2 s^{-1}$), which is about 0.04 μsec.

THE GRAMICIDIN CHANNEL AS A MODEL CHANNEL

The finding that gramicidin A, a hydrophobic peptide with known primary structure, forms alkali-ion permeable channels in lipid bilayer membranes (5) opened up the possibility of studying ion permeation through channels in a simple model system. Gramicidin A is a linear pentadecapeptide with the sequence HCO-L-Val-Gly-LAla-D-Leu-L-Ala-D-Val-L-Val-D-Val-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-$NHCH_2CH_2OH$. Evidence that gramicidin A forms channels (and does not act as a mobile carrier) has been obtained in experiments in which very small amounts of the peptide were added to a planar bilayer membrane (5). Under this condition the membrane current under a constant applied voltage fluctates in a step-line manner. The size of the single conductance step is about 90pS in 1 M Cs^+, corresponding to a transfer of 6×10^7 Cs^+ ions per sec. A structural model of the gramicidin channel has been proposed by Urry (11). According to this model which is now supported by many experimental findings, the channel consists of a helical dimer that is formed by head-to-head (formyl end to formyl end) association of two gramicidin monomers and is stabilized by intra-

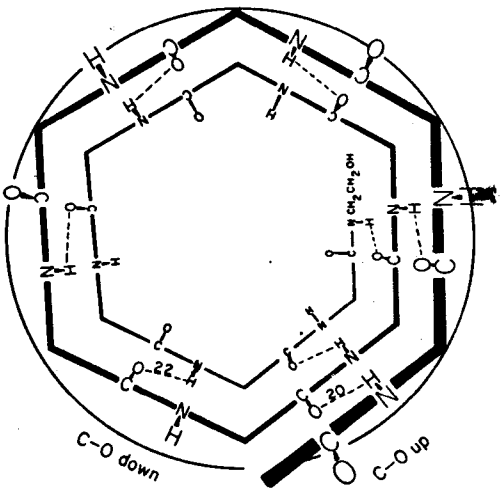

Fig.2. Structure of the π^6(L,D)-helix of gramicidin A [1] The hole along the helix axis has a diameter of 0.4 nm and is lined with oxygen atoms of the peptide carbonyls. Hydrophobic amino-acid residues located at the periphery of the helix are not shown. The transmembrane channel consists of two helices joined at the formyl end.

and inter-molecular hydrogen bonds (Fig.2). The central hole along the axis of the π^6 (L,D)-helix has a diameter of about 0.4 nm and is lined with oxygen atoms of the peptide carbonyls, whereas the hydrophobic amino-acid residues lie on the exterior surface of the helix. The total length of the dimer is about 2.5-3.0 nm, the lower limit of the hydrophobic thickness of thelipid bilayer.

CHANNELS WITH MULTIPLE CONFORMATIONAL STATES: A UNIFYING CONCEPT

A channel may be represented by a series of "binding sites" that are separated by energy barriers. The binding sites are the minima in the potential energy profile which result from interactions of the ion with one or several groups of the channel. Ion transport through the channel may be described as a series of thermally activated processes in which the ion moves from a binding site across an energy barrier to an adjacent site. In this treatment the energy levels of wells and barriers are usually considered to be fixed, i.e., independent of time and not influenced by the movement of the ion. This description, which corresponds to an essentially static picture of protein structure, represents a useful approximation in certain cases. Recent findings on the dynamics of protein molecules, however, suggest the use of a more general concept of barrier structure.

A protein molecule in thermal equilibrium may exist

in a large number of conformational states and may rapidly move from one state to the other (4). Evidence for fluctuations of protein structure comes from X-ray diffraction studies (4), NMR experiments (12) and from the kinetic analysis of ligand rebinding to myoglobin after flash photolysis (1). These and other studies have shown that internal motions in proteins occur in the time range from picoseconds to seconds. This suggests that the energy profile of a channel is subjected to fluctuations over a wide spectrum of times. Of particular interest is the possibility that transitions between conformational states of the channel protein may be coupled to the movement of the ion within the channel (8). Such a coupling may result, for instance, from electrostatic interactions between ion and ligand system. When an ion jumps into a binding site, the strong coulombic field around the ion tends to polarize the neighbourhood by reorienting dipolar groups of the protein. This reorientation is likely to shift the energy level of the binding site and the height of adjacent barriers. If the rate of conformational change induced by the ion is comparable to or smaller than the jump rate, the ion may leave the binding site before the protein structure has relaxed to the polarized state. Likewise, after the ion has left a binding site, a certain time is required to for the channel to return to the original conformation, and the next ion may find the structure still in a partly polarized state. Changing the ion concentration in the aqueous phase (and thus the average occupancy of the channel) may shift the distribution of conformational states of the channel and affect its permeability. A general treatment of multistate channels is, of course, rather complicated. In the following we discuss the simple case of a two-state channel with a single binding-site (Fig.3), which already exhibits some of the basic properties of channels with variable barrier structure (8). It is assumed that the channel molecule may exist in four distinct substates:

C_r conformation r, empty

C_r^* conformation r, occupied

C_s conformation s, empty

C_s^* conformation s, occupied

The rate constants for transitions between these substates are indicated in Fig. 4. Transitions between empty and occupied states may occur by exchange of an ion between the binding site and the left or right aqueous phase. Thus (Fig.3):