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COMPLEX MEMBRANE TRANSPORT SYSTEMS

A NON-MARKOVIAN APPROACH

Wolfgang STEPHAN

School of Biological Sciences, University of Sussex, Brighton BN1 9QG, U.K.

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This paper suggests a method of how to deal with complex membrane transport systems such as ion channels or ion pumps formed by proteins. The complexity of these systems results from the fact that proteins may undergo an internal dynamics of conformational changes and may thereby affect the transmembrane transport. Usually, complex transport systems are mapped into multi-state graphs and couched in terms of Markovian master equations. It is shown in this paper how the dimensionality of such multi-state systems can be reduced. The resulting description may be expressed in the form of a generalized master equation with a memeory function as integral kernel. The memory function reflects the protein's own dynamics and its overall effect on the transport. This formalism, non-Markovian in nature, is applied to describe the time-dependent action of ion pumps. A general model is constructed on the basis of the rate theory which contains all the essential parts of ion pumps such as a catalytic unit and a channel-like conduit for ion translocation and which is still analytically tractable. The short-time behaviour of the pumping process turns out to be of particular interest, since it reveals the dynamics of the catalytic unit itself. A strong correlation of the particle's motion over times less than a certain correlation time has been found. This result is compared with experimental findings on the proton pump of *Halobacterium halobium*. It is concluded that such a perfect short-time memory could be a generic property of active transport systems.

1. Introduction

The transport of ions through biological membranes depends on the presence of built-in proteins which provide for energetically favourable pathways through the apolar membrane core. In passive transport the ions follow an electrochemical potential gradient. Well studied examples for passive transport systems are the cation-permeable channel formed by the peptide gramicidin A [36] or the K^+ channel found in the membranes of nerve or muscle cells [10]. Apart from these systems, cellular membranes contain subunits which may drive transmembrane transport against an electrochemical gradient by means of an energy-supplying reaction. Well known examples of active transport systems are the Na^+/K^+ pump [30] or the Ca^{2+}-transport system in the sarcoplasmic reticulum [8] both depending on ATP hydrolysis. The proton pump [34] of halophilic bacteria is driven by light energy. Another major energy source consists of redox-potential energy differences which, for instance, allow for the active H^+ transport in chloroplast of green plants and in mitochondria [1,23].

The idea of this paper is to treat both types of transport systems in a unified form with special reference to the recent findings on the dynamics of protein molecules. A protein molecule may exist in a large number of conformational states and may change from one state to another. Evidence for fluctuations of the protein structure results from X-ray diffraction studies [5], NMR [37] and Mössbauer data [24]. These

studies have shown that internal motions in proteins may occur in a time range comparable with that of the transport itself. As several authors pointed out in their theoretical works, this can lead to coupling between protein dynamics and transport [4,16,18,27]. Also, experimental evidence has recently been reported by Sigworth through his studies of the open acetylcholine receptor channel [29]. The theoretical description can be made in the form of general barrier models. Here the channel is considered to be a linear sequence of activation barriers; 'general' only means that each conformational state of the protein has to be indicated by its own barrier structure. The concept of generalized channel models applies to both passive as well as active transport systems [6,15,17,18,21,26,35]. The justification for using barrier models is that at this time a strictly microscopic description is still impossible for most of the transport systems, especially for active ones, since their essential structural properties remain incompletely known.

In applying general channel models, the crucial point consists of how to find a minimum description of a transport system which still contains its essential characteristics.This means that when considering the transport through a channel under the influence of the protein's dynamics, one inevitably ends up with a multi-state model of a possibly complicated diagram structure. For instance, supposing the protein exists in m conformations, and the channels formed by that protein have n binding sites, then, if only one ion is allowed to be in the channel, the system consists of $(n+1)m$ different states. This is generally a very large number since, in accordance with protein dynamics, one has to assume that proteins exist in a quasi-continuum of conformations. In a previous contribution [18], we treated such a system in explicit Markov terms, but we were only able to discuss the case $n = 1$, $m = 2$. Since, of course, this is a rather rough approach to reality, the motivation for this paper has been to start with the full Markovian system and to subject this to a sensitive reduction.

The reduction procedure is based on the assumption that the transition steps of the full Markov scheme are not all of the same weight with respect to a given transport observable. Indeed, in electrical membrane transport systems transporting charged particles across membranes, chemical reactions, such as the binding of ATP to the channel protein, and the ensuing conformational changes of the channel are less relevant inasmuch as they do not contribute to a measurable charge displacement. The approximation, made here, is reasonable provided no concerted, vectorial motion of charged protein fragments has to be taken into account; i.e., the transmembrane current is generated by the transported particles only. Being not directly involved in charge displacement, however, does not mean that the conformation changes of the protein may be totally neglected, especially if the protein takes a long time to run through the conformations, while the particle is trapped, fixed more or less at the same position.

This is the situation the present paper wants to mimic. The above mentioned restrictions are not essential for the application of the reduction method, but they do determine the model to be dealt with. They could be eliminated in a more complex model by introducing additional transition steps. The paper is structured as follows: In section 2, the full model as well as the reduction procedure and the equations of the reduced scheme are presented. The effect of the channel's internal dynamics on the transport is studied for a barrier model with n sites allowing only one ion to be within the channel at the same time ('one-ion model', cf. ref. 32). It is shown that the internal dynamics can be described in an average way by a memory function. The multi-state model originally expressed by a Markovian master equation (of constant transition probabilities) transforms thereby into a generalized master equation (GME) with that memory function as integral kernel. This provides a non-Markovian description of the transport system which says that the process can looked upon as diffusion process with internal degrees of freedom. The number of equations of the original system is thereby reduced to $n + 1$ equations forming the GME. Though the GME represents a system of integro-differential equations, it can be attacked, at least to some extent, by analytical methods as done in the remainder of this paper. The stationary case of the one-ion model proves to be completely solvable (section 3) and, in a specific example, also the time-dependent one (section 4). In this example, the GME formalism is applied to an ion pump model. The ion pump is modelled here as basically consisting of a catalytic unit and a channel-like device for ion translocation. Both short- and

long-time aspects of the pumping process are of importance. Comparisons with normal diffusion behaviour can be drawn. Finally, we comment on the results, in particular, on whether and to what extent the reduction procedure and the ensuing non-Markovian approach could be useful in the field of membrane transport (section 5).

2. The model

2.1. The reduction procedure

The general model I will discuss in this paper is abstracted from the rate theory models often used to describe both passive ion diffusion through narrow channels in biological membranes as well as active transport systems [9,17]. The essential assumptions for these models are:

(i) The transport through the channels is considered as a one-dimensional hopping process over a sequence of activation barriers separated by the n minima of the ion's potential energy. The energy minima are usually referred to as binding sites where the ion is in an energetically favourable position with respect to the coordinating polar groups of the channel.

(ii) The diffusion domain is of finite size, i.e., mainly determined by the length d of the pore ($d \lesssim 10$ nm).

(iii) Channel proteins (both ion channels and, in particular, pump molecules) may exist in a large number, m, of conformational states and may move rapidly from one state to the other. In order to function as a pump or as a passive transport system the channels typically run through a cycle of conformational states [17].

Moreover, the channels are considered as non-interacting subunits within the membrane and may be occupied by one ion at most. The latter assumption contradicts the well known single-filing hypothesis allowing the pores to contain several ions at the same time. However, most of the qualitative features of channel systems, especially the transient ones, are already reflected by one-ion models [32].

As argued in section 1, the state representation of transport systems in biomembranes is in general very complex. Complexity is defined here as the size of the minimum description of the transport system, i.e., the number of states and the way in which these are interconnected. Due to the complexity of the system, it is often quite hopeless to understand the system's behaviour without structuring it in a certain manner. The usual way of doing this is to apply an appropriate reduction procedure, when the intention of which properties of the original model or of which data of the real system are to be contained has been clarified. For the case of complex transport systems, the viewpoint of this paper is to stress the transport mechanism of the original model and to describe all other processes coupled to the transport, as if they influence that indirectly. In this view, the original system consists of essentially two parts, the subsystem allowing for the ionic hopping process and the 'chemical' subsystem of the conformational transitions. The reduction thus follows the line that the transitions resulting from the hopping process are explicitly regarded whereas the conformational changes are described by means of some averaging procedure. As outlined in section 1, the underlying assumption here is that conformational changes are not connected with charge transfer.

The reduction method starts from an explicit graphical representation of the original system, or equivalently, from the corresponding Markovian master equation

$$\dot{P}_\mu = \sum_{\nu \neq \mu}^{\nu} \left(k_\nu^\mu P_\nu - k_\mu^\nu P_\mu \right), \qquad \mu = 1, \ldots, N \tag{1}$$

together with the normalization relation

$$\sum_{\mu=1}^{N} P_\mu = 1, \tag{2}$$

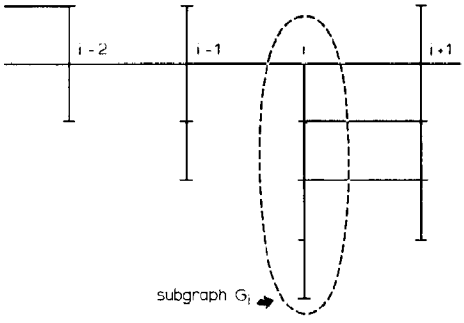

Fig. 1. Section of the original Markovian graph. The binding sites are compound, i.e., represented by subgraphs G_i of several states.

where $P_\mu(t)$ denotes the probability of finding the system in state μ, k_μ^ν is the constant transition probability from state μ to ν, and $N = (n+1)m$ is the total number of states.

Fig. 1 shows a section of the whole graph of the system. The transitions arising from the hopping process of the ion are designated by the horizontal edges of the graph whereas the vertical lines represent the possibly complex subgraph of the internal dynamics of the protein; for instance, G_i contains only the conformational states of the system belonging to the i-th binding site of the channel. The idea of the reduction procedure is now to represent each subgraph G_i by only one characteristic quantity. Since the ion sits at site i while the system may run through the whole subgraph G_i following the dynamics of the protein, the waiting time of the particle at site i seems to be most appropriate to account for the influences of the conformational changes on the transport. The waiting time distribution $\psi(t)$ is defined by the probability $\psi(t)\mathrm{d}t$ that the particle stays at a certain site after its arrival until t and jumps between t and $t + \mathrm{d}t$. For a transport system given by a graph or by the corresponding Markovian master equation, the waiting time of a particle sitting at site i can often be calculated explicitly be means of first passage times. To this end, random walks on the subgraph G_i are considered in the appendix. This procedure presents no problems for binding sites i which can be reached from neighbouring sites via exactly one substate of G_i. For instance, there is only one such state in the subgraph G_{i-1} of the above scheme (fig. 1) but three in the subgraph G_i. The problems arising for subgraphs with more than one state of entrance (exit) are discussed in section 5.

2.2. *Continous-time random walk formalism*

The hopping process of ions through channels can be treated as a random walk on a one-dimensional lattice of finite size. In order to apply the continuous-time random walk (CTRW) formalism [20], it would

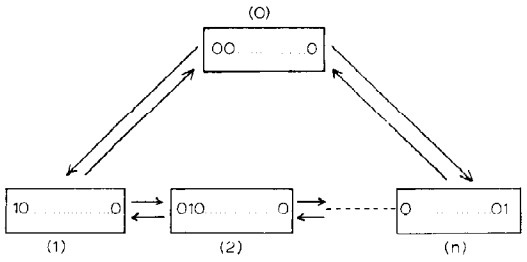

Fig. 2. State diagram of a channel with n binding sites. The channel is allowed to contain no more than one ion. The sequence of ones and zeros denotes the occupation pattern of the channel (1, occupied; 0, empty site).

be advantageous to map the finite lattice onto an infinite structure which is equivalent to that. This is possible in our case since the one-ion pore with n sites is graphically represented by a cycle consisting of $n+1$ nodes (fig. 2). Therefore, we obtain the probability density $P(i, t)$ of finding the particle at the i-th binding site of the channel by summing over the corresponding probability densities $P((n+1)j+i, t)$ of the infinite lattice

$$P(i,t) = \sum_{j=-\infty}^{\infty} P((n+1)j+i,t). \tag{3}$$

The successive jumps of the particle are described by the waiting time distributions $\psi^{\pm}(i, t)$ for the transitions from site i to $i \pm 1$. The waiting time distribution is normalized such that

$$\int_0^{\infty} \psi(i,t)\mathrm{d}t = 1 \tag{4}$$

with

$$\psi(i,t) \equiv \psi^{+}(i,t) + \psi^{-}(i,t). \tag{5}$$

The probability $h^{\pm}(i, t)\mathrm{d}t$ for the first jump ('first' refers to the initial condition at $t = 0$) to occur between t and $t + \mathrm{d}t$ has to be treated separately. $h^{\pm}(i, t)$ is in general different from $\psi^{\pm}(i, t)$, if a random initial time is chosen. $h^{\pm}(i, t)$ describes the average effect that the particle has made its last jump a certain time τ before $t = 0$ and is thus given by [12] *

$$h^{\pm}(i,t) = \frac{\int_0^{\infty} \mathrm{d}\tau \psi^{\pm}(i, t+\tau)}{\int_0^{\infty} \mathrm{d}t \int_0^{\infty} \mathrm{d}\tau \psi^{\pm}(i, t+\tau)}. \tag{6}$$

Note that the ψ and h functions are identical, only if ψ has an exponential form.

The CTRW problem can conveniently be approached by introducing the auxiliary quantities $R^n(i, t)$ [19]. $R^n(i, t)\mathrm{d}t$ is the probability that the particle has performed its n-th jump between t and $t + \mathrm{d}t$ and has thereby reached site i. $R^n(i, t)$ obeys the following recursion relation

$$R^n(i,t) = \int_0^t \{\psi^{+}(i-1, t-\tau) R^{n-1}(i-1,\tau) + \psi^{-}(i+1, t-\tau) R^{n-1}(i+1,\tau)\}\mathrm{d}\tau. \tag{7a}$$

This means that only transitions between next neighbours are considered in this model. The recurrence relation, eq. 6, is valid for $i \geqslant 2$. The first term is given by

$$R^1(i,t) = h^{+}(i-1,t)P(i-1,0) + h^{-}(i+1,t)P(i+1,0), \tag{7b}$$

where $P(i, 0)$ specifies the initial distribution. We now define $R(i,t)$ as the probability that site i is reached by a jump between t and $t + \mathrm{d}t$ as

$$R(i,t) \equiv \sum_{n=1}^{\infty} R^n(i,t). \tag{8}$$

* For choices other than the exponential form, the first jump, t = 0, in an ongoing stochastic process must be treated differently from succeeding jumps. For a most recent discussion of this point see ref. 12 and references cited therein.

Then we find by summation of the recursion relations, eqs. 7a and 7b

$$R(i,t) = R^1(i,t) + \int_0^t \{\psi^+(i-1,t-\tau)R(i-1,\tau) + \psi^-(i+1,t-\tau)R(i+1,\tau)\}\mathrm{d}\tau. \tag{9}$$

$R(i,t)$ is related to $P(i,t)$ through

$$P(i,t) = \int_0^t \mathrm{d}\tau \Psi(i,t-\tau)R(i,\tau) + H(i,t)P(i,0) \tag{10}$$

with

$$\begin{aligned} \Psi(i,t) &\equiv 1 - \int_0^t \mathrm{d}\tau\psi(i,\tau), \\ H(i,t) &\equiv 1 - \int_0^t \mathrm{d}\tau h(i,\tau). \end{aligned} \tag{11}$$

The first term of eq. 10 contains the probability $\Psi(i,t)$ that no jump has occurred after the last jump, and the second the probability $H(i,t)$ that no jump has occurred at all.

2.3. Generalized master equation

To derive the differential equation governing the dynamical behaviour of the system, we have to start from eq. 10 and to combine it with eq. 9. Moreover, it is convenient to employ a Laplace transformation over time with these equations

$$\hat{g}(u) \equiv \mathscr{L}\{g\} = \int_0^\infty e^{-ut} g(t)\mathrm{d}t.$$

Upon use of the convolution theorem eqs. 9 and 10 transform into

$$\hat{R}(i,u) = \hat{R}^1(i,u) + \{\hat{\psi}^+(i-1,u)\hat{R}(i-1,u) + \hat{\psi}^-(i+1,u)\hat{R}(i+1,u)\} \tag{12}$$

and

$$\hat{P}(i,u) - \hat{\Psi}(i,u)\hat{R}(i,u) = \hat{H}(i,u)P(i,0). \tag{13}$$

Using eq. 12 and carrying out simple algebraic manipulations we obtain eq. 13 in the following form

$$\begin{aligned} &\hat{H}(i,u)P(i,0) + \hat{\Psi}(i,u)\left\{\hat{R}^1(i,u) - \frac{\hat{\psi}^+(i-1,u)}{\hat{\Psi}(i-1,u)}\hat{H}(i-1,u)P(i-1,0)\right. \\ &\left. - \frac{\hat{\psi}^-(i+1,u)}{\hat{\Psi}(i+1,u)}\hat{H}(i+1,u)P(i+1,0)\right\} = \hat{P}(i,u) - \hat{\Psi}(i,u)\left\{\frac{\hat{\psi}^+(i-1,u)}{\hat{\Psi}(i-1,u)}\hat{P}(i-1,u)\right. \\ &\left. + \frac{\hat{\psi}^-(i+1,u)}{\hat{\Psi}(i+1,u)}\hat{P}(i+1,u)\right\}. \end{aligned} \tag{14}$$

The terms originating from the initial condition and the first jump are combined into the left-hand side of eq. 14. Multiplying this equation with u and using the abbreviations, eq. 11, we can write the right-hand side, $\hat{R}(i,u)$, of eq. 14 in a form which clearly reflects the transitions from site i to their next neighbours

$$\begin{aligned} \hat{R}(i,u) \equiv (1-\hat{\psi}(i,u))\left\{u\hat{P}(i,u) - u\left(\frac{\hat{\psi}^+(i-1,u)}{1-\hat{\psi}(i-1,u)}\hat{P}(i-1,u) - \frac{\hat{\psi}(i,u)}{1-\hat{\psi}(i,u)}\hat{P}(i,u)\right.\right. \\ \left.\left. + \frac{\hat{\psi}^-(i+1,u)}{1-\hat{\psi}(i+1,u)}P(i+1,u)\right)\right\}. \end{aligned} \tag{15}$$

The left-hand side, $\hat{L}(i, u)$, of eq. 14 can be converted into a similar form using eq. 7b

$$\hat{L}(i,u) \equiv (1-\hat{\psi}(i,u))\Big\{ P(i,0) + \frac{\hat{h}^{+}(i-1,u)(1-\hat{\psi}(i-1,u)) - \hat{\psi}^{+}(i-1,u)(1-\hat{h}(i-1,u))}{1-\hat{\psi}(i-1,u)} P(i-1,0) + \frac{\hat{h}^{-}(i+1,u)(1-\hat{\psi}(i+1,u)) - \hat{\psi}^{-}(i+1,u)(1-\hat{h}(i+1,u))}{1-\hat{\psi}(i+1,u)} P(i+1,0) - \frac{\hat{h}(i,u)-\hat{\psi}(i,u)}{1-\hat{\psi}(i,u)} P(i,0)\Big\}. \tag{16}$$

These two expressions can be combined into the following matrix equation

$$\{u\mathbf{E} - \hat{\boldsymbol{M}}(u)\}\,\hat{\boldsymbol{P}}(u) = \boldsymbol{P}(0) + \hat{\boldsymbol{A}}(u)\boldsymbol{P}(0), \tag{17}$$

where the matrix elements $\hat{M}_{ij}$ and $\hat{A}_{ij}$ are defined by eq. 15 and 16, respectively. $\hat{\boldsymbol{M}}$ and $\hat{\boldsymbol{A}}$ represent infinite tridiagonal matrices. In the case of regular lattices, i.e., when the waiting times $\psi^{\pm}(i,t)$ are equal to each other, our result reduces to that of Kehr and Haus [12].

We may now return to our cyclical channel system. By using eq. 3 and the $(n+1)$ periodicity of the waiting times $\psi^{\pm}(i,t)$, an equation is obtained which is formally identical to eq. 17 but has finite dimensional matrices $\hat{\boldsymbol{M}}$ and $\hat{\boldsymbol{A}}$. These matrices are also tridiagonal but with $\hat{M}_{1,n+1} = \hat{M}_{10} \neq 0$, $\hat{M}_{n+1,1} = \hat{M}_{n+1,n+2} \neq 0$ (and the same for $\hat{\boldsymbol{A}}$) in reflecting the periodic structure of the system. The obtained equation is equivalent to the following generalized master equation

$$\dot{\boldsymbol{P}}(t) = \int_0^t \boldsymbol{M}(t-\tau)\boldsymbol{P}(\tau)\mathrm{d}\tau + \boldsymbol{A}(t)\boldsymbol{P}(0), \tag{18}$$

where the matrices $\boldsymbol{M}(t)$ and $\boldsymbol{A}(t)$ have the Laplace transforms $\hat{\boldsymbol{M}}(u)$ and $\hat{\boldsymbol{A}}(u)$, respectively.

Both the memory kernel and the inhomogeneity of eq. 18 express the average effect of the conformational changes on the particle being transported. Eq. 18 represents a non-Markovian description of the transport, since it takes into account the whole past of the protein's internal dynamics. In case the waiting times are given by a single exponential

$$\psi^{\pm}(i,t) = k_i^{\pm}\exp(-k_i t), \qquad k_i \equiv k_i^{+} + k_i^{-}, \tag{19}$$

we no longer find memory behaviour but obtain a Markovian master equation (without an inhomogeneous term). This means that the memory-possessing transition rates in the GME arise from the reduction of the original Markov model because this was of higher dimensionality.

The total process can now be viewed as a random walk of a particle facing the average effect of the protein as a time-dependently interacting environment. From this standpoint a physical interpretation of the memory aspects of the process can also be given: The ion on its way through the membrane has an influence on the protein in affecting the conformational changes which then react upon it at later times. The impact the ion faces at a time t therefore depends on its behaviour at previous times.

3. Stationary behaviour

To characterize the stationary behaviour of the transport system we introduce the unidirectional fluxes from site i to $i+1$ in analogy to the Markovian case as

$$\Phi_i^{i+1}(t) \equiv \int_0^t M_{i+1,i}(t-\tau)P(i,\tau)\mathrm{d}\tau + A_{i+1,i}(t)P(i,0). \tag{20}$$

The net fluxes are then given by

$$\Phi_i(t) = \Phi_i^{i+1}(t) - \Phi_{i+1}^{i}(t). \tag{21}$$

The stationary fluxes

$$\Phi_i^{ss} \equiv \lim_{t\to\infty} \Phi_i(t) \tag{22}$$

can be determined by Laplace transformation and upon use of the Tauberian theorem in the limit $u \to 0$. For small u, i.e., $t \to \infty$, the stationary solution P_i^{ss} is thus given through

$$\hat{P}(i, u) = \frac{1}{u} \cdot P_i^{ss}. \tag{23}$$

By expanding the waiting time distributions up to first order

$$\hat{\psi}^{\pm}(i, u) = a_i^{\pm} - t_i^{\pm} u \tag{24}$$

and setting $t_i \equiv t_i^{+} + t_i^{-}$, for sufficiently small u, eq. 20 is transformed into

$$\hat{\Phi}_i^{i+1}(u) = \frac{1}{u} \cdot \frac{a_i^{+}}{t_i} \cdot P_i^{ss}. \tag{25}$$

This immediately yields the stationary fluxes

$$\Phi_i^{ss} = \frac{a_i^{+}}{t_i} \cdot P_i^{ss} - \frac{a_{i+1}^{-}}{t_{i+1}} \cdot P_{i+1}^{ss}, \qquad 0 \leqslant i \leqslant n. \tag{26}$$

This is simply the usual system of flux equations known from Markovian systems, because $a_i^{\pm}/t_i$ just represents the rate constants; t_i equals the mean waiting time defined by

$$\int_0^{\infty} t\psi(i, t)\mathrm{d}t \tag{27}$$

and a_i^{+} is the probability that the ion leaves site i to the right (with $a_i^{+} + a_i^{-} = 1$ because of eq. 2). If the graph of a reaction system consists of a single closed loop, the stationary fluxes must be pairwise equal; therefore, $\Phi^{ss} \equiv \Phi_0^{ss} = \ldots = \Phi_n^{ss}$. This means that the system of equations (eq. 26) can be easily solved for Φ^{ss} by successive elimination of the P_i^{ss} [25]. Summarizing now, the stationary problem can be discussed completely as done by Läuger in his study of the stationary transport through channels with a rigid barrier structure [14]. The reduction procedure as applied in the previous section has led to a model which displays the same stationary behaviour as the corresponding one-ion model without conformational transitions. However, we will obtain new features in a time-dependent analysis.

4. Transient behaviour of electrogenic ion pumps

The action of ion pumps is described on the basis of the general channel model derived in section 2. An ion channel functions as a pump if the channel molecule undergoes a cycle of conformational transitions during which the barrier structure is transiently modified. Absorption of light, energy from redox transitions or phosphorylation of the channel protein may alter the binding constant of an ion-binding site and the height of the adjacent barriers. Hence, by energy input an ion may leave a binding site where it has been trapped before.

This picture of trapping and release by means of an energy-supplying reaction plays the essential role in our pump model. Thus, the catalytic unit as the central part of the pump can be characterized by two states, an 'acceptor' state A (uptake of the ion) and a state of release R. A third component functioning as a device for ion translocation through the membrane is imagined as a channel-like conduit T [31]. A pictorial representation of our model is shown in fig. 3. The catalytic unit (for instance, imagined as a membrane-bound ATPase) is fairly widely exposed to the cytoplasmic side but it could also be located within the membrane [17]. More important is the assumption that the translocation device and catalytic unit are linearly arranged. The pump can therefore be viewed as a barrier model with a sequence of binding sites and wells. For simplicity, only one ion is allowed to be in the pumping system. The major assumption is that the catalytic unit is only accessible via the acceptor state and must be left via the release state. This means that passive leakage of the ions through the pump is negligible or, in other words, ion transport and energy input are completely coupled. The assumption of ideally irreversible pumping steps is supported by quantum-chemical calculations of the activation energy, for instance, for the retinal of the proton pump of *Halobacterium halobium* [28]. It can be understood with the pump's ability of storing energy.

In fig. 4 the model is formulated in terms of its Markovian state representation. This scheme provides for an extension of the theoretical treatment of ion pumps as the translocation steps are explicitly regarded. Uptake as well as release of the ions may occur in either direction. Nevertheless, an asymmetry of the pump providing that the ions are preferentially released to one side can be taken into account by an appropriate choice of the transport steps.

The aim of this paper is to give a qualitative survey over the time behaviour of the model by presenting analytical results. Following the lines of section 2 the whole state diagram (fig. 4) has first to be partitioned into the subsets $S_{AR} = \{A,R\}$, $S_{A^*R^*} = \{A^*,R^*\}$, $S_{T'} = \{T_i'\}$, $S_{T''} = \{T_i''\}$. To obtain subgraphs these sets must be completed by the respective transition probabilities; for instance, $G_{AR} = (S_{AR}, K_{AR})$ with $K_{AR} = \{k_A, k_R, k_{A^*}^+, k_T^-\}$. Note that each subgraph is constructed in the same way. Since it can be reached through exactly one state, we are able to calculate the waiting time of an ion within the subgraph on the basis of random walks (cf. appendix). For $G_{A^*R^*}$ this procedure leads in the Laplace domain to

$$\hat{\psi}^+_{A^*R^*} = \frac{k_{R^*} k_T^+}{k_{R^*}(k_T^+ + k_R^-) + u(k_{R^*} + k_T^+ + k_R^- + k_{A^*}) + u^2}, \qquad \hat{\psi}^-_{A^*R^*} = \frac{k_R^-}{k_T^+} \cdot \hat{\psi}^+_{A^*R^*}. \tag{28}$$

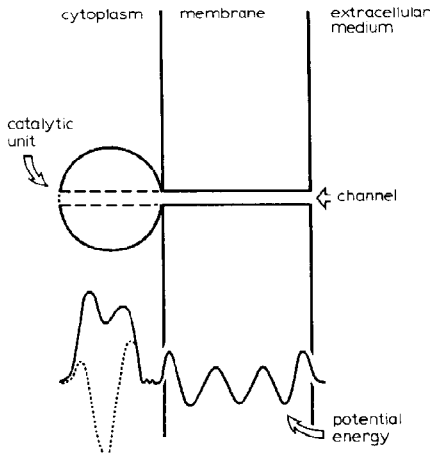

Fig. 3. Pictorial scheme of an ion pump. The pump consists of a catalytic unit at the cytoplasmic side and a channel-like conduit for the translocation of the ions through the membrane (upper part). The ion's energy profile is depicted in the lower part. The two conformational states of the pump are indicated by the dashed line.

Note that $\psi^{\pm}_{A^*R^*}$ is multiplicative such that

$$\psi^{\pm}_{A^*R^*}(t) = a^{\pm}\psi_{A^*R^*}(t) \tag{29}$$

where $a^+ = k_T^+/(k_T^+ + k_R^-)$ and $a^- = k_R^-/(k_T^+ + k_R^-)$ denote the probabilities that the particle is released to the right or left, respectively. $\psi_{A^*R^*}$ represents a biphasic curve of the form

$$\psi_{A^*R^*}(t) = A(e^{u_1 t} - e^{u_2 t}) \tag{30}$$

where u_1 and u_2 are just the roots of the denominator of eq. 28 with $u_2 < u_1 < 0$, and A is a positive constant determined by the normalization relation (eq. 2). The trapping of the particle in the acceptor state is expressed by $\psi_{A^*R^*}(0) = 0$. The electrodiffusive behaviour of the ions may be treated in the same way by indicating the translocation subgraphs $G_{T'}$ and $G_{T''}$ by their waiting time distributions. These are of the same form as $\psi^{\pm}_{A^*R^*}$. For simplicity's sake, let the waiting times of the whole system equal each other such that

$$\psi^+ \equiv \psi^+_{A^*R^*} = \psi^+_{AR} = \psi_{T'}, \qquad \psi^- \equiv \psi^-_{A^*R^*} = \psi^-_{AR} = \psi_{T''}. \tag{31}$$

Note that the second equality signs imply an approximation. To satisfy eq. 4 the subgraphs $G_{T'}$ and $G_{T''}$ must be combined into one, G_T. Finally, the non-vanishing elements of the memory function $M(t)$ are found to have the form of a single exponential (cf. [13]):

$$M_{i,i\pm1}(t) = a^{\pm}\omega_0^2 e^{-\lambda t} \qquad M_{i,i}(t) = \omega_0^2 e^{-\lambda t} \tag{32}$$

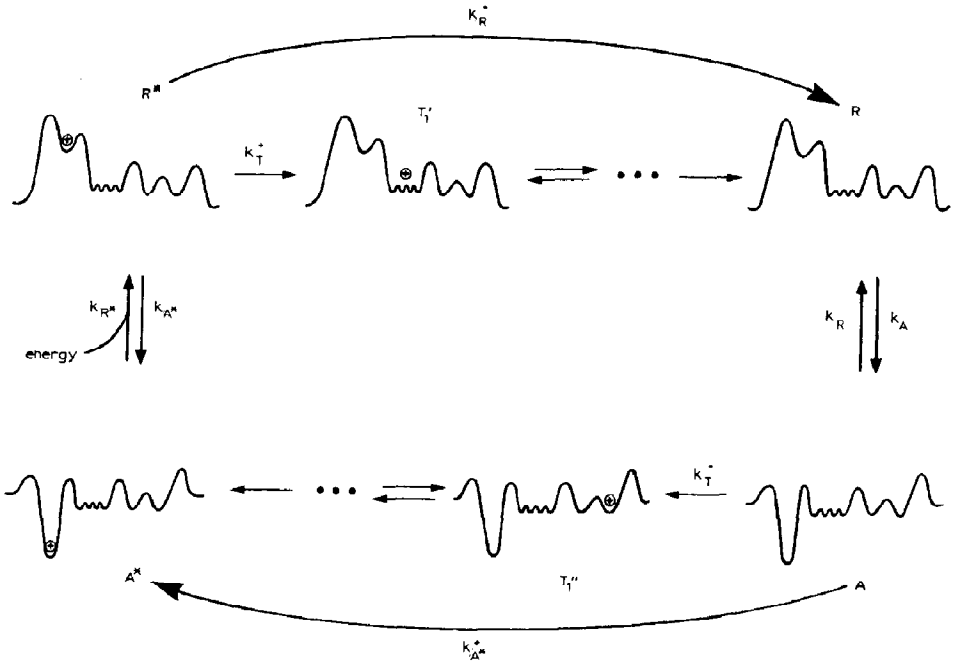

Fig. 4. Graphical representation of the ion pump of fig. 3. The graph consists of essentially three states – the acceptor states A* and A, the states R* and R of release, and the translocation states T_i' and T_i''. The k terms are rate constants indicating the translocation of the ion as well as the conformational changes of the pump. For technical reasons the transition between A and T_1' is assumed to be unidirectional. The other unidirectional transitions are biologically motivated (see text).

where ω_0 and λ can be expressed in terms of the original Markovian model as (cf. corresponding expressions for $a^{\pm}$)

$$\omega_0^2 = k_{R^*}(k_T^+ + k_R^-), \qquad \lambda = k_{R^*} + k_T^+ + k_R^- + k_{A^*}. \tag{33}$$

ω_0 plays the role of a characteristic frequency, and λ describes the relaxation of the channel.

As in section 2 it is again convenient to map the system onto an infinite lattice. When the GME (eq. 18) is then differentiated with respect to t and eq. 32 is substituted into the resulting expression the ensuing equation is

$$P_{tt}(i,t) + \lambda P_t(i,t) = \omega_0^2\{a^+ P(i-1,t) - P(i,t) + a^- P(i+1,t)\} + \{\lambda \boldsymbol{A}(t) + \dot{\boldsymbol{A}}(t)\}\boldsymbol{P}(0)|_i. \tag{34}$$

In order to discuss the qualitative behaviour of the pump, we may neglect the inhomogeneous term of eq. 34 because it only yields a particular solution of the system. The homogeneous part of eq. 34 can conveniently be discussed by proceeding to the continuum limit. Clearly, this is only an approximation since we cannot assume that the lattice spacings shrink to zero as in the case of rigid pores [7]. We make use here of the result that the continuum limit represents a rather good approximation even if the pore contains only a small number of binding sites [7]. Replacing the differences of the right-hand side of eq. 34 by the respective derivatives and going over to spatial probability densities $p(x,t)$ leads to a partial differential equation which is known as telegrapher's equation:

$$p_{tt}(x,t) + \lambda p_t(x,t) = v^2 p_{xx}(x,t) - a p_x(x,t) \tag{35}$$

with

$$v^2 = \tfrac{1}{2}\omega_0^2 l^2, \qquad a = \omega_0^2(a^+ - \tfrac{1}{2})l, \tag{36}$$

and l as the lattice spacing.

The difference between the Fokker-Planck equation governing the transport through continuous rigid pores and the telegraph equation can be understood by comparing eqs. 36 with the corresponding ones for rigid pores [33]. In the latter case the transition to the continuum equations has to be carried out such that $\omega_0 l^2$ remains finite; this means an infinite walk speed is implied in the limit $l \to 0$. However, carrying out the limit in eq. 36 would lead to a finite walk speed v. This makes the telegraph equation basically more realistic when applied to complex transport systems. In the limit $t \gg \lambda^{-1}$ the difference between the two equations is very small. In other words, as long as we consider times that are much longer than the duration of the walk correlation λ^{-1}, the telegraph equation (eq. 35) can be approximated by a Fokker-Planck equation with $v^2\lambda^{-1}$ as diffusion and $a\lambda^{-1}$ as drift coefficient. We omit here the discussion of the general solution of eq. 35 which can be found in standard textbooks on partial differential equations, and concentrate only on the other limiting case $t \ll \lambda^{-1}$. For this time range a wave-like equation governs the pumping of the ions, since the term λp_t of the left-hand side of eq. 35 can be neglected in comparison with the acceleration term. The picture of how the ions move through the pump is therefore as follows. On a large time scale, the transport shows the usual diffusion behaviour, on a fine time scale, however, the movement of the ions is strongly correlated which means that the transport process has a rather perfect memory.

Experimental evidence for such a short-time behaviour has been found in transient measurements of the photo-current generated by the proton pump of halophilic bacteria [2,22]. The retinal of the bacteriorhodopsin molecule from *H. halobium* undergoes *trans-cis* isomerization after photon absorption. The time behaviour of the flash-induced charge movements during the first steps in the photocycle can be interpreted in the light of our results, since the appearance of a large amplitude of the fast electric signal in response to a flash illumination indicates that the motion of the protons is strongly correlated over short time. To characterize the proton pump, no detailed information about the bacteriorhodopsin molecule has been

used. We may therefore conjecture that the appearance of a rather perfect memory in the transport process covers a wide class of active transport systems. The answer to this conjecture, however, must for the time being be kept open since the information available about ion pumps is still insufficient.

5. Conclusions

The aim of this paper has been to introduce a non-Markovian approach to complex membrane transport systems. To include the internal degrees of freedom of the transport system (protein) a memory function representation in combination with a GME has been used. In applying this formalism to any specific problem the decisive step is to obtain an appropriate expression for the memory kernel. It should be mentioned here that such an expression need not necessarily be derived from an underlying Markov scheme. Memory-possessing transition rates can also be obtained from microscopic theories as, for instance, has been done in the theory of excitation transfer [39], or they may be inferred from some ad hoc assumptions about the process in the form of simplified functions.

To make the non-Markovian formalism more applicable to practical modelling, further investigations are needed in at least two directions. First, time-dependent expressions for the transport observables (e.g., electric current) must be derived from the basic equations. This can be accomplished within the concept of discrete transport systems as developed by Frehland [38]. In this concept the observables have the general form (cf. eqs. 20 and 21 for definition of the fluxes)

$$T(t) = \sum_i \gamma_i \Phi_i \tag{37}$$

with the weights γ_i. The mathematical treatment can then best be done in the Laplace domain when starting from eq. 17 and applying a generalized moment expansion. This procedure employed by Schulten and co-workers [40] to Brownian dynamics in biological systems can immediately be extended to non-Markovian cases and is appropriate, in particular, for numerical studies. Since the moment expansion reproduces systematically the high as well as the low-frequency dependence of transport observables of the form given by eq. 37, it provides a useful tool for studying non-Markovian master equations in both short and long time ranges which are most interesting.

Second, the model GME must be extended to contain a wider class of transport systems. On the one hand, this is to cover also conformational transitions of the protein which are connected with macroscopic charge displacement but have been neglected in this paper (cf. section 1). On the other hand, one has to account for the fact that the graphical representation of both active and passive transport mechanisms typically contains closed loops. Such loops, however, are in general not exactly represented by a single waiting time distribution per binding site in the way they are approximated throughout this paper. This point is demonstrated in the following simple example of a channel with one binding site and two conformational states [18], which has been mentioned in section 1. Using the graphical representation of the system, the ohmic single-channel conductance Λ vs. the ionic concentration c of the baths can be calculated explicitly. For certain values of the rate constants one finds a maximum of Λ instead of a simple saturation behaviour. This property, however, typical for a two-state channel with concentration-dependent regulation, can no longer be observed if the channel's sites are described by one waiting time only (cf. appendix for calculation of the waiting time). In fig. 5 the single-channel conductance Λ resulting from the applied approximation procedure is plotted (dotted lines) and compared with the result derived from the Markovian model (full lines). The numbers by the curves denote the asymmetry of the channel system generated by conformational changes (cf. inset of fig. 5). Good agreement between both results is only obtained in the case $p = 1$ where both channel conformations have symmetric energy profiles.

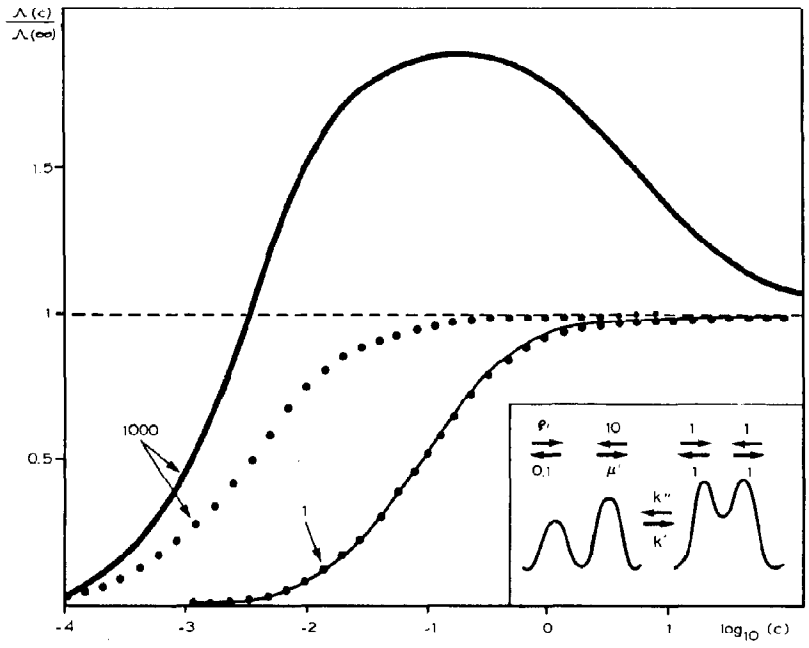

Fig. 5. Single-channel conductance Λ vs. ionic concentration c of the baths. (Full lines) Single-channel conductance for a channel with one binding site and two conformational states. The numbers by the curves indicate the asymmetry p of one of the channel states (cf. inset). The parameters ρ' and μ' take the values $\rho' = 10p$ and $\mu' = 0.1p^{-1}$. The transition between the two states depends on their occupation probability; i.e., $k' = 100\sqrt{p}$ and $k'' = 10$ when the channel is occupied, and $k' = 10$ and $k'' = 100\sqrt{p}$ when the channel is empty. (Dotted lines) Single-channel conductance of the same system calculated by means of the reduction procedure as described in the appendix.

This example cautions that not all systems can be reduced such that only the binding sites are left in the reduced scheme. In formal aspects, the reason why the reduction is not adequate in this example is that each subgraph contains two entries (exits). In physical terms, this means that the particle sitting in a binding site has two possibilities of moving forwards and backwards and therefore two states of motion. Apart from the spatial variable corresponding to the binding sites, this needs the introduction of a second variable which has to be accounted for in reduction. In continuum systems, this variable is the velocity of the particle. (The given example, therefore, provides the simplest discrete version of a Fokker-Planck equation which depends on both the spatial coordinate and the velocity [11].)

This example, however, represents no objection to the reduction method described in this paper. It only points out that the physics of a given process must be taken into account in order to perform an adequate reduction, and, in turn, the example also shows that a system can eventually be better understood by means of reduction.

Appendix: Calculation of the waiting time distribution

We calculate the waiting times only for those subgraphs of a given Markovian graph which are accessible through a single state. Moreover, since all the subgraphs used in the main text are linear a random walk problem on a one-dimensional chain with a reflecting and an absorbing boundary is considered in the following (cf. fig. 6).

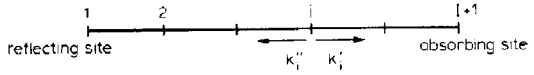

Fig. 6. Linear graph with a reflecting as well as an absorbing boundary. The k_i terms are dimensionless transition probabilities.

Let $\chi_i(n)$ be the probability that the random walker reaches the absorbing site $I+1$ in its nth jump when started at site i. Recursion relations for $\chi_i(n)$ can be set up following Feller [3].

$$\chi_1(n+1) = k_1'\chi_2(n)$$
$$\chi_i(n+1) = k_i'\chi_{i+1}(n) + k_i''\chi_{i-1}(n), \qquad 2 \leqslant i \leqslant I-1$$
$$\chi_I(n+1) = k_I'\delta_{On} + k''_I\chi_{I-1}(n) \tag{A1}$$

where δ_{On} is the Kronecker symbol and the k_i terms are dimensionless probabilities ($0 \leqslant k_i \leqslant 1$). On using

$$\hat{\chi}_i(u) \equiv \sum_{n=0}^{\infty} \chi_i(n)\left(\frac{1}{1+u}\right)^n$$

eq. A1 transforms into a set of equations which can be solved in the form of continued fractions. The inverse Laplace transform of $\hat{\chi}_i(u)$ then represents the waiting time distribution of a particle which entered the respective subgraph at site i at $t=0$. For a subgraph containing only two states $\hat{\chi}_i(u)$ is given as

$$\hat{\chi}_1(u) = \frac{k_2'}{k_1'k_2' + u(k_1' + k_2' + k_2'') + u^2}, \qquad \hat{\chi}_2(u) = (k_1' + u)\hat{\chi}_1(u). \tag{A2}$$

It follows from eq. A2 that the waiting time distribution $\chi_1(t) = \mathscr{L}^{-1}\{\hat{\chi}_1\}$ vanishes at $t=0$. This expresses that the particle cannot escape instantly after having entered the subgraph. In contrast, χ_2 takes a finite value at $t=0$ and decays monotonically for $t>0$.

When a certain subgraph, say G_i of site i, is accessible through two states, the formalism described above can no longer be applied, except that the system is in its stationary state. In this case only the distribution of the states within the neighbouring subgraph $G_{i\pm1}$ on its own is known. This information, however, is necessary to calculate the probability of each of the two acceptor states of G_i to be reached by the particle from the neighbouring site $i \pm 1$ by means of first passage times, as has been done above. The single-channel conductance curves of fig. 5 (dotted lines) are obtained by that procedure.

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