Mechanism of the translocation step of mediated transport across biomembranes: In favour of the allosteric model

ARTICLE · JUNE 2010

DOI: 10.1016/0307-4412(93)90139-Q

1 AUTHOR:

Michael Eze

The University of Winnipeg

29 PUBLICATIONS **270** CITATIONS

Mechanism of the Translocation Step of Mediated Transport across Biomembranes: in Favour of the Allosteric Model

MICHAEL O EZE

Department of Biochemistry
University of Nigeria, Nsukka
Nigeria

Introduction

Solute uptake in cells and organelles occurs by either non-energy-dependent (passive permeation and facilitated diffusion) or energy-dependent (active transport and group translocation) processes.[1-3] Passive permeation of solute through the membrane occurs by simple diffusion down a concentration gradient and its kinetics may be described by Fick's first law as modified to account for the presence of the membrane.[1-3]

The ability of the permeant to partition into the membrane lipid bilayer (ie its lipid solubility, or oil/water partition coefficient) is one of the factors which govern the passive permeation rate. This depends largely on (i) the number of methylene groups in the permeant molecule which are exposed to water, and (ii) the number of hydrogen bonds (with water) that must be broken.[4-6]

Facilitated diffusion is protein mediated but since it is not energy-coupled, results in equilibration just like passive permeation. Its rate is, however, generally much faster than the passive permeation rate for hydrophilic nonelectrolytes and ions. The activation energy (E_a) for mediated transport has been observed to be lower than that for the passive permeation of the same permeant molecule through the same cells,[7] or through liposomes.[8]

The energy-dependent transport systems, like facilitated diffusion systems, are mediated by proteins in the membrane and, for both types, the transport process obeys Michaelis–Menten kinetics. The initial transport rate (v) is therefore related to the maximum velocity (V_{max}) of transport attainable at saturation with substrate according to

$$v = V_{max}[S]/(K_m + [S])$$

One cycle of mediated transport comprises a number of steps: substrate recognition, binding, translocation, release and, finally, recovery of the mediator.

Mechanism of the Translocation Step

(*a*) *Fixed Pore (or Channel), and the Rotating (or Mobile) Carrier Models* The mechanism of the translocation step is controversial.[9,10] Earlier, two models were favoured: (i) the classical fixed 'pore' or 'channel' model in which lipid-insoluble substances, eg ions, are supposed to permeate the membrane through water-filled channels penetrating the membrane, ie through a hydrophilic environment (see Fig 1*a*), or (ii) the 'rotating' or 'mobile' carrier model (Fig 1*b*) which postulates that transport occurs by the attachment of the substrate to a carrier in the membrane which shuttles between opposite faces of the membrane.[2,3]

(*b*) *Evidence Drawn from Ionophoric Antibiotic Model Systems* Model systems which operate by these two possible mechanisms have been designed using the ionophoric antibiotics including pore-former antibiotic-mediated ion transport (eg gramicidin A-mediated K^+ transport), and mobile carrier antibiotic-mediated (eg valinomycin –, or nonactin-mediated) K^+ transport in lipid bilayers.[11,12] The experiments of Krasne *et al*[13] provided information on the temperature-dependence of ion transport by these antibiotics through lipid bilayers. According to

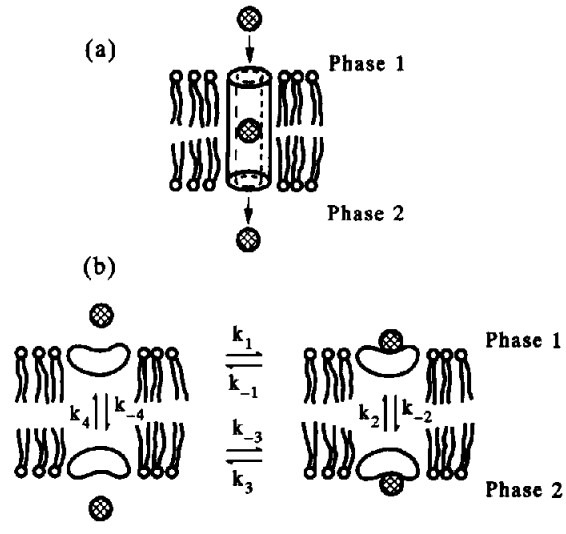

Figure 1a Depiction of the classical pore (or channel) through a lipid bilayer membrane. The polar transport substrate (⊕) passes through the fixed hydrophilic pore. 1b Depiction of the rotating (or mobile) carrier model of translocation mechanism. The carrier binds substrate (⊕) with a rate constant k_1. The translocation implies a flip-flop movement of the carrier-substrate complex with a rate constant k_2 from phase 1 to phase 2 of the membranes. Substrate release in phase 2 occurs with a rate constant k_3 and the empty carrier then returns to phase 1, again by flip-flop, with a rate constant k_4. The reverse process in each case has the reverse rate constant

others,[6,14,15] at high temperatures the hydrophobic core of the membrane polar lipid bilayer is fluid and relatively easy to penetrate, but at low temperatures, it exists as a gel and is practically impenetrable by lipophilic substances. Thus, the response of ion transport (in the presence of the ionophoric antibiotics) to the physical state of the membrane may be used to differentiate between the pore-former mechanism and the mobile carrier mechanism.

Gramicidin A is a linear peptide consisting of fifteen apolar amino acids[11] which forms water-filled transmembrane channels consisting of dimers of gramicidin A in a helical configuration (Fig 2). K^+ ions pass through this pore and the resulting K^+ conductance does not display a discontinuity as the membrane changes phase from liquid crystalline (fluid state) at high temperatures to gel at low temperatures, or *vice versa*.

In the case of valinomycin, this macrocyclic depsipeptide engulfs a K^+ ion and shields its charge (Figure 3) exposing an exterior that is entirely hydrophobic.[11] This complex easily traverses the fluid membrane, and brings about K^+ ion shuttling (Fig 3b). When the bilayer core is fluid (high temperatures) this process occurs with a low activation energy (E_a). In the gel phase

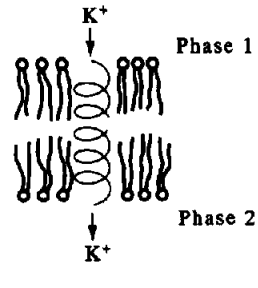

Figure 2 Two Gramicidin A molecules, each in helical configuration, form a hydrophilic pore through the membrane lipid bilayer. The K^+ conductance occurs through this resulting pore

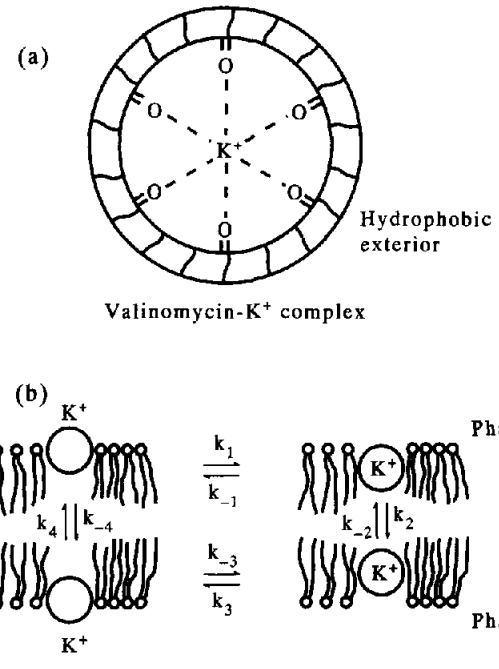

Figure 3a The valinomycin-K^+ complex which can easily traverse the membrane lipid bilayer due to its totally hydrophobic exterior, shielding the K^+ ion (Adapted from ref 25)

Figure 3b A K^+ ion shuttling across the membrane bilayer brought about by valinomycin and operating in the classical rotating (or mobile) carrier sense depicted in Figure 1b

(low temperatures) however, a higher (and usually prohibitive) E_a is required to allow the valinomycin/K^+ complex through the 'frozen' bilayer. A sharp discontinuity in the Arrhenius plot results.

(*c*) *The Natural Carriers* In biological membranes, the natural transport mediators are proteins (rather than antibiotics) whose integral location within the membrane bilayer inherently creates restrictions. In the case of transport requiring some periplasmic binding proteins (in bacteria for instance), these binding proteins direct the substrate to the integral protein(s) for the translocation step.[16,17] Now, an integral protein which completely traverses the bilayer possesses three domains — two hydrophilic ends and a middle portion which is hydrophobic.[17,18] It is known that the transfer of a polar moiety from water to a hydrophobic environment (or a nonpolar moiety from a hydrophobic to an aqueous phase) requires a large amount of free energy.[18,19] Consequently, while a valinomycin molecule can complex a K^+ ion and expose an entirely hydrophobic surface to the membrane hydrocarbon core,[11] integral membrane proteins catalysing transport are unlikely to expose an entirely hydrophobic surface. Thus the pore-former model[13] can apply in the natural membrane, but the classical rotating (or mobile) carrier is not feasible because it would involve transbilayer movement (ie flip-flop) of the amphipathic integral membrane proteins which is energetically expensive.[6,18]

(*d*) *The Utility of the Classical Mobile (Or Rotating) Carrier Formulation to Pioneers of Transport Kinetics* The classical mobile (or rotating) carrier model was useful and led to the formulation of mediated transport kinetics including the derivation of the rate constants for the individual substeps of the transport process.[2]

(*e*) *Aggregate Rearrangement (or Allosteric) Model* A third, and more attractive model for mediated transport translocation is the 'aggregate rearrangement' model proposed by Singer.[17] This model (Fig 4) was based on a consideration of thermodynamic principles and is mechanistically a hybrid of the pore-former and the mobile carrier models, but it is expected to respond like the mobile carrier model with respect to membrane lipid fluidity and phase state. An equivalent model named the 'allosteric' model has been proposed.[10]

The aggregate rearrangement model relies on conformational (allosteric) changes in the transport protein(s) triggered by the presence of substrate. Thus there are well coordinated co-operative subtle movements of subunits (monomers) that are parts of a whole (ie parts of the functional transport translocation system), bringing about transmembrane 'haulage' of substrate, in the absence of flip-flop. These subtle movements may be lateral, or rotational (about an axis perpendicular to the membrane bilayer plane), but not flip-flop. Where the transport protein is a single integral membrane protein, the subtle movements would be simply conformational changes within the protein.

(*f*) *Experimental Evidence Supporting the Absence of Flip-Flop during Translocation* Experiments have been designed to probe the possible contribution of flip-flop to transport.[6,20,21] Specific antibodies bound to purified Na^+, K^+-ATPase from dog kidney,[20] or to Ca^{2+}-ATPase purified from rabbit sarcoplasmic reticulum, neither inhibited the particular ATPase nor inhibited its associated specific ion transport in spite of the fact that a bulky hydrophilic protein, the antibody was attached to the transport system. This should have prevented transmembrane rotation (ie flip-flop) of the complex but did not. Recent experimental findings[22] have corroborated these conclusions.

(*g*) *The Merits of the Aggregate Rearrangement Model* The necessity to spell out the distinctions among these three models for the mechanism of the translocation step has arisen from the author's personal experiences teaching transport to undergraduate and postgraduate students within the past one and half decades. The merits of the aggregate rearrangement model derive from its verifiability exploiting the nature of Arrhenius plots of the temperature-dependence of transport by a facilitated

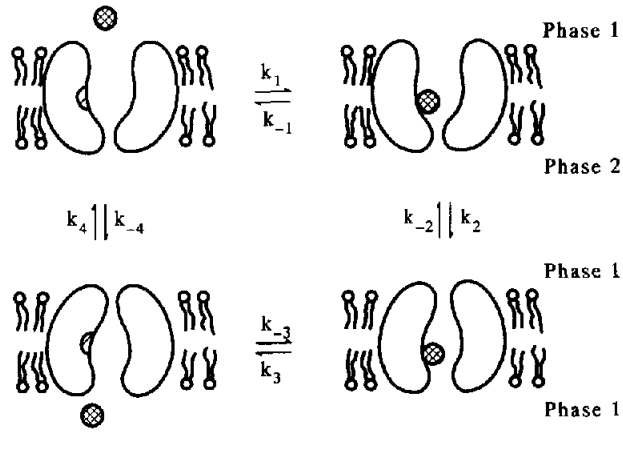

Figure 4 The Aggregate Rearrangement (or Allosteric) model of the mechanism of the translocation step of mediated transport across biological membranes. Substrate (⊕) binds to its site exposed to phase 1 on the transport protein with a rate constant k_1. A conformational change occurs in the absence of flip-flop *with a rate constant k_2, thus exposing the substrate to phase 2 into which it (the substrate) is released with a rate constant k_3. Recovery of the carrier occurs,* again in the absence of flip-flop, *with a rate constant of k_4, thus ending one cycle of translocation*

diffusion system (like that for glycerol in induced *E coli*[7]), or an energy-uncoupled active transport. Discontinuous Arrhenius plots may also be obtained for some active transport systems.[6,23,24] However, unequivocably interpretable results can be obtained only if the energy-uncoupled active transport system is studied. This is because the energy-uncoupling ensures that the temperature-dependence of the energy-coupling component itself does not present artifacts. Breaks or discontinuities in such Arrhenius plots of facilitated diffusion indicate abrupt activation energy (E_a) changes and prove the involvement of some subtle movement(s) (but certainly not flip-flop) as part of the rate-limiting step of the transport. The absence of changes in Ea may imply either that the system is a channel, or that it is a mobile carrier with a non-rate-limiting subtle movement of the carrier protein within the bilayer.[6] In such a case, further experiments such as assaying for the presence of 'counter-flow'[1-3] would delineate a channel (no counterflow) from a carrier (presence of counterflow).[10] In the Aggregate Rearrangement model, the thermodynamic restrictions imposed by the fluid mosaic model of biomembrane structure are not violated since flip-flop is not implied, or involved. In the extreme case where, *ab initio*, the channel dimensions of the transport protein(s) are large, the aggregate rearrangement model would behave like a fixed channel model.

References

[1] Christensen, H N (1975) 'Biological Transport', Second edition, Benjamin, Reading, MA

[2] Stein, W D (1967) 'The Movement of Molecules Across Cell Membranes', Academic Press, New York

[3] Kotyk, A and Janáček, K (1975) 'Cell Membrane Transport: Principles and Techniques', Second edition, Plenum Press, New York

[4] De Gier, J, Mandersloot, J G, Hupkes, J V, McElhaney, R N and Van Beek, W P (1971) *Biochim Biophys Acta* **233**, 610–618

[5] McElhaney, R N, De Gier, J and Van der Neut-Kok, E C M (1973) *Biochim Biophys Acta* **298**, 500–512

[6] Eze, M O (1990) *J Chem Educ* **67**, 17–20

[7] Eze, M O and McElhaney, R N (1981) *J Gen Microbiol* **124**, 299–307

[8] Read, B D and McElhaney, R N (1975) *J Bact* **123**, 47–55

[9] Simoni, R D and Postma, P W (1975) *Ann Rev Biochem* **44**, 523–554

[10] Harold, F M (1986) 'The Vital Force: A Study of Bioenergetics', W H Freeman, New York

[11] Grell, E and Funck, Th (1973) *J Supramolecular Structure* **1**, 307–335

[12] McLaughlin, S and Eisenberg, M (1975) *Ann Rev Biophys Bioeng* **4**, 335–366

[13] Krasne, S, Eisenman, G and Szabo, G (1971) *Science* **174**, 412–415

[14] McElhaney, R N (1982) *Current Topics in Membranes and Transport* **17**, 317–380

[15] Overath, P and Thilo, L (1978) *Int Rev Biochem* **19**, 1–44

[16] Prossnitz, E, Gee, A and Ames, G F L (1989) *J Biol Chem* **264**, 5006–5014

[17] Singer, S J (1974) *Ann Rev Biochem* **43**, 805–833

[18] Singer, S J and Nicolson, G L (1972) *Science* **175**, 720–731

[19] Tanford, C (1961) 'Physical Chemistry of Macromolecules', Wiley, New York, pp 129–131

[20] Kyte, J (1974) *J Biol Chem* **249**, 3652–3660

[21] Dutton, A, Rees, E D and Singer, S J (1976) *Proc Natl Acad Sci USA* **73**, 1532–1536

[22] Xu, K-Y and Kyte, J (1989) *Biochemistry* **28**, 3009–3017

[23] Eze, M O and McElhaney, R N (1987) *Biochim Biophys Acta* **897**, 159–168

[24] Eze, M O and McElhaney, R N (1989) *Microbios* **58**, 173–182

[25] Stryer, L (1988) 'Biochemistry', Third edition, W H Freeman, New York

Inexpensive Large-scale Molecular Models for Use in Developing Countries

OBI CHUDE

Department of Medical Biochemistry
College of Medicine
University of Nigeria
Enugu, Nigeria

Introduction

Molecular models are important in teaching and understanding of biochemistry: one is reminded of the use of models by Watson and Crick.[1] A recent paper[2] highlighted the role of models in teaching and a report on biochemical education in Zimbabwe and neighbouring countries[3] listed 29 problems that hinder the teaching of biochemistry in that part of the world. Three of the problems were (*a*) the problem of thinking in three dimensions, (*b*) lack of teaching aids, and (*c*) foreign exchange problems. Unfortunately, even simple plastic ball-and-stick or skeletal models are relatively expensive in many developing countries and computer models are out of the question in some of these countries because of the cost.

In order to overcome some of these problems we started to build very inexpensive molecular models. Models of simpler molecules such as purines, glucose, ATP, etc may easily be constructed using transparent adhesive tape, wires or broomsticks and colored paper or pieces of cloth. Some of these approaches have been published.[4–6] Broomsticks are not strong enough for the construction of larger molecules such as DNA. Here I describe how a model of a complex molecule such as DNA can be constructed inexpensively using only dry cleaners' hanger wire and a pair of pliers. It is a variation of earlier reported wire models[4,5] and we find it quite suitable for developing countries.

Model Construction Procedure

For this project, one needs only drycleaners' coat hanger wire, a pair of medium sized pointed pliers and a wooden support system. The wire can be obtained from your wardrobe or free as scrap. There is also a need for diagrams as shown in typical biochemistry texts.[7]

For fast construction, the principle used here involved the construction of nucleotide base-pair units which were then joined at specific points to produce the fully built up DNA model of any base-pair length. We chose to construct a B-DNA model containing ten base pairs. To facilitate construction, two base-pair units representing A = T and G ≡ C and which we call 'master base pair units' were first constructed very carefully using the textbook diagrams as guide. The scale used was 1 Å to 2.6 cm. The C–C, C–N and C–O bond lengths were approximated to 1.4 Å each, 1.6 Å was used for the P–O bond length and 3 Å was used for the hydrogen bond length.[8] Pieces of wire were first cut to the exact centimeter equivalents of the bond lengths and these pieces were then used as guide for bending the master base-pair units. The appropriate bond angles were also maintained.

Only the primary backbone bonds including hydrogen bonds were represented. This model is very close to the skeletal model. One unbroken length of wire was used for each base pair unit and a few internal bonds within the rings were added thereafter with short pieces of wire. The master units were subsequently used as guide for the fast production of base pair units. A typical base-pair unit is shown in Figure 1.

The base pair units could be linked either at the *N*-glycosidic bond or at the 5′ C–O bond. Linkage was achieved by simply twisting the two wires around each other in a double helical pattern using the pair of pliers. A major advantage of choosing