Semi-empirical Hartree–Fock calculations on the mechanism of enniatin B mediated transport of sodium ions

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The passage of sodium ions through enniatin B is studied by the CNDO method. Electronic dissociation energy of the enniatin B–Na^+ complex in water is 0.496 au for the open form (0.397 au for the closed form). The open complex is found to be more stable than the closed one in water. The dissociation energy changes when counterions representing charged heads of phospholipids are placed around the ionophore. Variation of counterion charges leads to different possibilities for the transport of Na^+ across a lipid membrane. These are (i) no transport, (ii) transport through channels and (iii) transport of the ion–ionophore complex. A pH dependence that relies on the nature of counterion charges is predicted for the transport.

1. Introduction

Ions, as they are charged, are less stable inside a hydrophobic biomembrane than in an aqueous medium. But an envelope providing a hydrophobic exterior and a hydrophilic interior can stabilize them. Some proteins satisfy this need [1]. Such proteins may be helical or cyclic. Helical proteins pack adjacent to each other, orienting themselves along antiparallel dipole moments [2–4]. This packing leads to an axial pore through which metal ions can move. Pullman et al. [5] have shown that the total energy changes in a favourable manner as the ions move through such pores.

Cyclic carriers have electron-donating atoms which can trap the ion inside the carrier. Such carriers, ionophores, are widely found in biological systems. Enniatin B is one such carrier that transports sodium and potassium ions across the membrane [6].

It is generally believed that ion transport occurs by two possible mechanisms. In the first case a channel is formed by end-to-end linkages of ionophores. These linkages are mostly in the form of hydrogen bonds. The channels can let ions pass along their axes [5,6]. Alternatively, the ion may be trapped at the centre of a single ionophore; in this case there is often a change in geometry since the ionophore wraps around the metal ion, and the complete unit (the ion–ionophore complex) makes its way across the membrane. This transport may occur under an electric field gradient or even by thermal agitations just as a dumpling moves through liquified cheese or butter.

This paper attempts to find the conditions for the different mechanisms of ion transport. This has been done by calculating the ionophore–metal ion potential energy surface by the CNDO method. Specifically, the enniatin B–sodium ion system has been studied here. Nevertheless the treatment is general enough to be followed for any other system. Influence of the aquation of metal ions upon the energetics of the complex has been studied in detail. Finally, the consideration of counterions representing the hydrophilic exterior of the phospholipid biomembrane has led us to several interesting but reasonable conclusions.

2. Method

2.1. Atomic coordinates

Atomic coordinates for the free enniatin B molecule (open structure) and the enniatin B–Na^+ com-

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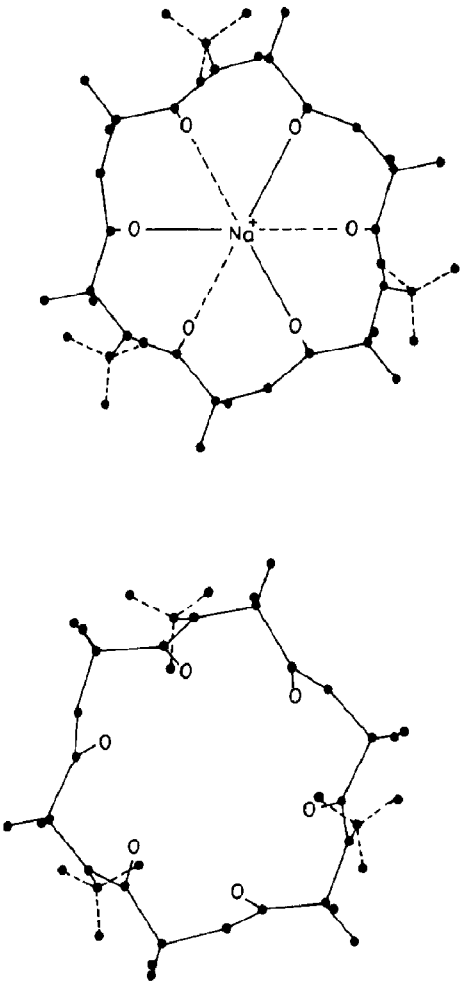

Fig. 1. Projected diagrams of the enniatin B–Na^+ complex (closed structure) and the free enniatin B molecule (open structure).

plex (closed structure) have been obtained from crystallographic studies of Dobler et al. [1,7]. We have adopted the basic skeleton of the ionophore as well as the coordinates of the methyl carbon atoms attached to the three skeletal nitrogen atoms from these crystallographic results. We have calculated the coordinates of the nine hydrogen atoms bonded to the three N-methyl carbon atoms. The lipophilic exterior of enniatin B is formed by six isopropyl side chains. For our calculations we have replaced these side chains by six hydrogen atoms. Thus we have six $-CH_2-$ linkages in the skeleton. All C–H bond lengths have been taken to be 1.10 Å. Projected diagrams of the resulting structures are shown in fig. 1. Using these atomic coordinates total energies of the ion–ionophore system have been calculated for various axial distances (R) of the sodium ion from the crystallographic center of the enniatin B moiety.

2.2. Aquation

The free ion, at a large (infinite) distance from the ionophore, should remain in an aqueous medium as a hydrated species. A variety of hydration numbers have been reported for Na^+, the more common ones being 4 ± 1 [8]. For our calculations we have assumed that the hydrated sodium ion has four water molecules tetrahedrally coordinated to it. For the sake of simplicity we have kept all Na–O–H angles and H–O–H angles in $Na(H_2O)_4^+$ equal to the tetrahedral angle (109°28′). The Na–O bond length in this species has then been varied to generate the minimum energy structure. In order to calculate the relative hydration energy we have similarly optimized the O–H bond length in the species H_3O^+ where again each H–O–H angle has been maintained at 109°28′.

2.3. Axial approach

We have assumed that the sodium ion approaches the center of the enniatin B moiety along the threefold axis of symmetry (the molecular axis or the z axis). At 10 Å separation it is considered to be in a hydrated state with four water molecules bonded to it and the ionophore residue has either the closed or the open structure. The situation is the same when the Na^+ ion is at a distance of 7, 6 or 4.5 Å from the origin. As the ion approaches further, it gradually loses the water molecules around it. At $R = 2.9774$ Å (or $R = 3.1874$ Å) it remains bonded to three water molecules and is weakly attached to three of the six active carboxyl oxygen atoms of the ionophore in its closed (or open) form, and the six Na^+–O directions point towards the corners of an octahedron. In the process of coming from $R = 4.5$ Å to $R = 2.9774$ Å (3.1874 Å) the ion loses one water molecule. Similarly two more water molecules are lost while the ion approaches the closed (open) ionophore from $R = 2.9774$ Å (3.1874 Å) to $R = 2.0802$ Å (2.2657 Å), the distance at which the sodium ion is bonded to the three carboxylic oxygen atoms and one water

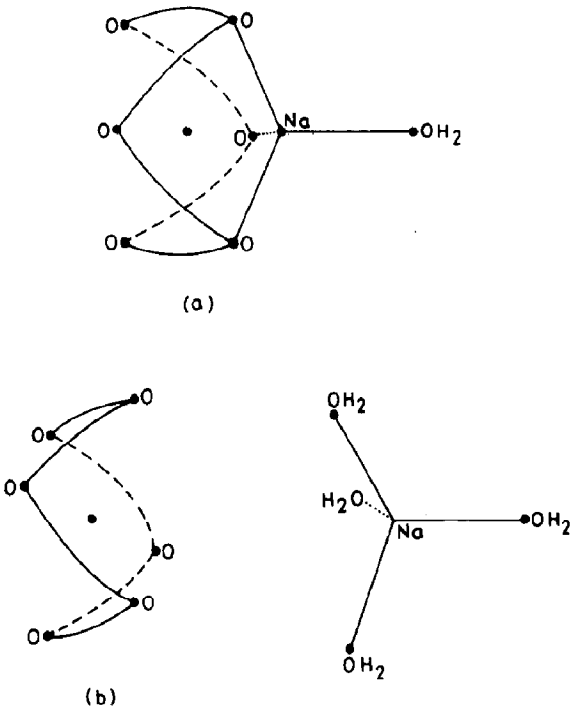

Fig. 2. Chosen geometry for the enniatin B–Na^+ system: (a) $R=2.080$ Å and (b) $R=7$ Å.

molecule along tetrahedral directions. The water molecule is attached from the positive side of the z axis. The ion is again in the state of "tetrahedral coordination" at $R=0.2858$ Å (0.4223 Å) but now the water molecule is bonded to it from the negative side. The $R=2.080$ Å and $R=7$ Å structures are shown in fig. 2. Energy curves for ion–ionophore complexes with solvated or unsolvated sodium ions and closed or open enniatin B residues are shown in fig. 3.

2.4. Transport

While considering channel transport we have taken the sodium ion to be solvated on the positive side of the z axis ($R>0$) and anhydrous on the negative side ($R\leqslant 0$). As the ion moves in axially the ionophore may or may not fold itself around the ion. We have assumed that when folding occurs the ionophore geometry remains open as long as R is greater than 7 Å and for R less than 7 Å the ionophore has the closed structure. This assumption is obviously not consistent with the slow folding of an ionophore when an

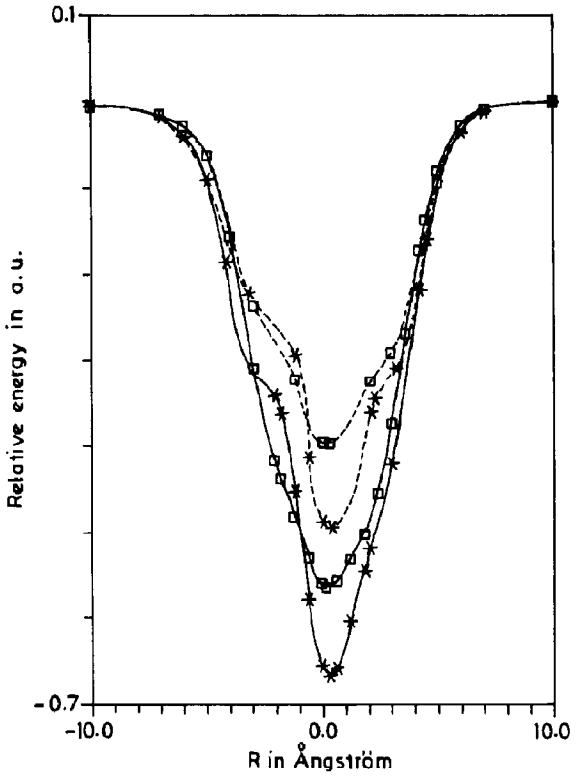

Fig. 3. The enniatin B–Na^+ energy curve: (1) (□) closed structure and (ii) (*) open structure. R is the distance of sodium from the crystallographic center of enniatin B. The sodium ion approaches along the z axis (molecular axis). Solid lines indicate that the incoming sodium ion is not hydrated. The dashed curves are for the aqua complexes with the number of bound water molecules varying with R as discussed in the text.

ion moves in and is trapped, but it does not in any way affect the calculation of electronic dissociation energy. It can only influence the exact shape of the energy curve on the right-hand side of the membrane–water interface. We have prepared energy curves with and without folding, and with and without aquation corrections. These curves are shown in fig. 4.

For carrier transport we have selected the open structure for the ion–ionophore complex in aqueous solution. The sodium ion is always at the equilibrium position and is attached to one water molecule which is on the negative side, this attachment being energetically favourable. The whole complex approaches the membrane from the right-hand side in an inverted configuration such that the water molecule is on the outer side and is easily detached when

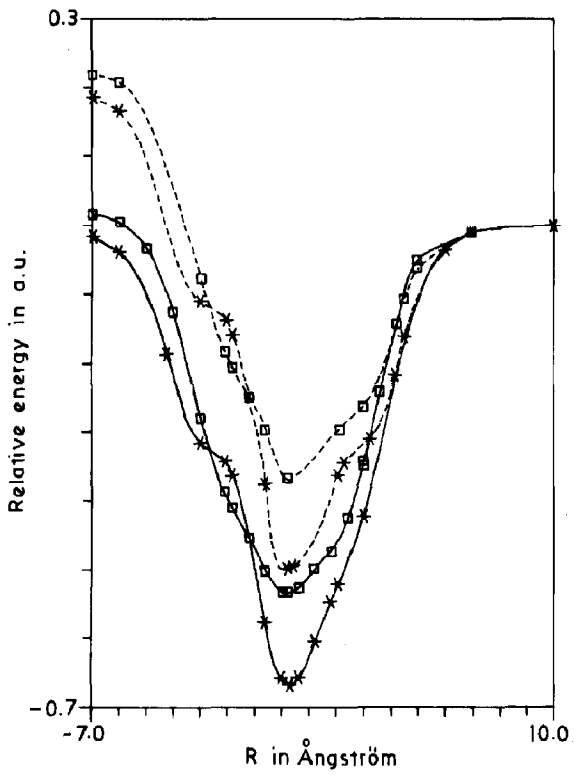

Fig. 4. Energy surface for enniatin B-Na^+ when the ionophore resides at the surface of the membrane. (i) (□) closed structure and (ii) (*) open structure. The complex is not hydrated when $R<0$. For $R>0$ a solid line represents a free sodium ion and a dashed line indicates aqueous ions.

the species penetrates the membrane. Both open and closed structures have been considered for the dehydrated complex inside the membrane.

2.5. Counterions

A biomembrane is made of phospholipids each containing a charged head and a neutral hydrophobic hydrocarbon tail. The charged head as well as any other ion trapped at the surface can provide an ionic surrounding to the ionophore present in this region, thus affecting the stability of the system. Bilayer lipid membranes also have charged heads which normally reside on the two sides of the membrane. These heads can be positively charged, zwitterionic or negatively charged as in the case of phosphatidyl serine bilayers immersed in an acidic, a neutral (or weakly acidic) or a basic solution. Phosphatidyl choline bilayers have zwitterionic heads when the membrane is in contact with a neutral solution. We have considered representative counterions around the ionophore. We have assumed that the interaction of counterion charges with the system is purely electrostatic in nature. Using the CNDO atomic charges we have calculated Coulombic corrections to the potential energy surface due to the presence of six counterions of alternative charges q_1 and q_2 placed symmetrically on a ring of radius twice the radius of the enniatin B chain. When the structure is open in the membrane phase, these six charges are placed as follows: $q_1(5.765,\ 0.417,\ -0.336+z_1)$, $q_2(2.407,\ 5.267,\ -0.336+z_2)$, $q_1(-3.244,\ 4.784,\ -0.336+z_1)$, $q_2(-5.765,\ -0.548,\ -0.336+z_2$, $q_1(-2.521,\ -5.200,\ -0.336+z_1)$ and $q_2(3.358,\ -4.718,\ -0.336+z_2)$. All coordinates are in Å. The charges are in atomic units. For channel transport we have chosen (i) $z_1=z_2=z=0$ and $q_1=q_2=q$ (with $q=1$ and 0.5 for positively charged heads, $q=0$ for no counterion, and $q=-0.5$ and -1 for negatively charged heads), and (ii) $z_1=-z_2=1$ with $q_1=-q_2=1$ (zwitterionic case). The following values have been chosen for carrier transport: (i) $z=0$ with $q=-0.5$ (negatively charged heads) and (ii) $z_1=-z_2=1$ with $q_1=-q_2=1$ (zwitterionic heads). Counterions with only positive charges have not been chosen here since these would strongly repel the positively charged complex. For carrier transport, assuming that the complex in the membrane phase is closed, counterions have been placed as follows: $q_1(3.232,\ -5.080,\ z_1)$, $q_2(-2.782,\ -5.339,\ z_2)$, $q_1(-6.014,\ -0.259,\ z_1)$, $q_2(-3.232,\ 5.080,\ z_2)$, $q_1(2.782,\ 5.339,\ z_1)$ and $q_2(6.014,\ 0.259,\ z_2)$. Here we have taken (i) $z_1=z_2=0$ with $q_1=q_2=-0.5$ and (ii) $z_1=-z_2=1$ with $q_1=-q_2=1$. Results of these calculations are discussed in section 3.

3. Discussion

3.1. The enniatin B-Na^+ energy surface

From the study of the axial movement of the free (unhydrated) sodium ion we obtain a potential energy surface with a depth of 0.563 au for the closed structure and another surface of depth 0.666 au for the open structure (fig. 3). The minimum occurs at

$R=0.204$ Å for the closed form ($R=0.295$ Å for the open form). This implies that the free ion can enter the pore as the process is energetically favoured, but once it is inside the pore and the complex is thermally stabilized the ion cannot easily come out of the ionophore shell. This would be the case if there were no (or very little) effect of the solvation of sodium ions.

3.2. Aquation corrections

The "free" sodium ion present in an aqueous medium is strongly hydrated. Table 1 shows the results for hydration number 4. The optimized Na–O bond length is 2.974 Å. The force constant for breathing vibration has been calculated by carrying out a cubic fit with the CNDO output. Stabilization energies have been calculated from CNDO total energies. Hydration energy of sodium ion has then been estimated by implementing standard corrections as per the Born model [8]. Calculated values of hydration energy of Na^+, proton hydration energy and the relative hydration energy are in good agreement with the observed values. The calculated hydration energies become slightly better when corrections due to the changes in entropy are taken into account. For the metal ion the entropy of hydration at 25°C has been calculated by using the Born formula where we have chosen the radius of the Na^+ ion as 0.97 Å [8]. Noyes has estimated the partial molal entropy of the hydrogen ion to be about -3.3 cal mol^{-1} [9]. We have adopted this value in our calculation of the hydrogen energy. Energy corrections due to the change in entropy are quite small, about 0.8 kcal mol^{-1} for the metal ion and 0.09 kcal mol^{-1} for solvated protons.

Aquation of the sodium ion irrevocably alters the enniatin B–Na^+ potential energy surface. The original and changed surfaces are plotted on a relative scale in fig. 3. Neither the closed structure nor the open one is exactly symmetric about $R=0$. Energy surfaces also are not symmetric. The plot for the free complex with open structure has a shoulder on the negative side of R. This arises from a rapid change in the coordination number of the sodium ion. This effect becomes more pronounced with aquation of the complex. Plots for both open and closed forms of the aqua complexes exhibit shoulders on both sides of R since in these cases a change in the coordination of the sodium ion is accompanied by a change of hydration. The dissociation energy changes to 0.397 au for the closed form and 0.496 au for the open form as shown in fig. 3. Interaction of the aqua complex of the sodium ion with the dielectric medium will reduce the dissociation energy by an additional amount. The Born model of ion–solvent interaction predicts this additional amount to be about 0.07 au while the interaction of enniatin B–Na^+ with the dielectric is neglected on account of the large ionic radius of the ion–ionophore complex. In any case the enniatin B–Na^+ complex, whether closed or open, is sufficiently stable in an aqueous medium. Our calculation also shows that the open structure of the complex is more stable than the closed structure. The main reason that the (KI) complex of enniatin B crystallizes in its closed form is that the crystal with the closed form of complex cations is associated with a much greater lattice energy (particularly when the

Table 1
Characteristics of the $Na(H_2O)_4^+$ complex

	Calculated [a]	Observed [b]
Na–O bond length (Å)	2.974	
breathing force constant (au)	1.024	
stabilization energy (kcal mol^{-1})	−126.5	
estimated hydration energy [c] (kcal mol^{-1})	−106.5 (−105.7)	−102.3
proton stabilization energy (kcal mol^{-1})	−265.4 (−265.3)	−266.0
relative hydration energy (kcal mol^{-1})	158.9 (159.6)	163.7

[a] The hydration energy has been estimated by adding to the stabilization energy the amount of 20 kcal mol^{-1} for cavity formation, structure breaking, cluster dissociation and condensation as per the Born model of ion–solvent interaction.
[b] Values in parentheses include corrections due to the change in entropy.
[c] Ref. [8].

Table 2
CNDO energies of the relevant species in au

Species	Total energy	Binding energy
H_2O [a]	−19.870	−0.511
H_3O^+ [b]	−20.293	−0.295
$Na(H_2O)_4^+$ [c]	−79.683	−2.047
enniatin B (free) [d]		
open structure	−323.485	−21.629
closed structure	−323.454	−21.597
enniatin B–Na^+ complex [e]		
open structure	−324.151	−22.097
closed structure	−324.017	−21.962
enniatin B–$Na(H_2O)^+$ complex [f]		
open structure	−344.054	−22.641
closed structure	−343.924	−22.511

[a] The O–H bond length is 0.96 Å and the H–O–H bond angle is 107.1°.
[b] The H–O–H bond angle is fixed at 109°28′. The optimized O–H bond length is 1.058(3) Å.
[c] All bond angles are equal to 109°28′. The O–H bond length is taken to be 0.96 Å. The optimized Na–O bond length is 2.974 Å.
[d] The enniatin B skeleton is chosen from ref. [1]. Side chains of isopropyl groups are replaced by hydrogen atoms.
[e] Equilibrium position of the sodium atom is (0.0, 0.0, 0.295) for the open structure and (0.0, 0.0, 0.204) for the closed structure.
[f] Sodium atom is in the previously mentioned equilibrium position and one water molecule is attached to it from the negative side of z.

isopropyl chains are retained by the ionophore).

3.3. Membrane-bound ionophore

The behaviour of the curve for large negative values of R ($R < -3.6$ Å) in fig. 4 will depend on whether a channel is formed or not. If a channel is formed, that is, if there is another enniatin B molecule behind the surface molecule then the curve will dip again. In case no channel is formed, the curve will become strongly repulsive whenever there is a non-polar molecule behind the surface enniatin B. In this case the Na^+ ion can be transported only if it is trapped by the ionophore and the resulting complex moves as a whole (carrier transport). As such fig. 4 indicates that due to aquation the "free" sodium ion is relatively more stabilized and consequently the formation of a channel becomes an energetically costly process. However, while drawing fig. 4 we have neglected the covalent interaction of the enniatin B molecule or of the enniatin B–Na^+ complex with the constituents of the membrane. Polar interactions between the ionophore and the membrane can be taken into account by considering the presence of counterions representing fractional charges on atoms or more specifically the charged heads. The effect of varying counterion charges (and locations) is shown in fig. 5 for channel transport and in fig. 6 for carrier transport.

3.4. Channel transport

While drawing fig. 5 we have assumed that enniatin B resides on the surface of the membrane and the sodium ion approaches from the aqueous side. For $q = 1$ a pair of large potential barriers is formed and there will be no transport. This is reasonable since positive counterions are expected to repel the approaching sodium ion. For intermediate values of q ($q = 0.5$) the energy surface is still wavy but the barrier height decreases. Therefore the ion may pass through if the barrier is reduced further due to covalent interactions between the complex and the membrane molecules or due to the presence of another ionophore behind the complex. If a barrier remains, the passage would be sluggish and the most likely mechanism of transportation would be the hopping of sodium ions from one ionophore to the next. If the barrier disappears, a channel would be created through which the metal ion can pass. The rather small dissociation energy of the complex for $q > 0$ may not favour a carrier transport. For counter zwitterions the barrier diminishes sufficiently but the dissociation energy of the complex bound to the

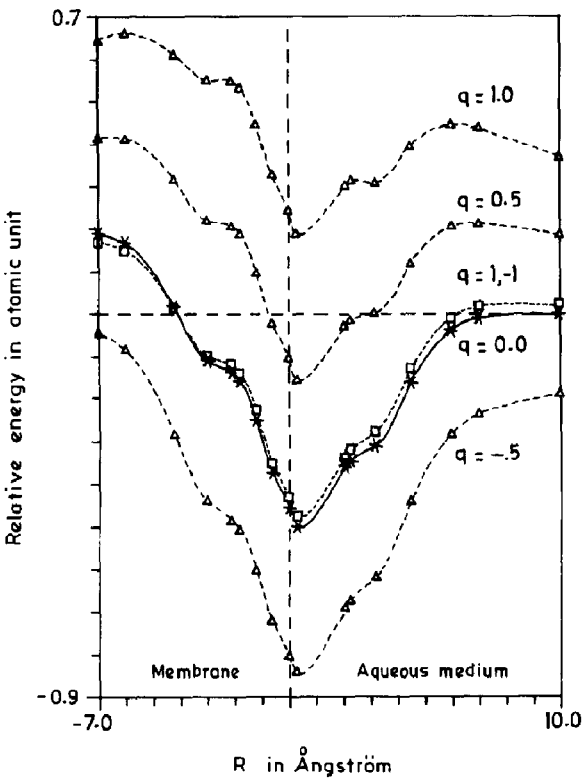

Fig. 5. Influence of counterion charges upon the energy curve for channel transport. The sodium ion is hydrated in the aqueous medium and anhydrous in membrane. The enniatin B molecule at the surface of the membrane has the open structure. The curve for $q = 1, -1$ corresponds to counter zwitterions.

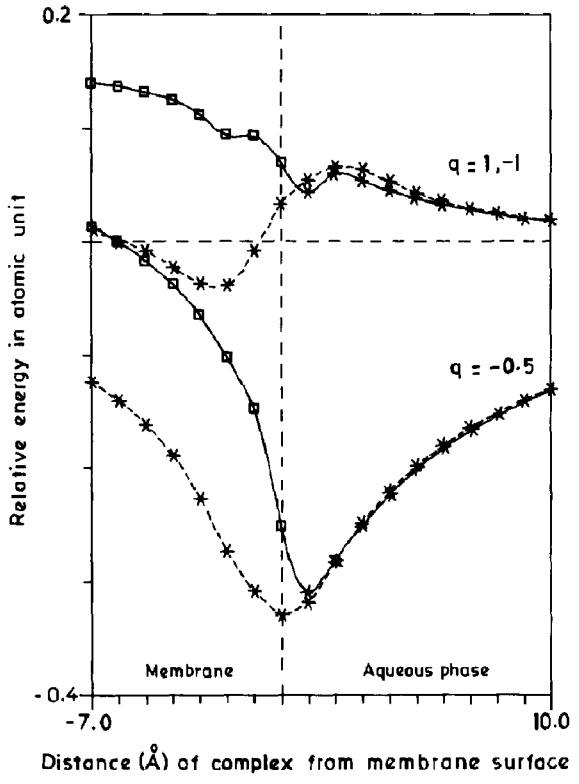

Fig. 6. Influence of counterion charges upon the energy curve for carrier transport: (i) (□) closed structure and (ii) (*) open structure. The sodium ion is always in the equilibrium position. The whole complex approaches the counterion array. The complex is an aqua complex in water but anhydrous in membrane. Energy of the complex at the membrane–water interface is taken as the average of energies for both solvated and unsolvated complexes in the counterion environment.

membrane increases considerably such that the bound complex is almost as stable as the complex in solution. So, in addition to the channel transport of excess sodium ions in solution, transport of the ion–ionophore complex as a whole aggregate would take place. If the concentration of "free" metal ions in solution is very small (as expected in the case of a significantly large dissociation energy of the aqua complex as in the present situation), translocation would mainly occur by the carrier mechanism. For still lower values of q ($q = -0.5$) the barrier altogether disappears but the dissociation energy enormously increases. So not only would there be channel transport (of excess metal ions) and carrier transport (of complexed metal ions) but also the ion–ionophore complex would be stuck in the membrane and eventually an equilibrium of solvated and "stuck" complexes would be established.

3.5. Carrier transport

Fig. 6 rules out carrier transport through a membrane with zwitterionic charge heads if the complex has the closed structure inside the membrane. With negatively charged heads the complex in both closed and open forms can pass across the membrane although the passage would be easier for the open structure.

4. Concluding remarks

One finding of this study is that in an aqueous medium the enniatin B–Na^+ complex will have the open

structure. The open conformation will in some cases lead to the formation of 2:1 complexes where the metal ions are sandwiched between enniatin B molecules. In fact 2:1 complexes of enniatin B and potassium ions are known to exist in equilibrium with the 1:1 complexes in solution [10].

Our investigation also predicts a pH-dependent transport of the complex. It is known that several toxins form channels in bilayer lipid membranes when a pH gradient is set up in such a way that the toxin fragment resides on the acidic side. Water, cations and several small molecules pass through these channels [11]. The pH dependence of the translocation of metal ions as discussed in this work would show a reverse trend: a higher pH increases the fraction of negatively charged heads which would be instrumental in stabilizing the positively charged ion–ionophore complex inside the membrane, and the chances of both channel and carrier transport would increase. This should happen whenever the ionophore–membrane covalent interaction is negligible. In the case of pH-dependent conformational changes of proteins like toxins in membranes the ionophore–membrane interaction obviously plays a greater role.

In this study we have considered only one ionophore and have assumed that it resides on the membrane–water interface. This would be a good starting point for the carrier mechanism. However, for the channel mechanism it is necessary to study the packing of ionophores so that the details of the energy curve in the bulk can be calculated. Besides, as discussed earlier, energy lowering due to covalent interactions between the enniatin B moiety or the complex and the membrane molecules needs to be investigated in detail. Improvements can also be made by considering counterdipoles instead of counterions around the surface ionophore. Finally, one needs to calculate explicitly the rate of channel transport and that of carrier transport. This work is in progress.

Acknowledgement

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