  Bull. Chem. Soc. Jpn., 66, 1490—1495(1993) [Vol. 66, No. 5

Effect of Cation Binding on the Conformation of Gramicidin A′ and Valinomycin in Monolayers

Tomoyoshi MITA
Department of Natural Science, Osaka Women's University, Daisen-cho, Sakai, Osaka 590
(Received November 30, 1992)

In order to obtain information concerning the conformational features of cation-complexed ionophores on biological membranes, surface-pressure studies of the monolayers of ionophores, such as gramicidin A′ and valinomycin formed at an air/water interface, were carried out. The effect on the films when various salts were present in the subphase was also studied. At a concentration of 4×10^{-1} mol dm^{-3} the π–A isotherms for gramicidin A′ on all of the salts studied exhibited a maximal expansion in the condensed state. Based on the concentration dependence on the expansion of the gramicidin A′ monolayer, the efficiency of monovalent cations was found to be in the order $NH_4^+ > K^+ > Na^+ > Li^+$. At a concentration of 4×10^{-1} mol dm^{-1}, although the π–A isotherms for a valinomycin monolayer on KCl were indistinguishable from that on water at the liquid-expanded state, the π–A isotherms on LiCl, NaCl, and NH_4Cl showed incremental shifts. On the other hand, by additional compression of a valinomycin monolayer on KCl over the plateau zone, an increase in the surface pressure appeared. However, the valinomycin monolayer on the other salts did not show such a behavior. This result indicates that valinomycin is specific for K^+ in a membrane.

The ionic permeabilities of alkali ions in both biological and synthetic membranes are strongly enhanced by certain antibiotics. Such antibiotics are roughly divided into two types of ionophores: channel and carrier. The prime distinction between the two types of action is that a carrier requires a liquid-like membrane interior through which it can diffuse; however, a channel former, since it forms stable transmembrane structure, does not.[1)]

Gramicidin A, one of the most-studied channel formers, is a naturally occurring pentadecapeptide produced by *Bacillus brevis*, and comprises 15 hydrophobic amino acids of alternating D and L configuration, the primary structure of which has been determined.[2)] The ionic selectivity in channel-mediated ion transport is generally poor compared that in the carrier-mediated ion type produced by macrocyclic actions.[3)] The permeation of monovalent cations through the gramicidin A channel appears to involve a binding of ions to sites near to the end of the channel and their translocation across a central barrier.[4)] Hladky and Haydon[5)] have found that the selectivity sequence is similar to that for the corresponding sizes of elctrolytes in aqueous solution. In addition, it has been proposed that the gramicidin A channel is divided into two compartments, and that the movement of ions through the pore occurs due to ions entering the nearer compartment, transferring from one to the other, and then leaving on the far side of the membrane.[4,6)]

On the other hand, valinomycin, one of the most-studied carrier formers, is a cyclodepsipeptide produced by *Streptomyces fulvissimus*. An increase in the K^+ uptake in mitochondria was first noted for valinomycin by Moore and Pressman.[7)] A striking selectivity was observed for the phospholipid membranes when valinomycin was introduced into the aqueous phase, e.g., valinomycin creats a K^+/Na^+ selectivity of about 400.[8)] The mode of action of this antibiotic can be interpreted in terms of a mobile carrier in which the most important step is complexation of a metal ion at the interface and the interior of membranes.[9)]

Accordingly, a number of studies have been directed at revealing the conformational features of free and complexed ionophores as well as the stability of complexes in solution, in order to obtain a clue for understanding such ion selectivity.[10)] Koeppe et al.[11)] reported that the binding of a cation leads to a large conformational change; in a crystal the channel becomes shorter and wider upon cation binding. On the other hand, Blout et al.[12—14)] studied the conformational feature of complexed valinomycin using synthesized valinomycin analogs. They reported that valinomycin possesses a conformation that is commonly referred to as a "bracelet" structure and a hydrophobic exterior around a hydrophilic cavity. This molecule is folded in such a way that all six ester carbonyl oxygens point toward the center, where they form the corners of an octahedron that encompasses the K^+ ion. Tabeta and Saito[10)] observed a conformational change of backbone carbons in valinomycin induced by metal-ion complexation by using high-resolution ^{13}C NMR.

However, since it may be that these structures more closely resemble the structures of molecules in an organic solvent than structures in a membrane, few studies have been carried out concerning the conformational feature of the complexed ionophores in membranes.[15—17)] Wallace et al.[18)] have pointed out that the conformation of ionophores adopted in membranes is not the same type as any of those adopted in organic solvents. To study the conformational feature of the complexed ionophores in membranes, the spread monolayer technique is useful, since the oriented ionophores at the air/water interface can be precisely understood by using this technique, and the area occu-

pied by each molecule can be determined over a wide limit by compression of the monolayers.

In the present study, we examined the conformational feature of the cation-complexed gramicidin A′ and valinomycin, as measured by the expansion in monolayers of the two ionophores, which are spread on the various cation-containing subphase.

Experimental

Materials. Gramicidin A′ and valinomycin were obtained from Sigma Chemical Co. and were used without further purification. Gramicidin A′ was a mixture of gramicidins A, B, and C. All of the salts and other chemicals were of the highest purity available. Water was distilled once, passed through a mixed ion bed exchanger, and distilled.

Apparatus. A Kyowa's surface-pressure apparatus (kyowa Surface Science Co. Japan) was used. The film balance is fully automated and provided with continuous recording of the surface pressure versus the film area. The inside dimensions of the Teflon-coated trough are 70×14×0.5 cm. The entire balance is surrounded by a water jacket, providing a constant temperature of 25±1°C. The film balance used for measuring the surface pressure has already been described in detail elsewhere.[17]

Surface Pressure. The surface pressure was measured by the Wilhelmy method, using a roughened glass plate. The films were compressed by the movable Teflon barrier. Chloroform was employed as a spreading solvent for the ionophores.[19] Gramicidin A′ and valinomycin were dissolved in the solvent at concentrations of 0.05 and 0.025%, respectively; these solutions were stored in a refrigerator and used after about 24 h. The salt-containing subphase at different concentrations was poured into the trough to a height of 1—2 mm above the rim. Surface cleaning was repeated until the surface pressure did not exceed 0.2 mN m^{-1}. A monolayer spreading was made by the direct application of numerous small drops (ca. 0.0005 cm^3 of spreading solution onto the surface of a subphase by using a microsyringe (Hamilton Co.), introduced by Ställberg and Teorell.[20] A time interval of 30 min was allowed for equilibration of the monolayers before it was compressed. Before compression, the surface pressure of the monolayers of ionophores did not exceed 0.2 mN m^{-1}, indicating no interaction between the ionophore molecules. Surface pressure-area isotherms were obtained by using compression velocities of 0.0567 and 0.0284 m^2 mg^{-1} min^{-1} for gramicidin A′ and valinomicin, respectively.

To obtain highly accurate data, each part of all experiments was repeated at least four times and then treated within an error of 3%.

Results

Influence of Monovalent Cations on the Gramicidin A′ Monolayer. Figure 1 shows surface pressure–area (π–A) isotherms for a gramicidin A′ monolayer spread on water and various saline solutions at 4×10^{-1} mol dm^{-3} at 25 °C. The π–A isotherm for a gramicidin A′ monolayer on pure water showed a transition from liquid-expanded through an intermediate at

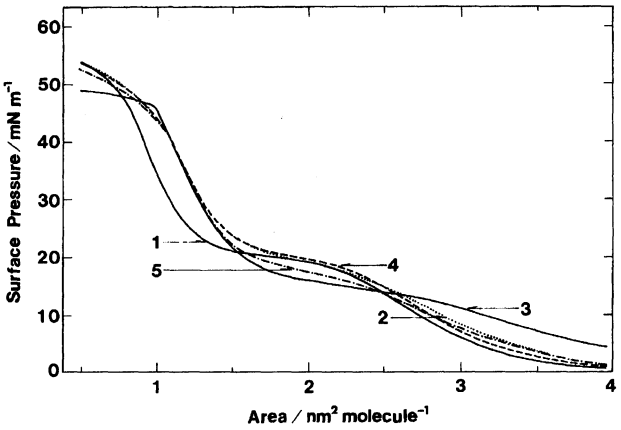

Fig. 1. π–A isotherms for a gramicidin A′ monolayer spread on water and various saline solutions at 4×10^{-1} mol dm^{-3} at 25 °C. 1) water, 2) LiCl, 3) NaCl, 4) KCl, 5) NH_4Cl.

around 20 mN m^{-1} to a condensed state, and reached the collapse pressure at around 55 mN m^{-1}. The π–A isotherms for the gramicidin A′ monolayer on all saline solutions exhibited an incremental shift at the condensed state above 20 mN m^{-1}. The effect of salts at surface pressures above 20 mN m^{-1} was not distinguished, although a large expansion on NaCl was observed at surface pressures below 20 mN m^{-1}. In addition, with several salt concentrations of between 1×10^{-3} and 4×10^{-1} mol dm^{-3}, the π–A isotherms on NH_4Cl at surface pressures above 20 mN m^{-1} were completely compatible with each other, indicating that they represented the maximal expansion on the gramicidin A′ monolayer due to the incorporation of cations. Such an expansion was not observed in the π–A isotherm for a monolayer of neutral phospholipid, such as phosphatidylcholine spread on the saline solutions at the same concentration (4×10^{-1} mol dm^{-3}), except in the case of a slight expansion on NaCl (data not shown).

It has been proposed that gramicidin A forms a NH_2-terminal to a NH_2-terminal helical dimer perpendicular to the plane at the air/water interface when the monolayer is compressed into a condensed state.[19,21] Then, the minimum packing area in which the curves at the condensed state above 20 mN m^{-1} were extraporated to $\pi=0$ is the corresponding cross-sectional area of the gramicidin A′ channel. Figure 1 shows that the minimum packing area of gramicidin A′ on water gave 1.54 nm^2. This value coincides with those of gramicidin estimated by Kemp and Wenner[21] (1.45 nm^2 and by Haydon and Hladky[22] (1.50 nm^2). On the other hand, the area of gramicidin A′ on saline solutions at 4×10^{-1} mol dm^{-3} gave 1.74 nm^2, corresponding to the maximal expansion. The expansion on a gramicidin A′ monolayer by various salts at different concentrations was therefore measured. Figure 2 shows the expansion on a gramicidin A′ monolayer as a function of the salt concentrations of electrolytes. The concentrations

which correspond to the half-maximal expansion on a gramicidin A′ monolayer are also 2×10^{-4}, 3.5×10^{-3}, 1.8×10^{-2}, and 8.5×10^{-2} mol dm^{-3} for NH_4Cl, KCl, NaCl, and LiCl, respectively. The alkali ions (K^+, Na^+, Li^+) did not affect the concentrations below 2×10^{-3} mol dm^{-3}. However, NH_4^+ showed stronger binding than did the alkali ions; the effect of the salt concentration was about 500-fold larger in NH_4^+ than in Li^+. Furthermore, from the concentration dependence on the expansion (Fig. 2), the efficiency of monovalent chlorides was found to be in the order $NH_4^+ > K^+ > Na^+ > Li^+$.

Influence of Monovalent Cations on a Valinomycin Monolayer. Figure 3 shows the π–A isotherms for a valinomycin monolayer spread on water and various saline solutions at a concentration of 4×10^{-1} mol dm^{-3} at 25 °C. In Fig. 3, the minimum packing area of valinomycin on water gave 3.10 nm^2.

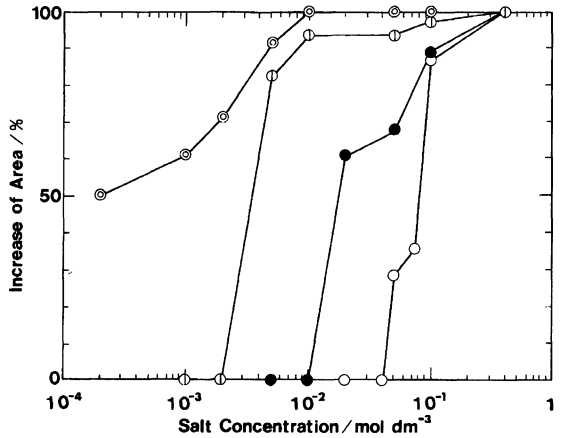

Fig. 2. Expansion on a gramicidin A′ monolayer as a function of the salt concentrations (logarithmic scale). The increase in the area in percentage represents the relative expansion to the maximal expansion. ○: LiCl, ●: NaCl, ◑: KCl, ◎: NH_4Cl.

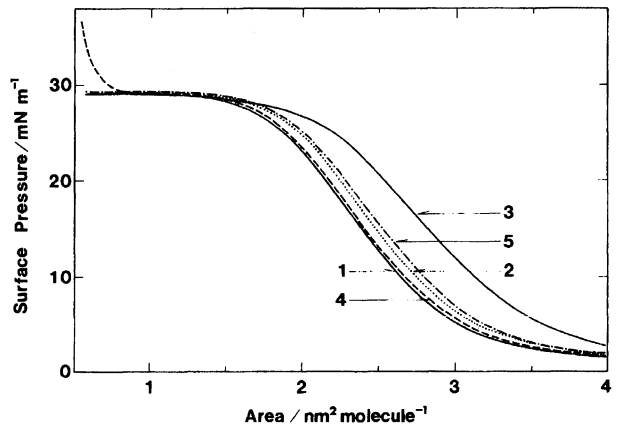

Fig. 3. π–A isotherms for a valinomycin monolayer spread on water and various saline solutions at 4×10^{-1} mol dm^{-3} at 25 °C. 1) water, 2) LiCl, 3) NaCl, 4) KCl, 5) NH_4Cl.

This value coincides with those of valinomycin estimated by Colacicco and Gordon[15] (3.2 nm^2) and by Peng et al.[23] (3.1 nm^2). At surface pressures below 30 mN m^{-1}, the π–A isotherm for a valinomycin monolayer on KCl was indistinguishable from that in the absence of salt. The isotherms on LiCl and NH_4Cl showed a slightly incremental shift within the liquid-expanded state below 30 mN m^{-1}. On the other hand, the isotherms on NaCl exhibited a larger incremental shift than the other salts. When the valinomycin monolayer on KCl was followed by compression over the plateau zone, an increase in the surface pressure appeared again, as shown in Fig. 3. However, valinomycin monolayers on LiCl, NaCl, and NH_4Cl did not show such behavior, even at a concentration of 1 mol dm^{-3} (data not shown except for NaCl in Fig. 4).

The above-mentioned increase in surface pressure was then measured as a function of KCl concentrations, as shown in Fig. 4. This increase was not detected at concentrations below 2.5×10^{-1} mol dm^{-3}. However, this second increase appeared at concentrations above 3×10^{-1} mol dm^{-3}, showing an incremental shift with increasing KCl concentrations and attaining a maximal value at concentrations above 8×10^{-1} mol dm^{-3}. Furthermore, although the valinomycin monolayer on KCl at concentrations above 6×10^{-1} mol dm^{-3} underwent an abnormal phase transition at about 25 mN m^{-1}, that on the other salts did not show this transition. The surface pressure at this transition decreased with increasing KCl concentrations.

The second minimum packing area in which the curves of the second increase in the surface pressure were extrapolated to $\pi=0$ was determined from Fig. 4. Figure 5 provides the plots of the second minimum area as a function of the KCl concentration. A curve-fit-

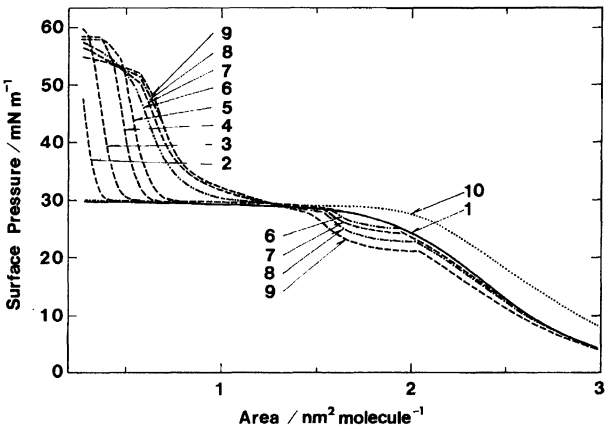

Fig. 4. π–A isotherms for a valinomycin monolayer spread on KCl at the different concentrations and on NaCl at 1 mol dm^{-3} (25 °C). 1) water, 2) 3×10^{-1} mol dm^{-3}, 3) 3.5×10^{-1} mol dm^{-3}, 4) 4×10^{-1} mol dm^{-3}, 5) 5×10^{-1} mol dm^{-3}, 6) 6×10^{-1} mol dm^{-3}, 7) 7×10^{-1} mol dm^{-3}, 8) 8×10^{-1} mol dm^{-3}, 9) 1 mol dm^{-3}, 10) NaCl at 1 mol dm^{-3}.

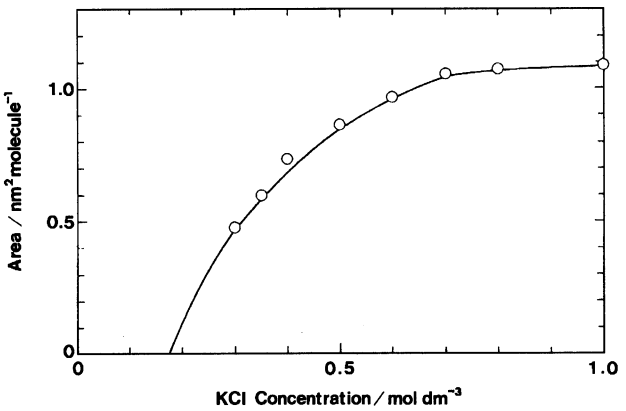

Fig. 5. Plots of the second minimum area of a valinomycin monolayer as a function of the KCl concentrations. The solid line represents the curve using the equation of an orthogonal hyperbola.

ting procedure using the equation of an orthogonal hyperbola was carried out. The solid line represents the curve fitted using this equation. Figure 5 shows that the second packing area appears at concentrations above 2×10^{-1} mol dm^{-3} increases with increasing KCl concentrations, and becomes saturated at the concentrations above 7×10^{-1} mol dm^{-3}.

Discussion

The structure of gramicidin in membranes is thought to be formed by the head-to-head (N-terminus to N-terminus) dimerization of two gramicidin monomers, stabilized by six intramolecular hydrogen bonds, to form a continuous pore through a lipid bilayer.[24] It forms ion channels in membranes that are specific for small monovalent cations. Conductance and fluorescence measurements have demonstrated that the conducting species in the membrane is the N-terminus to N-terminus dimer.[11,25,26] X-Ray crystallographic studies by Koeppe et al.[27] have indicated that the structure of gramicidin in crystals formed from methanol and ethanol is a helical channel with a diameter of around 0.5 nm and a length of 3.2 nm. On the other hand, Stankovic et al.[24] have reported that this dimer is approximately 2.6 nm in length and has a pore diameter of approximately 0.4 nm, although it is slightly different from the value found by Koeppe et al.[27] These dimensions are just sufficient to accommodate a column of water molecules hydrogen-bonded together, through which monovalent cations may pass in single file. In addition, Koeppe et al.[11,27] have found that the binding of such cations as K^+ and Cs^+ leads to a large conformational change; the length of the channel decreases from 3.2 to 2.6 nm, whereas the diameter of the cylinder formed by the peptide main chain atoms increases from about 0.5 to 0.68 nm. In the π–A isotherms for the gramicidin A′ monolayer on all saline solutions at a concentration of 4×10^{-1} mol dm^{-3}, the minimum packing area of gramicidin A′ increased from 1.54 to 1.74 nm^2, corresponding to the maximal expansion (Fig. 2). This suggests that in the gramicidin A′ monolayer the binding of cations leads to a large conformational change, such as channel widening, as does studies of crystals formed from an organic solvent.

A consequence of such a conformational change would be a change in the pitch of helix, which should be detectable by circular dichroism (CD). However, CD studies[18,28] have demonstrated that in membranes the helical pitch (and, therefore, the width and length) of the gramicidin A molecule remains unaltered upon the binding of ions, suggesting that the ion-channel binding mechanism must involve only small, local changes, such as the position of side chains or hydrogen bonds near to the cation-binding site. Based on the above-mentioned results, Wallace et al.[18] pointed out that for this polypeptide, which spans the membrane, organic solvents are not good models for the lipid bilayer. However, the result of the gramicidin A′ monolayer demonstrates that in a membrane the channel widens considerably upon the binding of cations. It may consequently be that due to cation binding the channel foreshortens, but that no, definite transition from π_6(L,D) to the π_8(L,D) helix takes place, which is detectable by CD.

It is well-known that the ion selectivity of a phospholipid membrane modified by gramicidin A is in the order $H^+>NH_4^+>Cs^+>Rb^+>K^+>Na^+>Li^+$,[3,29] depending upon the hydration size of the cations. Some of these membranes modified by the above-mentioned component show ionic selectivity, as observed by the transmembrane potential as a function of the salt gradients. Urban et al.[4] have shown that the movement of an ion through a pore occurs due to an ion entering the nearer compartment, transferring from one to the other and then leaving on the far side the membrane. They have also demonstrated that NH_4^+ shows a stronger binding than do the alkali ions, and also seems to interact more strongly with a second ion in the pore. We then examined the ion-binding potential, as observed by the expansion of the gramicidin A′ monolayer as a function of the salt concentrations. As shown in Fig. 2, NH_4^+ showed stronger binding than did the other monovalent cations. In addition, the efficiency of monovalent cations was in the order $NH_4^+>K^+>Na^+>Li^+$, consistent with the known specificity of monovalent cations.[3,29] Accordingly, the above-mentioned results suggest that the ion selectivity of a membrane modified by gramicidin A′ depends not only upon the hydration size of the cations, but also upon the ion-binding potential of the channel.

By measuring the salt gradient of identical ions across a phospholipid membrane modified by valinomycin, the monovalent chlorides showed the order of increasing permeability to be $Rb^+>K^+>Cs^+\gg Na^+>Li^+$.[30—32] Accordingly, valilnomycin is defined as a K^+-specific ionophore regarding biological function. On the other hand, CD and nuclear magnetic resonance (NMR)

studies on valinomycin and its cation complexes have shown that the selectivity of a particular cation by this molecule depends upon the nature of the ligands and the conformational states of the molecule.[33,34] X-Ray crystallographic studies on valinomycin have shown that there is a clear difference in the conformation, unit cell size, and space group between complexed and uncomplexed crystalline compounds.[35,36] This mechanism requires minor displacements on the atoms involved and is consistent with the observation that, when K^+ is complexed at the valinomycin monolayer, a conformational change occurs that decreases the surface area by approximately 0.25—0.3 nm^2.[37]

The π–A isotherms for the valinomycin monolayer on LiCl, NH_4Cl, and NaCl at 4×10^{-1} mol dm^{-3} showed an incremental shift at surface pressures below 30 mN m^{-1}, whereas that on KCl did not show this behavior (Fig. 3). From the above-described viewpoint, it is considered that the valinomycin monolayer results in contraction, rather than expansion, due to the complexation of valinomycin with monovalent cations. A similar behavior was positively observed in a valinomycin monolayer on KCl below 30 mN m^{-1} at concentrations above 4×10^{-1} mol dm^{-3} (Fig. 4); the opposite behavior, however, was observed in valinomycin monolayers on other salts. It is thus considered that the lateral expansion of the valinomycin monolayer is attributable to cations interacting with the carbonyl groups of valinomycin and, thereby, affecting the packing of valinomycin in the monolayer. However, the reason why the expansion of the monolayer differs from a variety of cations is still obscure.

As shown in Figs. 3 and 4, due to an additional compression of the valinomycin monolayer over the plateau zone, an increase in surface pressure of over 30 mN m^{-1} appeared; this increase showed an incremental shift with increasing KCl concentration. However, valinomycin in monolayers on LiCl, NaCl, and NH_4Cl did not show such behavior. The above-mentioned result suggests that the valinomycin monolayer interacts specifically with K^+. Vishwanath and Easwaran[34] have reported that valinomycin forms a dimer, such as a 2:1 (peptide–ion–peptide) sandwich complex, with Ca^{2+}. If such a valinomycin dimer forms on the monolayer due to the complexation with K^+, a second increase in the surface pressure may occur. However, it is considered that valinomycin does not form a sandwich complex through complexation with K^+.[14,34]

We thus consider the following mechanism: The gross conformational feature of the valinomycin molecule due to complexation with K^+ is observed in crystal structures, i.e., when K^+ is brought into full coordination, the molecule rounds out.[37] If in an air/water interface the above-mentioned structure is formd by complexation with K^+, the valinomycin monolayer would become labile. Evidently, the π–A isotherms for the valinomycin monolayer on KCl have a decremental shift with increasing KCl concentrations below 30 mN m^{-1} (Fig. 4). On the other hand, by additional compression over the plateau zone an increase in the surface pressure of over 30 mN m^{-1} appeared. This behavior suggests that by complexation with K^+ the valinomycin monolayer occurs through a transition from horizontal into perpendicular to the plane at the air/water interface. In this case, such an increase in the surface pressure would occur with an incremental shift as the amount of valinomycin-K^+ complex increases.

It has been observed that with several electrolyte concentrations of between 1×10^{-1} and 5×10^{-1} mol dm^{-3}, the π–A isotherms for valinomycin monolayers on NaCl and KCl are not distinguished, as is also the case of the surface potential–pressure isotherms; however at higher electrolyte concentrations the surface potential of valinomycin on KCl is considerably higher than that on NaCl.[15] The above-mentioned result suggests that valinomycin in the monolayer is complexed with K^+ at concentrations above 5×10^{-1} mol dm^{-3}. This is consistent with our result. As shown in Fig. 5, it should be noted that the increase in the second minimum packing area derived from the increase of the surface pressure over 30 mN m^{-1} follows Michaelis–Menten's equation, which is applied in order to facilitate the diffusion of carrier ionophores.

References

1) G. Eisenman, G. Szabo, S. Ciani, S. Mclaughlin, and S. Krasne, *Prog. Surf. Membr. Sci.*, **6**, 139 (1973).
2) R. Sarges and B. Witkop, *J. Am. Chem. Soc.*, **87**, 2011 (1965).
3) S. Ohki, *Prog. Surf. Membr. Sci.*, **10**, 117 (1976).
4) B. W. Urban, S. B. Hladky, and D. A. Haydon, *Biochim. Biophys. Acta*, **602**, 331 (1980).
5) S. B. Hladky and D. A. Haydon, *Biochim. Biophys. Acta*, **274**, 294 (1972).
6) S. H. Heinemann and F. J. Sigworth, *Biochim. Biophys. Acta*, **987**, 8 (1989).
7) C. Moore and B. C. Pressman, *Biochem. Biophys. Res. Commun.*, **15**, 562 (1964).
8) P. Mueller and D. O. Rudin, *Biochem. Biophys. Res. Commun.*, **26**, 398 (1967).
9) B. C. Pressman, *Ann. Rev. Biochem.*, **45**, 501 (1976).
10) R. Tabeta and H. Saito, *Biochemistry*, **24**, 7696 (1985).
11) R. E. Koeppe, II, J. M. Berg, K. O. Hodgson, and L. Stryer, *Nature*, **279**, 723 (1979).
12) V. Madison, M. Atreyi, C. M. Deber, and E. R. Blout, *J. Am. Chem. Soc.*, **96**, 6725 (1974).
13) V. Madison, M. Charles, and E. R. Blout, *J. Am. Chem. Soc.*, **99**, 4788 (1977).
14) B. Baron, L. G. Pease, and E. R. Blout, *J. Am. Chem. Soc.*, **99**, 8299 (1977).
15) C. Colacicco and E. E. Gordon, *J. Colloid Interface Sci.*, **63**, 76 (1978).
16) V. A. Howarth, M. C. Petty, G. H. Davies, and J. Yarwood, *Langmuir*, **5**, 330 (1989).
17) T. Mita, *Bull. Chem. Soc. Jpn.*, **62**, 2299 (1989).

18) B. A. Wallace, W. R. Veatch, and E. R. Blout, *Biochemistry*, **20**, 5754 (1981).
19) T. Mita and Y. Sairyo, *Biosci. Biotechnol. Biochem.*, **56**, 1971 (1992).
20) S. Ställberg and T. Teorell, *Trans. Faraday Soc.*, **35**, 1413 (1939).
21) G. Kemp and C. Wenner, *Arch. Biochem. Biophys.*, **176**, 547 (1976).
22) D. A. Haydon and S. B. Hladkey, *Quart. Rev. Biophys.*, **5**, 187 (1972).
23) J. B. Peng, B. M. Abraham, P. Dutta, J. B. Ketterson, and H. F. Gibbard, *Langmuir*, **3**, 104 (1987).
24) C. J. Stankovic, S. H. Heinemann, and S. I. Schreiber, *Biochim. Biophys. Acta*, **1061**, 163 (1991).
25) W. Weinstein, B. A. Wallace, E. R. Blout, J. S. Morrow, and W. Veatch, *Proc. Natl. Acad. Sci. U.S.A.*, **76**, 4230 (1979).
26) D. W. Urry, T. L. Trapane, and W. U. Prasad, *Science*, **221**, 1064 (1983).
27) R. E. Koeppe, II, K. O. Hodgson, and L. Stryer, *J. Mol. Biol.*, **121**, 41 (1978).
28) D. C. Shungu, J. F. Hinton, R. E. Koeppe, II, and F. S. Millett, *Biochemistry*, **25**, 6108 (1986).
29) V. B. Myer and D. A. Haydon, *Biochim. Biophys. Acta*, **274**, 313 (1972).
30) T. E. Andreoli, M. Tieffenberg, and D. C. Tosteson, *J. Gen. Physiol.*, **50**, 2527 (1967).
31) G. Eisenmann, G. Szabo, S. Mclaughlin, and S. M. Ciani, *Bioenergetics*, **4**, 93 (1972).
32) G. A. Davidson and M. C. Berman, *J. Biol. Chem.*, **260**, 7325 (1985).
33) G. D. Davis and D. C. Tosteson, *Biochemistry*, **14**, 3962 (1975).
34) C. K. Vishwanath and K. R. K. Easwaran, *Biochemistry*, **21**, 2612 (1982).
35) G. D. Smith, W. L. Daux, D. A. Langs, G. T. de Titta, J. W. Edmonds, D. C. Rohrer, and C. M. J. Weeks, *J. Am. Chem. Soc.*, **97**, 7242 (1975).
36) K. Neupert-Laves and M. Dolber, *Helv. Chim. Acta*, **58**, 432 (1975).
37) G. Kemp and C. E. Wenner, *Biochim. Biophys. Acta*, **282**, 1 (1972).