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INTERACTION OF VALINOMYCIN WITH CATIONS AT THE AIR–WATER INTERFACE

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SUMMARY

A high surface potential is obtained when a valinomycin monolayer is spread upon a subphase containing KCl, RbCl or CsCl at concentrations > 0.5 M. The observed selectivity pattern $Rb^+ \geqslant K^+ > Cs^+$ is the same as observed for valinomycin in bilayer conductance and partition experiments.

Force–area curves of valinomycin at the air–water interface in the presence of 1.0 M K^+ or Rb^+ show a surface pressure transition that coincides with the rise in surface potential. This translation which is presumed to represent the association of valinomycin with the cation occurs at successively lower surface pressures as the cation concentration is increased. Collapse occurs at 165 Å^2 and 26–28 dynes/cm. In the absence of the cation, the force–area curve lacks the transition plateau and collapses at 24.5 dynes/cm and 185–190 Å^2. These data suggest that when valinomycin complexes a cation at the membrane–water interface, it undergoes a rearrangement that is evident from a decrease in surface area of approximately 20–25 Å^2.

INTRODUCTION

Valinomycin is one of a number of macrocyclic antibiotics that have been shown to act by increasing membrane cation permeability by as much as a factor of 10^8. Valinomycin acts on both natural mitochondrial[1], erythrocyte[2], and chloroplast[3] membranes, as well as model membrane systems such as liposomes and black lipid membranes[4,5]. The conformation of valinomycin has been studied and quite well defined from information gained from a number of techniques, including infrared spectroscopy[6], optical rotatory dispersion[6], nuclear magnetic resonance[6,7], and X-ray crystallography[8]. The structural properties and the known effects of valinomycin are consistent with a model which proposes that valinomycin forms a complex with a cation from one side of the membrane, transports the cation across the membrane, and releases it to the other side. Valinomycin shields the cation, which is normally highly insoluble in the hydrophobic interior of the membrane, while hydrophobic side chains solubilize the complex within the membrane. A striking feature of this process is the remarkable selectivity shown between cations; K^+ is transported three orders of magnitude more rapidly than Na^+ (refs 4, 5). It is not yet completely clear to what extent the ion selectivity is determined by equilibrium properties such

as the stability constants or by kinetic parameters such as the rate of complex formation or dissociation but a recent observation of Colacicco[9] offered a promising approach to obtain insight into the mechanism of complex formation at the membrane interface. Thus, Colacicco reported that valinomycin monolayers spread over a subphase containing concentrations of KCl greater than 0.5 M gave rise to a surface potential that increased to 1.2 V at 30 dynes/cm with increasing compression of the film and with increasing K^+ concentration. Therefore, further study of this phenomena was made to obtain information on the structural rearrangements which occur at the membrane-solution interface when a cation is sequestered from the aqueous phase by a valinomycin molecule at the air–water interface.

MATERIALS AND METHODS

Valinomycin (Calif. Biochem. Corp.) was dissolved in either chloroform or ethanol such that the total volume of solvent did not exceed 6 μl. Samples were added to the surface with a 200-μl Gilmont microsyringe.

Egg lecithin was obtained from either Supelco, Bellefonte, Pa., or as a gift from Dr Papahadjopoulos, and stored prior to use at -10 °C. Although rigorous comparison of the phospholipid from the two sources was not made, there was no apparent difference in the results obtained. Inorganic salts were analytical grade and were roasted prior to use at 400 °C for 4 h to remove traces of organic material. All water was double distilled, the second distillation was done in an all-quartz apparatus. Surface pressure and potential measurements were made in a sliding barrier teflon Langmuir trough measuring 30 cm × 5 cm × 2 cm as described previously[10].

Surface pressure was measured with a "platinized" platinum wire immersed in the solution and connected to a C. I. Electronics Micro-force balance. Surface potential was measured with an air-ionizing electrode containing a 1 cm × 1.5 cm piece of Americium foil[11].

RESULTS

Fig. 1 shows the change in valinomycin surface potential with molecular area. The initiation of the increase in ΔV is dependent on the concentration of K^+, and a potential as high as 1.2 V was reached at concentrations of K^+ greater than 1.0 M.

The cation selectivity was examined next, and K^+ in the aqueous phase was replaced by the other alkaline monovalent cations. Fig. 2 demonstrates that the changes in surface potential correspond to the established values for the cation selectivity sequence of valinomycin by solvent extraction procedures[1] as well as by biionic potentials[2]. Cs^+ (3.0 M) fails to elicit the full surface potential change exhibited by K^+ and Rb^+, while 3.0 M Na^+ or Li^+ show no effect.

Fig. 3 shows the force–area plot of valinomycin in the presence of varied concentrations of KCl. When the KCl concentration approaches 1.0 M, an inflection in the valinomycin force–area curve becomes evident. As the KCl concentration is increased the change occurs at successively lower surface pressures. It can be seen that the surface pressure transition takes place at the same KCl concentrations and states of compression that the sharp rise in surface potential occurs. This correspondence is

highly indicative that both are a consequence of the same phenomenon, presumably the association of valinomycin with a cation.

In Fig. 4, a comparison of the force–area curves with several monovalent cations is made. The initiation of the transition occurs at the lowest packing pressure with Rb^+, then K^+, and then Cs^+. In contrast, Na^+, Li^+ and H^+ showed no transition. The established cation selectivity sequence is again obtained.

It was next of interest to see if additional information was available from mixed phospholipid–valinomycin monolayers. Figs 5 and 6 show the additivity of

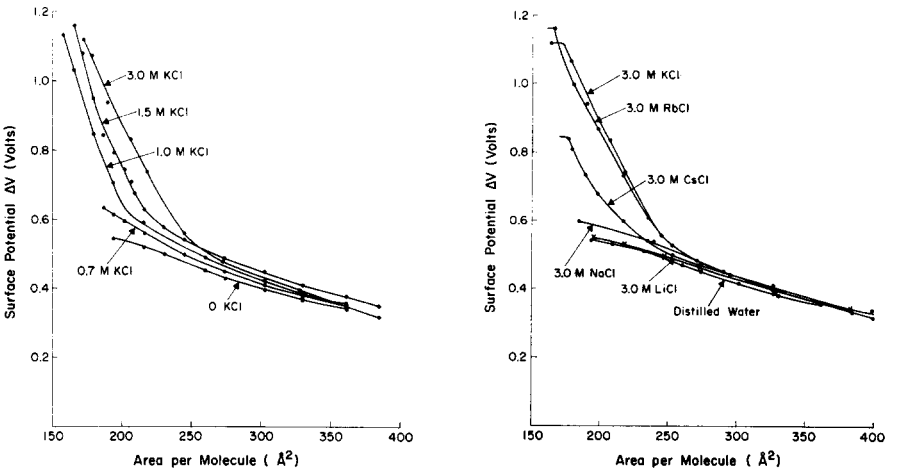

Fig. 1. Dependence of valinomycin surface potential on packing area and KCl concentration. Valinomycin was added to the surface with ethanol or chloroform as spreading solvent.

Fig. 2. Comparison of monovalent cation ability to induce surface potential changes. Conditions are the same as in Fig. 1.

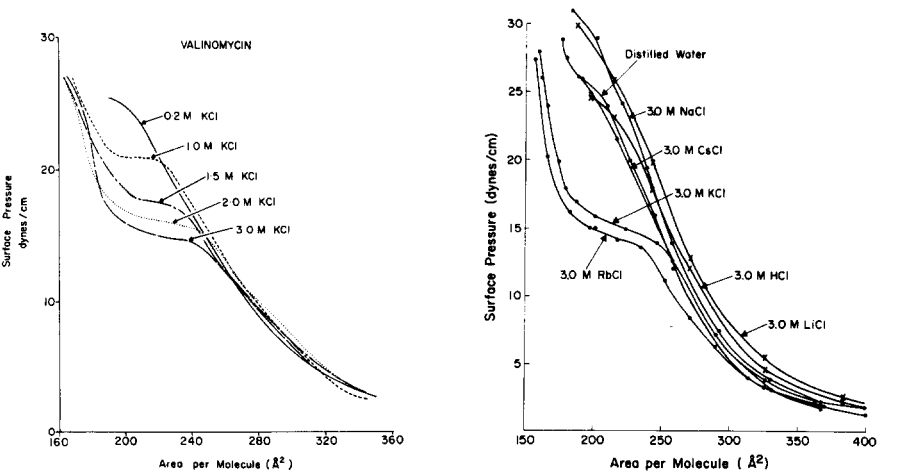

Fig. 3. Force–area plot of valinomycin showing the effect of KCl on the cation-induced phase transition. Conditions are the same as described in Fig. 1.

Fig. 4. Force–area curves of valinomycin showing the effect of monovalent cations on the cation-induced phase transition. Conditions are the same as in Fig. 1.

molecular areas of mixed valinomycin-egg lecithin films. Fig. 7 shows that when sufficient KCl is present in the suphase to cause valinomycin to undergo the transition, the transition occurs at the same pressure as in the pure valinomycin film, and additivity is retained past the transition. Additivity of molecular areas, the collapse of valinomycin from mixed monolayers at its own collapse pressure, and the observation that valinomycin will not penetrate lecithin monolayers at pressures above its own

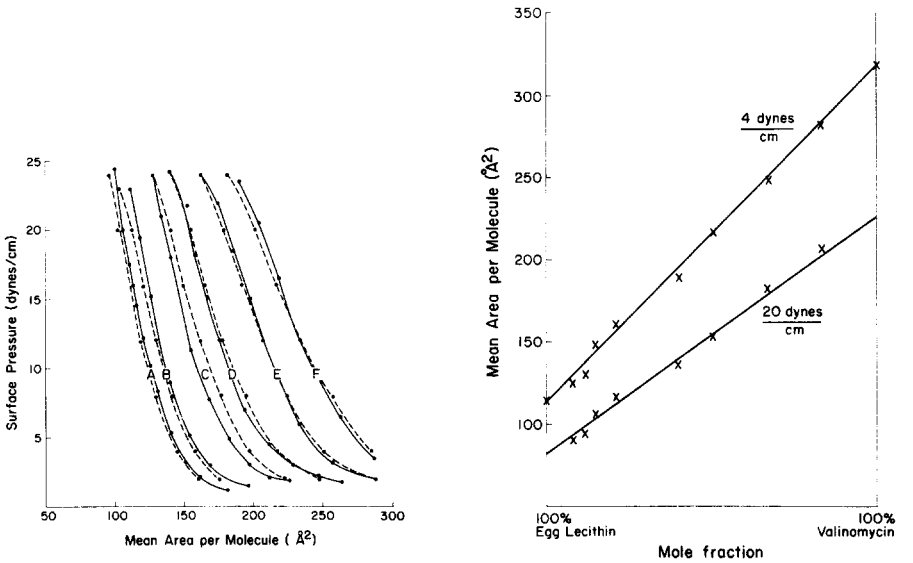

Fig. 5. Valinomycin–egg lecithin mixed monolayers additivity of molecular areas. The dotted line is predicted from force–area curves of components measured alone. The solid lines are the experimentally obtained values of mixed films. The subphase was distilled water. Mole fractions of valinomycin in each case: A, 15%; B, 22%; C, 40%; D, 50%; E, 67.5%; F, 83%.

Fig. 6. Additivity of different mole fractions of egg lecithin–valinomycin monolayers at 4 and 20 dynes/cm. The line is predicted assuming additivity from the two components measured alone.

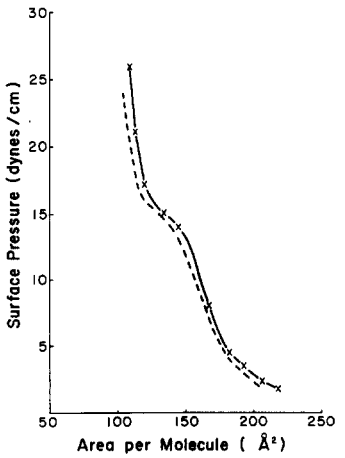

Fig. 7. Valinomycin–egg lecithin mixed monolayers additivity of molecular area and surface potential through the phase transition. The mole fraction of valinomycin was 33%. The subphase was 3.0 M KCl. The dotted line is predicted from force–area curves of components measured alone.

collapse pressure[6] provide compelling evidence that valinomycin and lecithin do not form miscible monolayers at the air–water interface.

DISCUSSION

Surface properties of valinomycin films

A compressed valinomycin monolayer containing 1.0 M or greater K^+ or Rb^+ collapses at a molecular area of 160 Å^2. If Na^+, Li^+, or H^+ is substituted for K^+ or Rb^+ in the subphase or in the absence of cations, the film collapses at an area of 185–190 Å^2. It has been established that uncomplexed valinomycin and its K^+ complex have a different molecular conformation[6], and our results suggest that valinomycin undergoes a rearrangement upon ion complexation such that its molecular area at the membrane interface decreases by 25–30 Å^2. Examination of the force–area plot of Cs^+ reveals why 3.0 M Cs^+ fails to yield the full surface potential of 1.2 V. The Cs^+ complex is less favored than Rb^+ and K^+, and the transition begins to occur just prior to collapse of the monolayer. The transition is thus not complete, and the molecular area at collapse is 175–180 Å^2, indicating that a mixture of complexed and uncomplexed forms is present.

A molecular area of 150 Å^2 for the valinomycin–K^+ complex is predicted from a space filling model of the complex, when projected on a surface such that the plane of the ring is parallel to the plane of the surface. This is in excellent agreement with the 160 Å^2 figure obtained at the air–water interface, assuming 10 Å^2 to be lost in packing. If the counterion is positioned at the base of the cavity, as the aurichloride

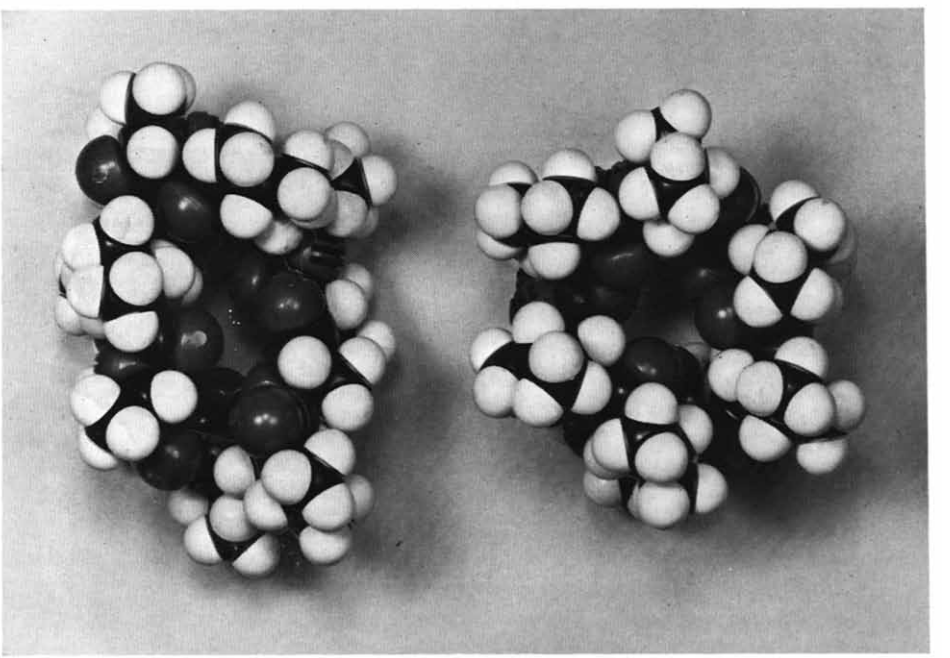

Fig. 8. A photograph of CPK models of valinomycin and its K^+ complex. The uncomplexed form as reported by Dr William Duax is represented on the left-hand side.

anion was reported in the structure determined by X-ray crystallography[8], then this dipole not only gives rise to a valinomycin–K^+ surface potential but also provides a means of anchoring the molecule to the interface. Duax *et al.*[13] have recently determined the crystal structure for uncomplexed valinomycin with the use of a direct methods technique. The complexed and uncomplexed forms are compared in Fig. 8. When a space filling model is oriented at the interface such that it will sequester a cation from the solution, it projects an area of 170–175 Å^2. This figure may be compared with the 185–190 Å^2 that is obtained from surface data presented in this paper. The uncomplexed model occupies an area 20–25 Å^2 larger than in the model of the complexed state, in excellent agreement with the 25–30 Å^2 expansion predicted at the air–water interface. A summary of these molecular areas is shown in Table I.

TABLE I

COMPARISON OF VALINOMYCIN MOLECULAR AREAS

	Area per molecule (A^2)	
	K^+ complex	*Uncomplexed*
Air–water interface at collapse pressure	160	185–190
Space filling model	150	170–175

Relevance of interfacial properties to membrane transport

A remaining question is to explain the dependence of the association on the surface pressure. It is known that the affinity of valinomycin for cations is dependent upon the dielectric of its environment, and that in aqueous systems complex formation is minimal[6,13]. At low packing pressures, there is little affinity between valinomycin and the cation. As the monolayer is compressed, it is possible that the monolayer itself becomes a hydrophobic region and thus the cation becomes associated. Although this explanation is not substantiated, it does provide a means for explaining the dependence of the association on the surface pressure of the monolayer.

It was hoped that by placing valinomycin in a mixed lecithin monolayer that the lipid would further reduce the dielectric of the environment, and that a lower cation concentration would be able to elicit the transition. Such was not the case, because valinomycin proved immiscible with the lecithin in the monolayer. However, it is possible that the affinity of valinomycin for cations at the air–water interface is in accord with the affinity in membranes. Stark *et al.*[14] have noted that the association constant for the cation–valinomycin complex at the interface is several orders of magnitude lower than in ethanolic solution.

If, as Stark *et al.*[14] have proposed, the equilibrium constant K_{eq} = [K^+–valinomycin]/[valinomycin] [K^+] is 0.1 M^{-1}, then for K^+ = 1.0 M, [K^+–valinomycin]/[valinomycin] = 0.1. Here the valinomycin is largely in the uncomplexed state, and if the transport rate is proportional to the amount of complexed valinomycin at the interface, then the transport rate will be sensitive to changes in K^+ concentration up to as high as 1.0 M, as it is[15].

Stark *et al.*[14] have found that at concentrations of KCl above 1 M, the association step is no longer rate-limiting. This is in agreement with our finding that at concentrations of KCl greater than 1.0 M, complex formation becomes essentially complete. So, in fact, the affinity of valinomycin for cations at the membrane solution

interface may be in accord with that observed at the air–water interface*. It should be noted that a low binding constant favors release of the cation at the opposite interface where the complex again encounters a hydrophilic region.

The present results which demonstrate that the ability of cations to develop surface potentials decreases in the order $Rb^+ \geqslant K^+ > Cs^+ \gg Na^+$ or Li^+ are also in accord with the conclusion that cation selectivity results as a consequence of binding at the interface, since in monolayers, no transport steps are involved.

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* In contrast, if the binding constant were as high as that reported by Shemyakin *et al.*[6] with K^+ in ethanol: $K_{eq} = 2 \cdot 10^6\ M^{-1}$, then the association of the cation and the ionophore would be essentially complete at cation concentrations greater than about 10^{-5} M. In this case, the transport rate would be relatively insensitive to changes in ion concentration above about 10^{-6} M or to the nature of the ion. Indeed, in heptane layers containing valinomycin, a system where transport would be dominated by the diffusion of the complex across the partition, the bulk resistivity was only 1.5–2.5 times greater when the partition separated solutions of 0.1 M KCl as compared to solutions of 0.1 M NaCl[16].