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CATION BINDING BY VALINOMYCIN AND TRINACTIN AT THE AIR–WATER INTERFACE

COOPERATIVITY IN CATION BINDING BY VALINOMYCIN

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SUMMARY

A previous communication reported the uptake of monovalent cations by a valinomycin monolayer at the air–water interface (Colacicco, G., Gordon, E. E. and Berchenko, G. (1968) *Biophys. J.* 8, 22a). A similar study has been done with trinactin. As in the case of valinomycin, an elevated surface potential is obtained when the cation–ionophore complex is formed. A surface potential of 0.82 V was obtained for the trinactin–cation complex, as compared with 0.54 V for uncomplexed trinactin. The observed cation selectivity $NH_4^+ > K^+ > Rb^+ > Cs^+$, Na^+ and Li^+ is in agreement with partition and bilayer conductance experiments.

A minimum packing area of 130 $Å^2$ obtained for the trinactin–cation complex was in excellent agreement with the 125 $Å^2$ predicted from space filling models, reinforcing the suggestion that area-per-molecule calculations obtained at the air–water interface can provide useful information on the molecular dimensions of these hydrophobic, relatively low molecular weight transport antibiotics.

Comparison of the data obtained previously with valinomycin and with trinactin revealed two striking differences: (1) a large inflection in the force–area curve concurrent with cation binding and indicative of a conformational change was obtained with valinomycin, but no evidence was found with trinactin; (2) the uptake of cations by trinactin could be predicted by simple equilibrium expressions, but the uptake of cations by valinomycin was strongly cooperative. Possible mechanisms for this cooperative association of cations are discussed.

INTRODUCTION

Following the report of a very high surface potential of 1.2 V when a valinomycin monolayer was spread upon a subphase of > 0.5 M KCl[1] this phenomenon was studied in detail[2]. Significant features of this work were: (1) the cation selectivity sequence $Rb^+ \gg K^+ > Cs^+ \gg Na^+$ and Li^+ obtained in the study was in agreement with that obtained by other techniques, indicative that the high surface potential was a consequence of cation binding by valinomycin in the monolayer; (2) a plateau was found in the valinomycin force–area curve corresponding to the development of the

high surface potential resulting in a decrease in surface area of approx. 25 Å^2. This was interpreted to be a consequence of a conformational change induced by cation binding; (3) the minimum packing areas of the valinomycin–cation complexes, obtained with a subphase of 1.0 M KCl or RbCl, and the uncomplexed state, obtained with a subphase of NaCl, LiCl, or distilled water, were in excellent agreement with molecular dimensions predicted from space filling models. A molecular area of 160 Å^2 was obtained for the cation complex and 185–190 Å^2 for the uncomplexed state. This compares favorably with the 150 -Å^2 value predicted from a space filling model of the established valinomycin-K^+ complex. The conformation of uncomplexed valinomycin in membrane systems has not been established.

In order to determine whether there was general agreement between the calculated minimum packing areas at the air–water interface and molecular dimensions predicted from space filling models, we wished to make comparisons of these two parameters with other ionophores. Further, it was desirable to learn whether additional examples of conformation changes induced by cation binding could be found. In so doing, we hoped to extend the applicability of monolayer techniques in studying the properties of these and similar hydrophobic peptides, depsipeptides and low molecular weight polymers. We report here the results of that study, the surface properties of trinactin, its interaction with cations, and a comparison of the properties of trinactin and valinomycin at the air–water interface.

MATERIALS AND METHODS

Trinactin was obtained as a gift from Dr H. Bickel and Dr K. Scheibli, Ciba-Geigy, Ltd, Basel. All inorganic salts were roasted at 400 °C for 4 h to remove traces of organic material, except NH_4Cl, which was heated to a temperature slightly below its decomposition temperature of 340 °C. The measurement of surface pressure and surface potential is described elsewhere[3].

Monolayers of valinomycin and trinactin are stable for at least a period of 6 h and perhaps much longer. There appears to be some tendency for some loss of film material at surface pressures very near the collapse pressure of the monolayer in the presence of a low ionic strength subphase, but in the presence of 0.1 M or greater salt solutions the monolayers are quite stable. The force–area compressions were continued until collapse was noted, the minimum packing area indicated on the force–area and ΔV–area curves is the point at which collapse began to occur.

RESULTS

Fig. 1 shows the surface potential–area curves of trinactin obtained in the presence of 3.0 M solutions of monovalent cations. Elevated surface potentials were obtained with NH_4^+, K^+, and Rb^+. The development of the high surface potentials was pressure dependent, occurring only at higher packing pressures. The cation selectivity sequence $NH_4^+ > K^+ > Rb^+ > Cs^+$, Na^+ and Li^+ is in agreement with partitioning studies of Eisenman *et al.*[4], strongly suggesting that the elevated surface potential, as in the case of valinomycin, is a consequence of cation binding by the ionophore.

Fig. 2 shows the corresponding force–area curves obtained with 3.0 M solutions

and the same monovalent cations. The curves obtained in the presence of K^+, Rb^+, and NH_4^+, cations presumed capable of complex formation, are very similar to those obtained with 3.0 M Li^+, Na^+, Cs^+, and distilled water subphases. This result was rather surprising in view of the large differences in the valinomycin force–area curves produced by the introduction of suitable concentrations of cations which are capable of eliciting elevated surface potentials.

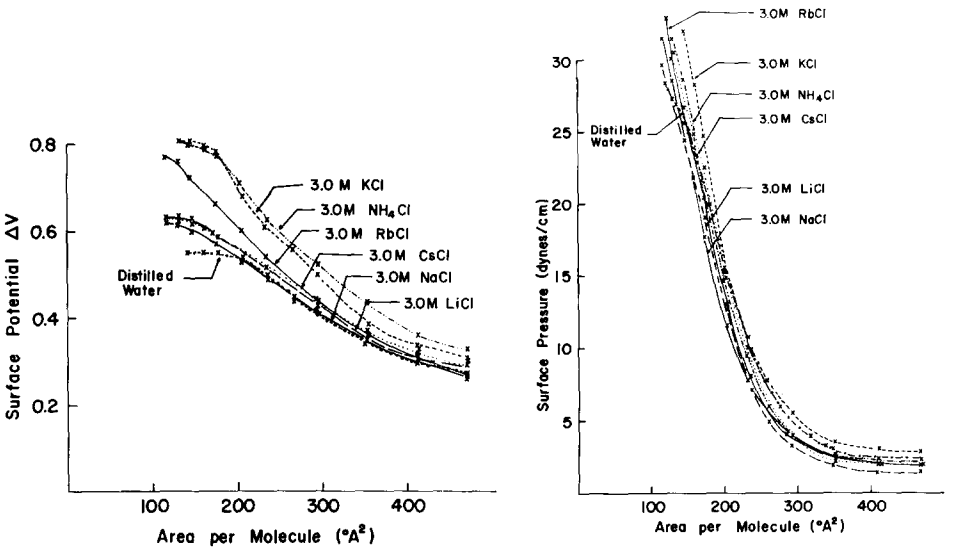

Fig. 1. Surface potential–area curves of trinactin in the presence of 3.0 M monovalent cations.

Fig. 2. Surface pressure–area curves of trinactin in the presence of 3.0 M monovalent cations.

The structure of the nonactin has been determined by X-ray crystallography, and since the trinactin structure is presumed to be identical except for the addition of three peripheral methyl groups, it was possible to build a space filling model of trinactin for the purpose of measuring its dimensions. Assuming the molecule to orient itself at the interface in a manner suitable for loading and unloading of cations, the space filling model projects a molecular area of approx. 125 $Å^2$. This orientation appears to be the only possible one allowing sufficient hydrophilic interaction to anchor it to the aqueous phase. This is in good agreement with minimum packing area obtained in these studies. The minimum packing area of trinactin in the presence of Na^+, Cs^+, or Li^+, cations that do not form complexes at the air–water interface, is slightly less, perhaps 115–120 $Å^2$. The force–area curves of the complexed and uncomplexed trinactin are almost identical, differing only at high surface pressures, and it is not possible to determine whether this difference is due to a difference in the molecular structure of the two forms or simply due to the complex having greater resistance to collapse. It would appear, therefore, that there is no striking change in the molecular architecture of trinactin detectable at the air–water interface, and that conformational changes induced by cation binding are minor.

It is not unreasonable to expect that the complexation of cations might be described by a simple equilibrium expression:

$$K_a = \frac{[\text{trinactin–}NH_4^+]}{[\text{trinactin}]\,[NH_4^+]} \tag{1}$$

Although one cannot properly discuss molar concentrations of materials in monolayers, the ratio $[\text{trinactin–}NH_4^+]/[\text{trinactin}]$ can be approximated by examination of the magnitude of the increase in surface potential due to the presence of the complexed cation. In these calculations, the surface potential used was always the maximum obtained for a given subphase and thus was the value obtained at a minimum packing area. In making this calculation, it is necessary to make the assumption that the surface potentials of the two forms of trinactin are additive. This is analogous to assuming additivity of surface potentials in any two component mixed film, and although the additivity assumption may be unjustified, precedent from other systems suggests that deviation from additivity will probably not be greater than 20–30%, an error, as will be apparent shortly, of insufficient magnitude to alter the conclusions in this paper.

The surface potential obtained in the presence of 3.0 M solutions of monovalent cations is shown in Fig. 1. Assuming the surface potential of the trinactin–cation complex (ΔV_{M^+}) to be 0.82 V and using the Li^+ (or Cs^+ and Na^+) data of Fig. 1 as a measure of the surface potential of the uncomplexed form (ΔV) at the appropriate subphase concentrations, the following expression is obtained:

$$\frac{\text{trinactin–}M^+}{\text{trinactin–}M^+ + \text{trinactin}} = \frac{\Delta V_{obs} - \Delta V}{\Delta V_{M^+} - \Delta V} \tag{2}$$

where ΔV_{obs} is equal to the value obtained experimentally at a designated subphase concentration. This can be rearranged to:

$$\frac{\text{trinactin–}M^+}{\text{trinactin}} = \frac{\Delta V_{obs} - \Delta V}{\Delta V_{M^+} - \Delta V_{obs}} \tag{3}$$

Since $K_a = \dfrac{[\text{trinactin–}M^+]}{[\text{trinactin}]\,[M^+]}$, substitution of the ratio of complexed trinactin to uncomplexed trinactin into the equilibrium expression yields:

$$\frac{\Delta V_{obs} - \Delta V}{(\Delta V_{M^+} - \Delta V_{obs})\,[M^+]} \tag{4}$$

Fig. 3 tests the applicability of these calculations in describing the association of cations by trinactin at the air–water interface. From the lack of dependence of the association constant upon subphase concentration, it is apparent that despite the several approximations such an approach involved, there is at least rough agreement between model and theory (including the additivity assumption described earlier as

well as the use of concentrations rather than activities to describe the subphase concentrations). It is recognized that this approach involves several approximations.

It is possible to make similar assumptions for valinomycin using previously published data[2]. Calculations are included in Fig. 3. Similar results are obtained for the complex of valinomycin with Rb^+. In order to plot calculated values for Cs^+, it is necessary to in addition assume that the maximum surface potential obtained with Cs^+ is the same as that obtained with K^+ and Rb^+. If such an assumption is made, then these conclusions may be applied to the Cs^+–valinomycin complex as well. It is readily apparent that the association of cations from the subphase by valinomycin, in striking contrast to trinactin, cannot be described by simple equilibrium expressions. Indeed, when the observed surface potential is plotted against the subphase cation concentration, a sigmoidal relationship is obtained, suggestive of a strongly cooperative interaction between valinomycin molecules.

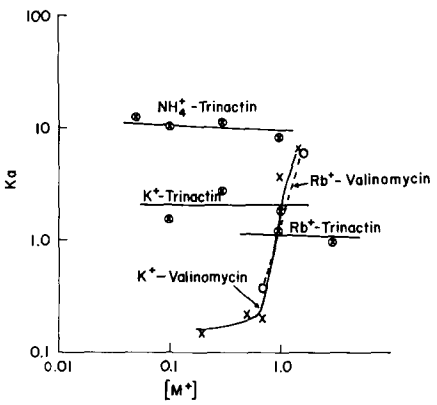

Fig. 3. Test of the simple equilibrium assumption with monolayers of trinactin and valinomycin. Surface potentials were used to calculate the association constants at different cation concentrations. Surface potentials were obtained at surface pressures of 23 dynes/cm for valinomycin and 26 dynes/cm for trinactin.

CONCLUSION

The minimum packing area of the trinactin–K^+ complex, approx. 130 Å^2, is in excellent agreement with the 125 -Å^2 value obtained from a space filling model based upon the structure of the nonactin–K^+ complex[5] obtained by X-ray crystallography. This prediction assumes trinactin to be oriented at the interface in a manner allowing loading and unloading of the cation. Such an orientation would allow anchoring of the molecule to the interface by hydrophilic interaction with the aqueous solvent. Monolayer techniques, at least in the cases of trinactin and valinomycin, thus appear to yield useful information on the molecular dimensions and thus the conformations of such small hydrophobic peptides and macrotetrolide antibiotics. Trinactin does not undergo a large conformational change upon cation binding, as is indicated by the similarity in the force–area curves of the complexed and uncomplexed molecules.

The basis for the cooperative uptake of cations by valinomycin is not at present understood. However, it is possible to propose at least two possible mechanisms that could at least in part contribute to the effect. These include:

(1) The cooperativity may be a consequence of the classical allosteric model, in which a conformational change induced by substrate binding alters the affinity for a substrate at a second binding site.

(2) The cooperativity may result at least in part from an induced change in surface dielectric constant resulting from the valinomycin conformational change. Such a mechanism was suggested previously[2] to explain the dependence of the association of cations on the extent of compression of the film. Thus an increased hydrophobicity of a molecule upon undergoing conformational change might be propagated to and experienced by neighboring molecules as a change in interfacial dielectric thereby increasing the ease with which neighboring molecules may form cation complexes.

Examples of cooperativity in membrane systems already exist. Gordon and Haydon[6] have indicated from bilayer conductance experiments that the peptide alamethicin, a channel-forming transport antibiotic, may form two-dimensional aggregates on the surface of a membrane in which channels situated adjacent to each other interact to yield cooperativity between channels.

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