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Monolayer characteristics of valinomycin in the presence of various salts in aqueous subphase

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The surface pressure–molecular area, surface potential–molecular area and Brewster reflectivity–molecular area isotherms for valinomycin monolayers (states II and III) at various aqueous salt subphases were measured. Two additional states of valinomycin monolayers were observed in the presence of K^+ and Rb^+ as compared to the other alkali metal cations. This phenomenon correlates with the strong complexation between the valinomycin and these cations in bulk. State II corresponds to the very special 'bangle' conformation of the polypeptide ring of valinomycin, in which all carbonyl and carboxyl groups of the amino-acid residues are coordinated to the cation inside the polypeptide ring. State III corresponds to the vertical orientation of the 'bangle' conformation of valinomycin molecules with respect to the interface. An influence of the anion size on the stability of valinomycin-cation complexes at the interface was found.

Introduction

Valinomycin is one of the most interesting and widely investigated ionophores, possessing high membrane activity, including the ability to selectively increase the cation permeability through biological and synthetic membranes [1,2]. Since the character of the membrane interfaces plays a main role in membrane transport, it is important to study the ionic selectivity of valinomycin directly at hydrophilic/hydrophobic interfaces, such as water/air. The monolayer technique is particularly suited for model studies because it provides simple membrane-like systems containing molecules at known distance, concentration and orientation with respect to the interface and enables one to observe the interaction between the molecules at the interface and those in the aqueous subphase. In spite of these obvious advantages of the monolayer technique, there exist only few reports [3–10] concerning valinomycin monolayers at the water/air interface. Taking into consideration the importance of valinomycin for model membrane studies and its wide applicability for ionoselective electrodes and sensors, it is necessary to investigate in detail the properties of valinomycin in monolayers at the water/air interface.

For this purpose not only traditional methods of surface pressure and surface potential measurements vs. molecular area [11], but also the recently developed technique of Brewster angle reflectivity and microscopy of monolayers at the water/air interface [12–14] were chosen. Reflectivity measurements under the Brewster angle of incident light with respect to the pure water surface had provided extremely important information about the molecular organization of lipid and dye molecules in monolayers [13,14]. By Brewster angle microscopy, a transparent monolayer can be visualized without addition of fluorescent probes. Therefore, it was very interesting to apply such a new technique to the investigation of rather complicated biologically active molecules in a membrane-like system at the interface.

This study is devoted mainly to the observation of the interaction of valinomycin in monolayers with various alkali metal cations present in the aqueous subphase.

Experimental

Valinomycin (Sigma, USA) was used as 1.0 mM solution in chloroform (HPLC grade, Fluka, Germany). All inorganic salts and organic solvents were analytical grade.

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Monolayers of valinomycin were prepared and investigated on a commercial film balance (Lauda, Germany) and self-made rectangular Teflon trough (area, 355 cm^2; depth, 1 cm) with a vibrating plate condensor for surface potential measurements, respectively. The principle of the surface potential measurements, using a vibrating plate condensor, was described in a previous paper [11]. 10–20 μl of the valinomycin solution were spread with a microsyringe onto distilled water or aqueous salt subphases. As a standard procedure, monolayers were rested 5 min before compression to allow sufficient solvent evaporation and to reach equilibrium of initial surface pressure and surface potential. Then, the surface pressure (π)–molecular area (A) and surface potential (ΔV)–molecular area (A) isotherms were recorded simultaneously during continuous compression of the monolayer by a movable barrier at a constant rate of 20 cm^2/min. The temperature was 18.0 ± 0.5°C. The values reported are the averages of at least three runs with separately prepared films.

The reflectivity $R = I/I_0$ (I = reflected intensity, I_0 = incident intensity) of the water surface at the Brewster angle under p-polarization was recorded simultaneously with the surface pressure (π) as a function of the area per molecule (A). The reflected light of a laser diode (power < 1 mW, wavelength 670 nm, light spot diameter about 1 mm) in combination with a polarizer was detected with a photomultiplier (Hamamatsu R1635). The angles of incidence and observation were fixed to the Brewster angle for the pure water subphase (53.3°). The whole system was fixed onto a tripod. By simply tilting the tripod, small angle adjustments were possible. The optimal position was found by minimizing the background signal of the pure water. The system was mounted above a rectangular Teflon film balance. All data were recorded and processed by IBM-compatible personal computer (type 386). A more detailed description of the system and technique was published previously [12].

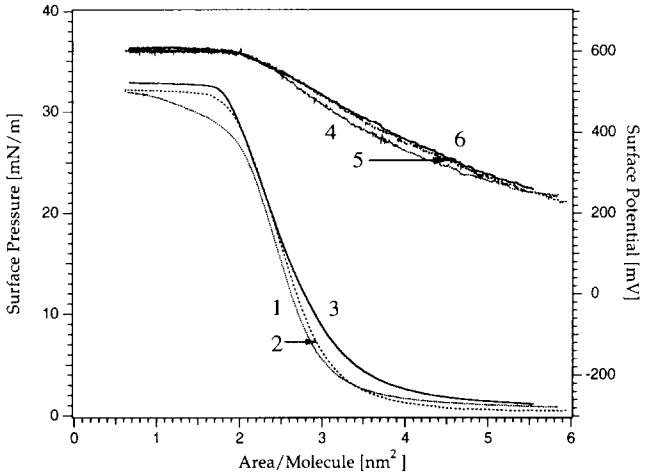

Fig. 1. Surface pressure–area (1–3) and surface potential–area (4–6) isotherms of valinomycin monolayers on water (1,4) and 1.0 M solutions of LiCl (2,5) and NaCl (3,6) at 18°C.

Results and Discussion

Valinomycin forms stable monolayers on water and various aqueous salt subphases. Surface pressure and potential isotherms for valinomycin on LiCl, NaCl, CsCl and NH_4Cl are very similar to each other and close to the same isotherms on distilled water (Figs. 1, 2). Only the liquid-expanded state (I) of valinomycin monolayer is observed in all these cases. The area per valinomycin on water, extrapolated from the linear part of the π–A isotherm to zero surface pressure, is about 3.20 nm^2/molecule, in a good agreement with the previously reported data [3].

Surface pressure and potential isotherms for valinomycin on aqueous KCl and RbCl subphases (Fig. 2)

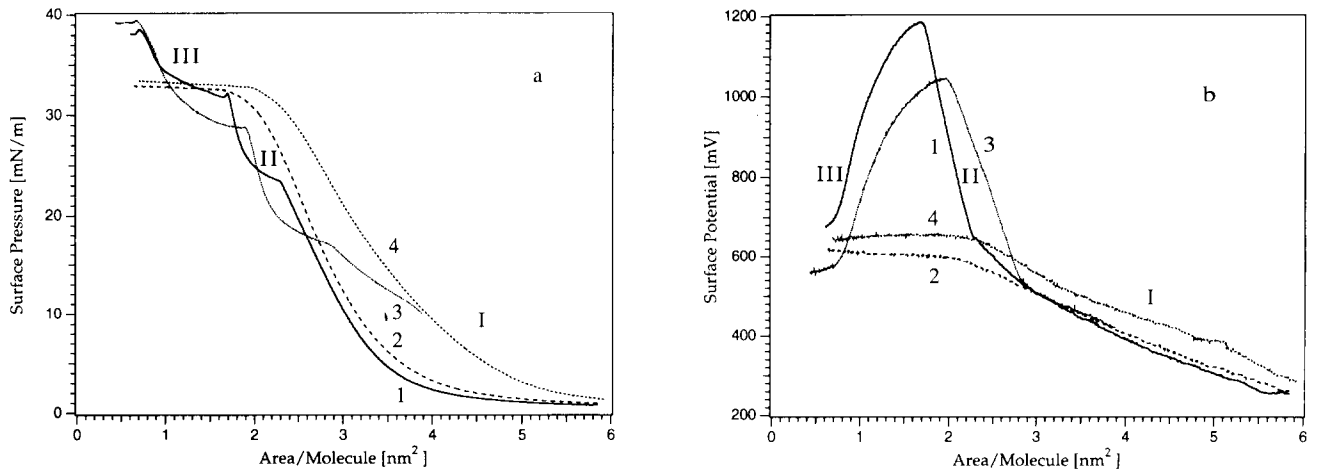

Fig. 2. (a) Surface pressure–area and (b) surface-potential–area isotherms of valinomycin monolayers on 1.0 M solutions of KCl (1), NH_4Cl (2), RbCl (3) and CsCl (4) at 18°C.

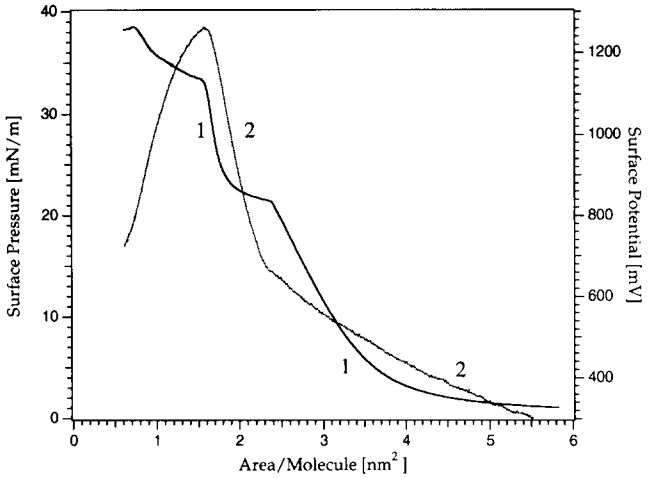

Fig. 3. Surface pressure–area (1) and surface potential–area (2) isotherms of valinomycin monolayers on 1.0 M mixture of KCl/NaCl = 1:1 at 18°C.

TABLE I

Cation size and stability constants

Area per molecule (A) at surface pressure 10 mN/m, maximal surface potential (ΔV) and effective dipole moment (μ) of close-packed valinomycin monolayers at aqueous subphases with various alkali cations (1.0 M) in comparision with cation radii and stability constants of the valinomycin-cation complexes in ethanol (from Ref. 1, p. 6 and p. 224)

Subphase	Cation radii (Å)	Complex constant	A ($\pi = 10$) (nm^2/mol)	ΔV_{max} (V)	μ_{max} (D)
Water	–	–	2.70	590	3.75
LiCl	0.68	< 5	2.78	600	3.95
NaCl	0.98	< 50	2.90	610	4.30
KCl	1.33	2600000	3.05	1170	5.65
RbCl	1.49	2900000	3.88	1050	5.55
CsCl	1.65	650000	3.95	660	4.95
NH_4Cl	1.46	< 47	3.15	620	4.35

have some similarity to those in Fig. 1 only at low surface pressures and high areas (state I), being completely different upon further compression. On 1.0 M solutions of these salts, both isotherms have a special region at surface pressures 20–30 mN/m with corresponding areas 3.0–1.7 nm^2/molecule (state II) as well as at 30–40 mN/m and 1.7–0.6 nm^2/molecule (state III), see Fig. 2. It is important to stress that all three break points in the π–A isotherms, which can be assigned to the transition between states I–II, II–III and III–collapse of the monolayer, occur in the same areas as in the ΔV–A isotherms (Fig. 3). The break point between states I and II can be explained as the onset of the transition from an 'expanded' conformation of the polypeptide ring of valinomycin, when the hydrophilic groups are facing to the aqueous subphase, to the very special 'bangle' conformation, in which all carbonyl and carboxyl groups of the amino-acid residues are coordinated to the cation inside the polypeptide ring. The 'bangle' conformation is very rigid and can be formed spontaneously even in solution in the presence of K^+ or Rb^+, as was shown first by Ovchinnikov et al. [1]. It is correlated with the liquid-condensed character of the state II of valinomycin monolayers, in particular with its low compressibility. The surface pressure of the I–II transition depends strongly on the concentration of K^+ or Rb^+ in the aqueous subphase, while the pressure of the II–III transition is always the same in the range of 32.0–34.0 mN/m for KCl and 28.0–30.0 mN/m for RbCl at various concentrations of these cations in the aqueous subphase. The transition II–III may be attributed to a reorientation of the valinomycin molecules with the 'bangle' conformation in the monolayer from parallel to the perpendicular position with respect to the interface. The monolayer in state III has a collapse in the range of 38.0–40.0 mN/m for both K^+ and Rb^+. This unusual behaviour of valinomycin monolayers on KCl and RbCl subphases

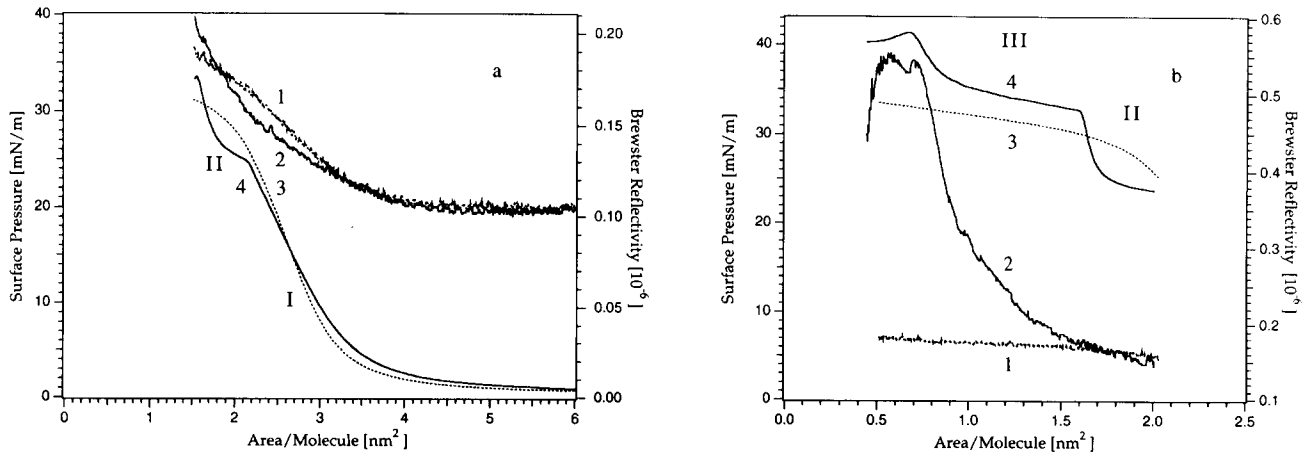

Fig. 4. Brewster reflectivity–area (1,2) and surface pressure–area (3,4) isotherms of valinomycin monolayers on distilled water (1,3) and 1.0 M solution of KCl (2,4) at large (a) and small (b) molecular areas (18°C).

can be explained as complex formation of ionophore at the interface with a cation from subphase.

The obtained data are in a good agreement with the known sizes of the alkali metal cations and differences of stability constants of the valinomycin-cation complexes for various cations in organic solvents (Table I). The area per valinomycin molecule at 10 mN/m (this surface pressure was chosen as reference) increases with increasing cation radius, probably because of the strong electrostatic repulsion between the valinomycin-cation complexes. The maximal surface potentials and dipole moments for valinomycin on KCl and RbCl subphases are higher than for the other cations. This observation correlates with the high complexation constants for these cations.

The differences in reactivity between the cations are clearly seen in the case of valinomycin monolayers on a mixed equimolar KCl-NaCl subphase (Fig. 3). The similarity of the surface pressure and potential isotherms of valinomycin on this mixture with those on KCl (Fig. 2a, 2b), as well as pronounced differences with those on NaCl (Fig. 1) is evidence for strong binding of K^+ by valinomycin in the presence of the same concentration of Na^+ in aqueous subphase. This experiment demonstrates the ionic selectivity of valinomycin at interfaces.

The Brewster reflectivity–molecular area isotherms of valinomycin monolayers (Fig. 4) are rather different compared to the known data for fatty acids or lipids [12–14]. The reflectivity isotherms of fatty acids and some lipids show a sharp and high increase upon compression long before the surface pressure starts to increase, whereas the reflectivity isotherm of valinomycin on distilled water shows only a small and gradual increase, which coincides perfectly with the beginning of the increase in surface pressure (Fig. 4). The shape of a very small increase in reflectivity ($< 10^{-7}$) for valinomycin monolayers in the area range of 3.5–1.5 nm^2/molecule at distilled water perfectly correlates with the shape of the π–A and ΔV–A isotherms (Fig. 4). Thus, valinomycin monolayers on water show only a liquid-expanded state without domain structure in contrast to the monolayers of fatty acids, which have big domains even at very high molecular areas. The same structure is typical for monolayers of valinomycin at aqueous subphases in the presence of NaCl. Direct evidence of such differences in structure of valinomycin and fatty acid monolayers was obtained by using the Brewster angle microscopy (BAM). No domains were detectable in valinomycin monolayers, whereas for fatty acids and lipids the domain structure was clearly observed in agreement with already published results [13,14].

A different shape of the Brewster reflectivity–molecular area isotherms of valinomycin monolayers was observed in the presence of the KCl in aqueous subphase (Fig. 4). At high areas and low surface pressures (state I) a small increase in the reflectivity also correlates with the increase of surface pressure (Fig. 4a, curves 2 and 4, respectively). But at further compression (state II) the reflectivity continues to increase whereas in the absence of K^+ the signal becomes constant (Fig. 4a, curve 1). At small areas and high pressures (state III) the reflectivity increases sharply and reaches constant values (about 4-times higher than for valinomycin on water) near the collapse point of these monolayers (Fig. 4b). As known from the investigation of the reflectivity change of the water surface by monolayers of fatty acids, methyl esters and alcohols with various chain length [13–15], reflectivity increases with increasing thickness of the monolayer film. Therefore, the thickness of the valinomycin monolayers at distilled water and various salts, including KCl (at state I and II), is not significantly changed during compression. In contrast, the thickness of valinomycin monolayers on KCl at high compression (state III) is changed

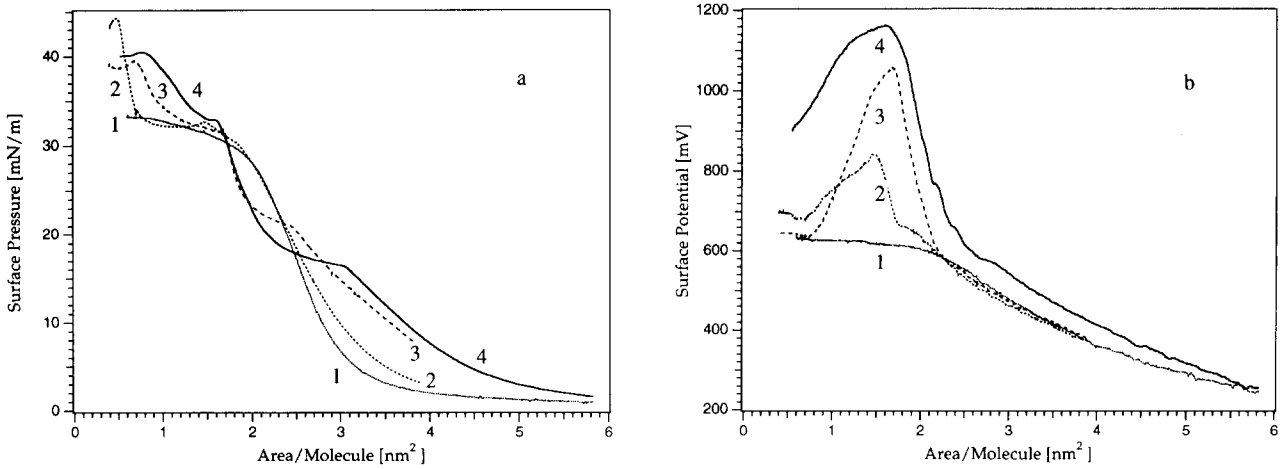

Fig. 5. (a) Surface pressure–area and (b) surface potential–area isotherms of valinomycin monolayers on KCl solutions with various concentrations: 0.1 M (1), 0.5 M (2), 1.5 M (3) and 3.0 M (4) at 18°C.

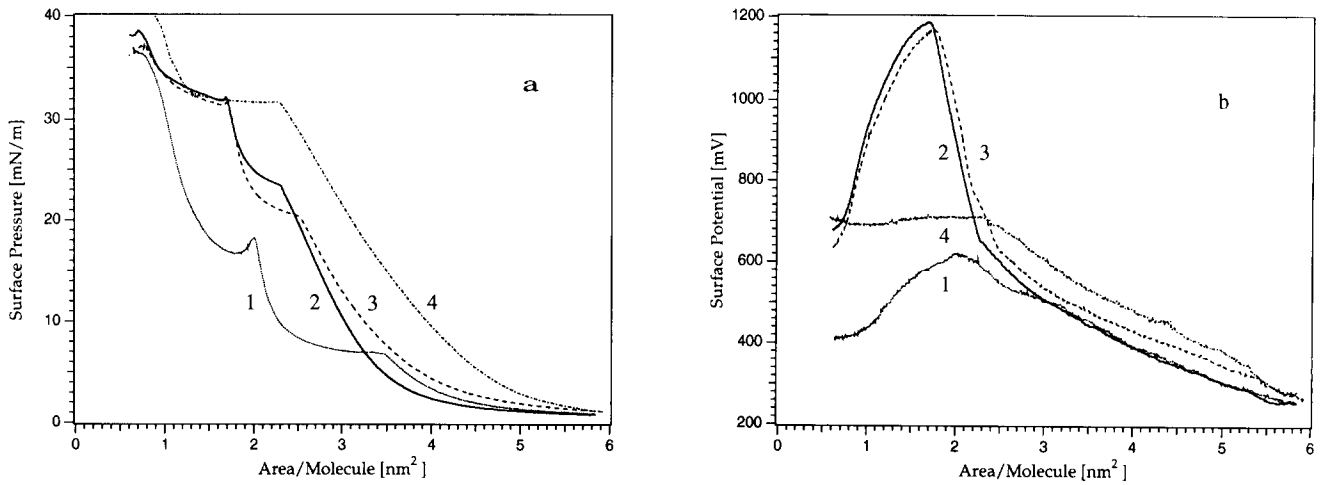

Fig. 6. (a) Surface pressure–area and (b) surface potential–area isotherms of valinomycin monolayers on 1.0 M solutions of KI (1), KCl (2), KBr (3) and KF (4) at 18°C.

drastically. This can be explained as reorientation of the valinomycin molecules in the monolayer from parallel to the perpendicular position with respect to the interface. The valinomycin molecules have high asymmetry in plane and vertical, especially in the 'bangle' conformation [1]. Thus the pronounced changes on all types of the isotherms (π–A, R–A and ΔV–A) in the state III of valinomycin monolayers on aqueous subphases with KCl can be explained simply by reorientation of the valinomycin molecules.

TABLE II

Monolayer characteristics

Surface pressure (π), area per molecule (A), surface potential (ΔV) and effective dipole moment (μ) of close-packed valinomycin monolayers at aqueous subphases with various NaCl concentrations (*, maximum values of surface pressure; **, maximum values of surface potential).

[NaCl] (M)	π (mN/m)	A (nm^2/mol)	ΔV (mV)	μ (D)
0.001	10.0	2.55	520	3.56
	32.1 *	1.05	637 **	3.13
0.010	10.0	2.56	513	3.75
	32.0 *	0.82	624 **	2.60
0.100	10.0	2.62	519	3.73
	31.4 *	1.00	603 **	2.90
0.500	10.0	2.79	560	4.34
	32.2 *	0.93	664 **	3.40
1.000	10.0	3.06	503	4.30
	32.7 *	1.65	606 **	3.84
1.500	10.0	2.94	485	3.85
	33.8 *	1.51	612 **	3.43
2.000	10.0	2.99	496	4.08
	33.4 *	1.31	606 **	3.41
3.000	10.0	3.14	514	4.34
	34.3 *	1.51	620 **	3.54

The observed break points in the π–A and ΔV–A isotherms of valinomycin monolayers on a KCl subphase depend strongly on the salt concentration of the aqueous subphases (Fig. 5a, b). Up to a KCl concentration of 0.1 M in the aqueous subphase, both isotherms are similar to those obtained on water or, for example, in the presence of NaCl. Only at KCl concentrations higher than 0.5 M the changes in the isotherms (assigned to the states II and III), especially for the surface potential, become more pronounced. The magnitude of these changes increases with increasing KCl concentration in aqueous subphase and reaches almost constant values at the KCl concentration of 3.0 M. This effect is additional evidence of strong complexation between ionophore and K^+. In contrast, the shape of the isotherms π–A and ΔV–A of valinomycin on NaCl subphases are almost independent of the salt concentration in the aqueous suphase. Only the absolute values of area per molecule increase about 15% with increase of the NaCl concentration in aqueous subphase from 1.0 mM to 3.0 M (Table II). This effect can be explained as non-specific binding of Na^+ by valinomycin without conformational changes of the molecule.

The transition from state II to state III of valinomycin monolayers in the presence of K^+ depends on the nature of counter-anion (Fig. 6). For very small anions like fluoride, this transition disappears and the surface potential remains constant, probably, because this anion can enter the cavity in the ring together with the cation and thereby prevents the formation of the 'bangle' conformation. For the big anions (bromide and especially, iodide), which can not enter the cavity of the ring together with the cation the transition II–III appears at essentially larger pressures and higher areas. It is interesting that the surface potential–area isotherms of valinomycin on KCl and KBr are very similar showing a pronounced maximum, while the isotherms for KI gives only small changes. As was

shown in our previous publication [9], the nature and size of the anion did not influence the isotherms of the valinomycin monolayers on Na^+, because of the low affinity of the valinomycin to this cation.

We hope that further experiments can clarify the conformational changes of the valinomycin in monolayers in the presence of various cation-anion pairs. The understanding of this phenomenon will be useful for future application of such thin films in ionoselective membrane electrodes.

Conclusions

The behaviour of valinomycin monolayers depends strongly on the nature and size of the cation-anion pairs in aqueous subphases. Additional two monolayer states of valinomycin exist in the presence of K^+ and Rb^+ as compared to the other alkali metal cations, due to strong complexation of valinomycin at the interface with these cations from aqueous subphase. The anion size influences the stability of valinomycin-cation complexes at the interface.

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