BBA 75117

DIFFERENTIAL EFFECT OF SODIUM AND POTASSIUM ON CALCIUM ADSORPTION TO STEARIC ACID MONOLAYERS

A. YAMAUCHI, A. MATSUBARA, H. KIMIZUKA AND L. G. ABOOD

Department of Chemistry, Kuyushu University, Fukuoka (Japan) and Center for Brain Research, University of Rochester, Rochester, N.Y. (U.S.A.)

(Received September 26th, 1967)

SUMMARY

The effects of cations on the adsorption of Ca^{2+} to stearic acid monolayers was investigated by the radiotracer method. The addition of either Na^+ or K^+, once the monolayer had formed in the presence of Ca^{2+}, caused the desorption of Ca^{2+} from the monolayer. The rate of desorption of Ca^{2+} brought about by K^+ was greater than that by Na^+; whereas, the equilibrium adsorptions were almost the same. The free energy of the exchange reaction was the same for both ions. Mg^{2+}, Ba^{2+} and Sr^{2+} all bind to the monolayer to the same extent as Ca^{2+} but to a considerably lesser extent than the transition metal ions. The difference in the interfacial effect of Na^+ and K^+, which may be related to the time needed to desolvate the ions prior to interaction, may in part account for the K^+ selectivity of cellular membranes.

INTRODUCTION

The investigation of interactions between surface monolayers and the solute in the underlying solution is essential to an understanding of the reactions occurring at the interface of cellular membranes. In recent years considerable evidence has accumulated to show that Ca^{2+} is involved in regulating permeability of ions across the cell membrane[1–3,6]. It is of importance, therefore, to examine the interactions between Ca^{2+} and other ions within the monolayer. The adsorption of Ca^{2+} to the stearic acid monolayer has become quantitatively measurable by applying the radiotracer technique[4–8]. The aim of the present study is to compare the static and kinetic behaviors of the adsorption of radioactive Ca^{2+} to monolayers of stearic acid in the presence of other inorganic cations.

METHODS AND MATERIAL

Materials, the methods and the apparatus were described in preceding papers[7,9]. Surface pressure–area diagrams and data on surface adsorption of Ca^{2+} were obtained with monolayers of stearic acid in the presence of $3.6 \cdot 10^{-5}$ M $^{45}CaCl_2$ (specific activity, 10 mC/mole) and $2.5 \cdot 10^{-5}$ M metal chloride salt. For the experiments requiring a constant surface concentration of the stearic acid monolayer, the stearic acid was spread to an occupied area of 26.8 Å^2 per molecule on the surfaces of the solutions containing $CaCl_2$ with a fixed concentration of $1.62 \cdot 10^{-5}$ M and varying concentrations of NaCl or KCl. The pH of the solutions was adjusted to 8.0–8.2 with either $NaHCO_3$ or $KHCO_3$, the adsorption of Ca^{2+} approaching saturation at this pH (ref. 6). It was established that the adsorption of Ca^{2+} was not significantly affected by the addition of the small quantities of Na^+ or K^+ used for adjusting pH. The experiments were carried out at temperatures near 10°.

RESULTS

The degree of adsorption of radioactive Ca^{2+} was measured by means of the Geiger–Müller tube and conventional scaler and expressed as the surface fraction covered by calcium stearate Φ which is given by a relation $\Phi = I/I°$ where I denotes the increase in the intensity of radiation (counts/min) in the presence of the added salt and $I°$, the saturation value.

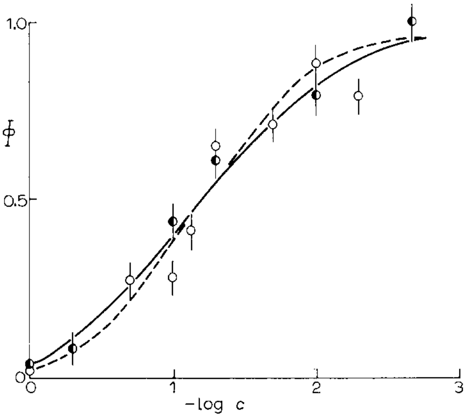

Fig. 1. Effect of alkali ions on the adsorption of Ca^{2+} to stearic acid monolayers. $[Ca^{2+}] = 1.62 \cdot 10^{-5}$ M (pH 8–8.2); ○---○, NaCl; ◐—◐, KCl. The full and dotted lines are calculated according to Eqn. 2. Abscissa is the negative log of the molar concentration of NaCl and KCl; the ordinate is the fraction (Φ) of initial Ca^{2+} remaining in the monolayer.

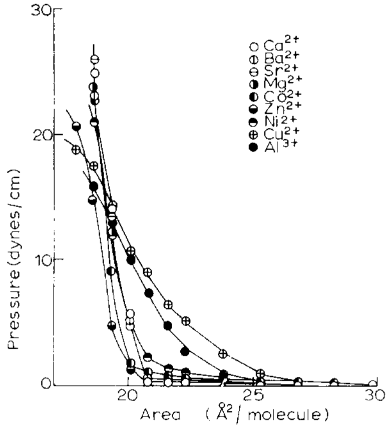

Fig. 2. Effect of added salts on the surface pressure of stearic acid monolayers as a function of surface area. Substrate containing $3.6 \cdot 10^{-5}$ M $^{45}CaCl_2$ and $2.5 \cdot 10^{-5}$ M metal chloride salt, at pH 8.0 ± 0.1.

The equilibrium values of Φ's were measured with varying concentrations of added monovalent ions and the results are shown in Fig. 1. There is an increased adsorption of Ca^{2+} to the stearic acid monolayer which is increasingly replaced by Na^+ or K^+ with the increase in their concentrations.

The effects of added salts on the surface pressure–area diagram of stearic acid monolayer and on the adsorption of Ca^{2+} are shown in Fig. 2 and Table I, respectively. As seen in Fig. 2, in the presence of Cu^{2+} or Al^{3+}, there occurs an expansion of monolayer, presumably due to the complex soap formation[9]. The reason why the films do not exhibit the expansion in the presence of Co^{2+}, Ni^{2+}, and Zn^{2+} may be due to the low concentrations of these ions and the effect of condensation by Ca^{2+}. In the presence of various metal ions there is a diminished adsorption of Ca^{2+} (Table I), pre-

TABLE I

FRACTION OF SURFACE OCCUPIED BY CALCIUM STEARATE, Φ, AND APPARENT EQUILIBRIUM CONSTANT, K

Parameter	*Added ions*							
	Ba^{2+}	*Sr*$^{2+}$	*Mg*$^{2+}$	*Co*$^{2+}$	*Ni*$^{2+}$	*Zn*$^{2+}$	*Cu*$^{2+}$	*Al*$^{3+}$
Φ	0.56	0.61	0.63	0.25	0.18	0	0	0
K	0.88	1.08	1.18	0.23	0.15	—	—	—

sumably because of competition by the metal ion. In Table I are presented the apparent equilibrium constants for the competitive adsorption between Ca^{2+} and metal ions, calculated according to the equation

$$K = \frac{\Phi c_{\mathrm{M}^{2+}}}{(1 - \Phi)\, c_{\mathrm{Ca}^{2+}}} \tag{1}$$

where $c_{\mathrm{Ca}^{2+}}$ and $c_{\mathrm{M}^{2+}}$ denote the concentrations of the Ca^{2+} and the metal ions, respectively, and Φ, the fraction of monolayer occupied by calcium stearate at 20 Å^2 per stearyl group. It may be seen in Table I that Mg^{2+} and the alkali earth metal ions have an adsorptivity comparable to that of Ca^{2+}, while transition metal ions have a greater adsorptivity.

An interesting property of the cellular membrane is its selective permeability to certain alkali earth metals. It has been convenient to discuss this property in relation to adsorptivity of ions. Since the phenomenon of permeability is kinetic in nature and may be more intimately related to the rate of adsorption or desorption rather than to any static property such as the equilibrium adsorption[7], the following experiment was done. When the equilibrium adsorption of Ca^{2+} to the stearic acid monolayer was attained, various concentrations of Na^{+} or K^{+} were injected into the subsolution by means of a syringe and the time course of the desorption of Ca^{+} measured (Fig. 3). Under such conditions bound Ca^{2+} was desorbed more rapidly by K^{+} than by Na^{+}. It should be noted that the desorption of Ca^{2+} did not occur

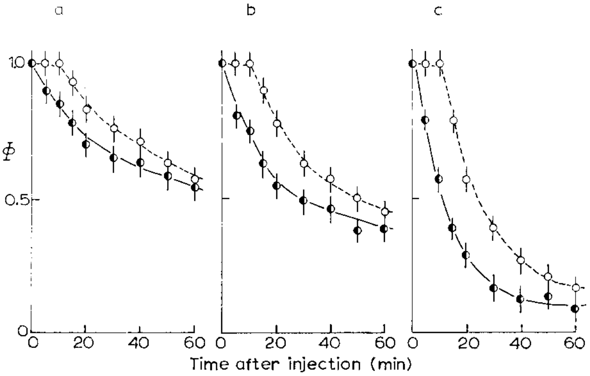

Fig. 3. Desorption of bound Ca^{2+} with time. [Ca^{2+}] = $1.62 \cdot 10^{-5}$ M (pH 8.0–8.2); ○---○, NaCl; ◐—◐, KCl. 1 ml of 0.4 M (a), 1.0 M (b) and 4.0 M (c) alkali chloride solution, respectively, was injected into the subsolution. Φ = fraction of initial Ca^{2+} remaining in the monolayer; abscissa is time following injection of NaCl or KCl.

immediately after the injection of Na^+, while the desorption curve exhibited an induction period of about 10 min. On the other hand, the injection of K^+ caused an immediate desorption of Ca^{2+}. The same phenomenon has been observed with lecithin films[7].

DISCUSSION

In the present case, the following equilibrium holds for the surface adsorption of Ca^{2+} to the stearate monolayer:

$$2\ \mathrm{M(St)} + \mathrm{Ca}^{2+} = \mathrm{Ca(St)_2} + 2\ \mathrm{M}^+ \tag{2}$$

where St represents the stearyl group and M^+, the monovalent ion. Disregarding the very small fraction of undissociated stearic acid, the monolayer formed at the surface consists of calcium stearate and monovalent metal stearate. If such mixed monolayers are assumed to be a non-athermal mixture of monomer and dimer, the adsorption isotherm can be expressed by the following equation[7]:

$$\frac{a_{\mathrm{Ca}^{2+}}}{a^2_{\mathrm{M}^+}} = \mathrm{e}^{\frac{\Delta\mu^0 - RT}{RT}} \cdot \frac{\Phi}{(1 - \Phi)^2} \cdot \mathrm{e}^{\frac{2W}{RT}(1 - 2\Phi)} \tag{3}$$

where $\Delta\mu^\circ$ is the standard free energy change of the reaction (2), W the interchange energy and a's, the activities of the respective ions.

Assuming that the activity coefficients of ions in the substrate are all unity, the linear relation was obtained between the ln $[(1-\Phi)^2/x^2_{\mathrm{M}^+}\cdot\Phi]$ and Φ at the fixed concentration of Ca^{2+}, where x_{M^+} is the mole fraction of the ion M^+ in the bulk solution. The values of W and $\Delta\mu^\circ$ can be evaluated by using the slopes and the intercepts of the ordinate, respectively. All their values, together with the equilibrium constant K, calculated from $\Delta\mu^\circ$, are shown in Table II. By the use of these values, the $\Phi - c_{\mathrm{Na}^+}$ and $\Phi - c_{\mathrm{K}^+}$ curves are well reproduced with Eqn. 2, as shown by full and dotted lines in Fig. 1. From the values for the equilibrium constants it can be concluded that the difference between the equilibrium adsorptivity of Na^+ and K^+ is very small.

TABLE II

CONSTANS FOR EQN. 2 AND EQUILIBRIUM CONSTANT K

Parameter	*Added ions*	
	Na^+	*K^+*
W (kcal/mole)	—0.23	—0.50
$\Delta\mu^\circ$ (kcal/mole)	—0.68	—0.70
K	3.4	3.5

The fact that the rate of interfacial exchange of Ca^{2+} and K^+ exceeds that for Ca^{2+} and Na^+ implies that factors other than ionic charge and radius are responsible for the differential effect. One possible explanation is that the removal of water dipoles from the hydrated Na^+ or K^+ as interaction occurs may be a rate-determining step in the exchange reactions; so that more time is required in the case of the more hydrated Na^+. A further test of this hypothesis would require the use of other alkali metals, such as Li^+ which is more hydrated than Na^+. It is obvious from the present studies that ion transport processes across the cell membrane could be better understood by a consideration of the kinetics of adsorption rather than adsorptive phenomena at equilibrium. This difference between the interfacial behavior of Na^+ and K^+ in ion exchange kinetics could account for the K^+ selectivity so characteristic of most cellular membranes.

ACKNOWLEDGEMENT

This research has been supported in part by a grant in aid for special project research on biophysics from the Ministry of Education, Japan.

REFERENCES

1 L. G. Abood, *Intern. Rev. Neurobiol.*, 9 (1966) 223.
2 F. Brink, *Pharmacol. Rev.*, 6 (1954) 243.
3 J. A. Tobias, *J. Cellular Comp. Physiol.*, 52 (1958) 89.
4 H. Kimizuka and K. Koketsu, *Nature*, 196 (1962) 995.
5 H. Kimizuka and K. Koketsu, *J. Gen. Physiol.*, 47 (1963) 379.
6 L. G. Abood, H. Kimizuka, G. Rogeness and J. Biel, *Ann. N.Y. Acad. Sci.*, 107 (1963) 1139.
7 A. Matsubara, R. Matuura and H. Kimizuka, *Bull. Chem. Soc. Japan*, 38 (1965) 369.
8 H. Kimizuka, T. Nakahara, H. Uejō and A. Yamauchi, *Biochim. Biophys. Acta*, 137 (1967) 549.
9 R. Matuura and H. Kimizuka, *Bull. Chem. Soc. Japan*, 28 (1955) 668.