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KINETICS OF Ca^{2+} ADSORPTION AND CATIONIC SELECTIVITY WITH A SYNAPTIC MEMBRANE PROTEIN

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SUMMARY

A study was conducted on the adsorption of $^{45}Ca^{2+}$ to a surface film of a hydrophobic protein derived from synaptic membranes isolated from bovine cerebellum. A kinetic analysis of Ca^{2+} displacement from the protein by various metal and organic cations could be described by a rate law based on diffusion and displacement. The relative rate constants for the displacement of bound Ca^{2+} were in the order $Li^+ < Na^+$, $Rb^+ < Cs^+ < K^+$, NH_4^+. Among the alkaline earth series the sequence was Mg^{2+}, $Sr^{2+} < Ba^{2+}$. Ca^{2+} adsorption could be described by a theoretical formulation which takes into account an interfacial energy and potential barrier as well as the diffusional process. An attempt was made to consider the effect of energy of hydration of the cations, surface charge, and the chemical environment at the interface on catonic selectivity. The behavior of the cations in this system significantly resemble their behavior in natural membranes, particularly excitatory ones. The structural and physicochemical environment of the protein at the interface is discussed in relation to Ca^{2+} binding and cationic selectivity.

INTRODUCTION

It is now established that bioelectric phenomena are associated with differential fluxes of alkali metal ions across excitable membranes; and although a great deal is known about the kinetics of the ionic fluxes, little is known of the chemical components of the membrane involved. A variety of approaches ranging from artificial ion-exchange systems [1] to intact muscle and nerve [2] have been used to investigate ionic adsorption, exchange, and permeability. Although such studies have yielded valuable information on the selectivity sequences of inorganic ions and the physicochemical factors governing them, they have focused on the role of phospholipids to the neglect of proteins and macromolecular complexes. A logical approach to this problem has been to utilize interfacial films of lipids and proteins derived from natural excitatory membranes; and, by measuring such properties as surface potential, pressure and adsorption, to study interactions with inorganic ions, ATP and various psychotropic drugs [3–5]. A particularly useful preparation has been a "hydrophobic" protein isolated from synaptic membranes of rat and beef brain.

In the present study a monomolecular film of the synaptic protein at an air–water interface was used to investigate the kinetics of Ca^{2+} adsorption and the ability of various metal ions to exchange with bound Ca^{2+}. By application of a rate law based on diffusion of the cation from the bulk phase to the interfacial protein, one could adequately account for both Ca^{2+} adsorption and the selectivity sequence of the cations. An attempt is made to relate the adsorption kinetics of cations to the protein by applying a theoretical treatment, previously developed for lipid monolayers [6], which presupposes both an adsorption potential and energy barrier to adsorption.

METHODS

Preparation of nerve ending membranes from calf brain

Approx. 100 g of cerebellar gray matter from the brains of freshly killed calves were homogenized thoroughly in large glass homogenizers (capacity, 50 ml; clearance, 0.2 mm) in 10 times its volume of 0.32 M sucrose. After removal of the heavier particulates by centrifugation twice at $1000 \times g$ for 10 min, a lighter pellet was obtained by centrifugation at $15000 \times g$ for 20 min. This pellet was then homogenized in 30 ml of 0.32 M sucrose and applied equally to 12 cellulose nitrate centrifuge tubes containing equal volumes of 0.8 M sucrose layered on 1.2 M sucrose. Centrifugation at $80000 \times g$ for 2 h yielded three layers; and the intergradient level, which contained nerve endings, was removed by gentle suction and retained. The nerve ending pellet was then resuspended in 100 ml of water quickly frozen and thawed twice, and centrifuged at $15000 \times g$ to yield a pellet (Fraction M). After homogenization in 100 ml of 0.1 M Tris buffer, pH 7.5 and recentrifuged at $20000 \times g$, Fraction M was ready for protein fractionation.

Preparation of hydrophobic protein from membrane fraction M

The membrane preparation M was homogenized in enough 0.1 M Tris buffer, pH 7.5 to make a final protein concentration of about 20 mg/ml. To the suspension was added 30 mM dodecylsulfate to make a final concentration of 0.3 mM dodecylsulfate and the suspension was carefully homogenized, so as to avoid foaming, in a glass homogenizer with a tight-fitting teflon pestle. Centrifugation at $100000 \times g$ for 30 min yielded a clear slightly yellow supernatant, Fraction Sm, which was ready for fractionation by gel filtration on a Sepharose 6-B column.

Approximately 5 ml of Fraction Sm containing 15–18 mg protein/ml was applied to a Sepharose 6-B column (100 cm $\times$ 2 cm) which had been previously equilibrated with 0.1 M Tris, pH 7.5, and 0.3 mM dodecylsulfate. Collection was made in 6-ml portions at the rate of 12 ml/h, maintained by a proportioning pump. Essentially three fractions were obtained: the initial 30 ml near the exclusion limit, an intermediate fraction of comparable volume, and the last fraction of approx. 50 ml, and containing about one-half of the original protein. This last fraction was passed through a Sephadex G-25 column to remove most of the dodecylsulfate, lyophilized, resuspended in 5 ml of Tris buffer, pH 7.5, and applied to the Sepharose 6-B column. No additional dodecylsulfate was necessary, since the lyophilized preparation still contained sufficient dodecylsulfate to solubilize the protein. Fractionation with the 0.3 mM dodecylsulfate–Tris buffer, pH 7.5 yielded a minor fraction of high molecular

weight (about 20%) and major fraction (about 60–70%), Fraction Pm, in the molecular weight range of bovine albumin (69 000). After passing through a Sephadex G-25 column and dialysis first against running distilled water for 48 h, Fraction Pm was lyophilized and stored at −20 °C. A precipitate which formed after removal of dodecylsulfate by dialysis against 0.5 M NaCl was combined with the supernatant prior to lyophilization. An analysis revealed this fraction to consist of 98% protein, 1.2% lipid and 0.4% polysaccharide (mostly neuraminic acid) by weight. Described elsewhere are other properties of the protein: behavior in acrylamide gel electrophoresis, molecular weight, and amino acid composition, including end group analysis and peptide composition [3].

Measurement of $^{45}Ca^{2+}$ adsorption and exchange in protein films

The adsorption of Ca^{2+} to surface films of proteins was measured by the radiotracer technique [7]. Measurements were carried out in teflon planchets (2.0 cm diameter, 0.5 cm deep) containing a final volume of 1.2 ml and radioactivity determined by means of a scaler and Geiger–Müller tube (mica end window, 1.0 mg/cm^2) mounted 0.5 cm from the surface of the fluid. After radioactivity was determined in a solution containing 0.01 μCi $^{45}CaCl_2$ in 10^{-6} M $CaCl_2$, pH 8.0, 4 μl of the protein solution was applied carefully to the surface and the time course of radioactivity measured. The protein solution was prepared by homogenizing the lyophilized Fraction Pm in 1 mM Tris, pH 7.5, and adding enough 0.1 mM dodecylsulfate to bring to a final concentration of 0.002 mM dodecylsulfate. Radioactivity was measured in 1–2-min intervals until equilibrium was attained (usually within 20 min). At this point 30 μl of either an electrolyte solution adjusted to pH 8.0 or buffer was injected slowly into the subsolution and the sample was again counted. The pH of all solutions was adjusted with Tris, the final concentration of Tris being about 1 mM. By using a Langmuir trough (teflon) and obtaining surface pressure–area curves for Fraction Pm [8], it could be determined that the surface pressure within the planchet was about 20 dynes/cm corresponding to a surface area of 0.6 m^2/mg protein. The small amount of dodecylsulfate used to solubilize the protein did not by itself exhibit detectable Ca^{2+} adsorption; similar results being obtainable when the protein was solubilized in non-polar detergents. If kept at 5 °C, the protein solution was good for at least 3 days. The amount of radioisotopes adsorbed to the monolayer, Γ, was calculated according to the expression [7]

$$\Gamma = (I - I_b)/S \cdot A$$

where I=final radioactivity in cpm, I_b=cpm before protein was applied, S=specific activity of $^{45}Ca^{2+}$, A=surface area.

Displacement of the adsorbed Ca^{2+} by various metal ions was measured as follows. After the radioactivity from the adsorbed Ca^{2+} had attained equilibrium (usually 15–20 min) 30 μl of the various electrolyte solutions were carefully injected into the subsolution using a microsyringe with a 30-gauge needle. The decrease in radioactivity was measured until equilibrium adsorption was reached; the times varying from 10–30 min.

Surface potential

Surface potential measurements were carried out as described elsewhere [4] utilizing the technique of Yamins and Zisman [9].

RESULTS AND DISCUSSIONS

The following discussion is divided into two major parts: the first dealing with the kinetics of Ca^{2+} adsorption to the protein film, and the second to the kinetics of displacement of bound Ca^{2+} by various inorganic and organic cations. Finally the results are discussed in terms of the physicochemical and structural features of the interfacial protein in relation to adsorption and displacement.

Adsorption of Ca^{2+} to the protein film

By taking into account the diffusional process and presupposing an energy barrier to adsorption, a theoretical treatment of the adsorption kinetics of a substance adsorbing at an interface yielded the expression

$$k_0 = \frac{k}{\Gamma_\infty} = \frac{2}{d}\left(\frac{D}{\pi}\right)^{\frac{1}{2}} e^{-E/RT} \tag{1}$$

where k_0 is the rate constant of adsorption, k, the adsorption rate, Γ_∞, the thickness of the adsorbed layer, D, the diffusion coefficient in the bulk solution and E, the energy barrier to adsorption [6]. Eqn 1 was shown to be applicable to the adsorption of Ca^{2+} to a stearic acid monolayer; and was generally valid for ions interacting at a charged interface [5]. A kinetic analysis of the adsorption of Ca^{2+} to stearic acid revealed that the value for E was the same regardless of the presence of excess NaCl in the bulk solution. The adsorption followed a rate law given in integrated form by Eqn 2.

$$\int_0^t (I - I_b)dt = \tfrac{2}{3}k(t^{\frac{3}{2}} - t_0^{\frac{3}{2}}) = J - J_b \tag{2}$$

A plot of Ca^{2+} adsorption to the protein vs $t^{3/2}$ yielded a series of straight lines for various concentrations of Ca^{2+} (Fig. 1). As was found in the case of stearic acid [5], by utilizing the data in Fig. 1 and plotting k against the increased radioactivity at equilibrium for each concentration of Ca^{2+} a linear relationship was obtained; the

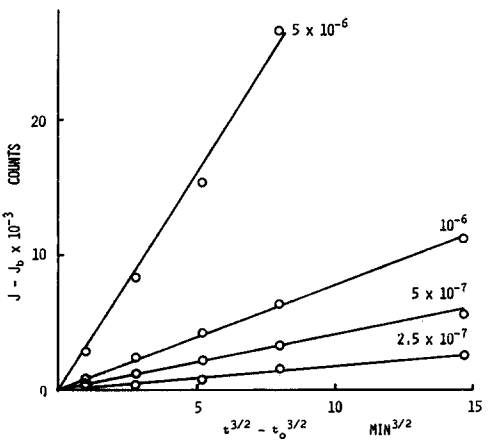

Fig. 1. Kinetics of Ca^{2+} adsorption to Fraction Pm. Counting began approx. 6 s after the application of the film as described in the Methods. The various Ca^{2+} concentrations are noted on the figure. The data was plotted according to Eqn 2.

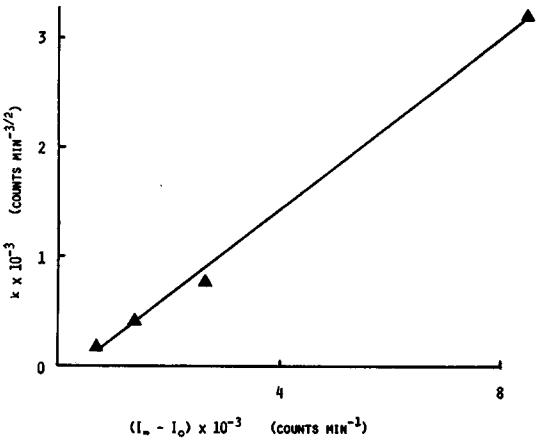

Fig. 2. Relation between the rate of Ca^{2+} adsorption to Fraction Pm, k, and the equilibrium amount of adsorption $(I_\infty - I_0)$. The values of k were determined from the slopes of the lines in Fig. 1. The rate constant k_0 determined from the slope was 0.075 $s^{-1/2}$.

slope, k_0, being equal to 0.07 $s^{-1/2}$ (Fig. 2). The value for stearic acid was 0.02 $s^{-1/2}$, which is 3 times less than the k_0 for the protein. If the assumption is made that the adsorbed Ca^{2+} is surrounded by a monomolecular thickness of water (hydrated diameter = 4.74 Å [5]) and $D = 8 \cdot 10^{-6}$ cm^2/s, from Eqn 1 a slightly lower value for E is obtained for Ca^{2+} adsorption to a protein film; 7 ± 1 kcal/mole as compared to 9.0 for stearic acid. This difference indicates that the energy barrier for Ca^{2+} adsorption to the protein is less than that for stearic acid.

Displacement of Ca^+ by alkali metal cations

The displacement of Ca^+ by two monovalent cations, M^+, from the protein surface can be represented by Eqn 3.

$$P\text{–}Ca + 2M^+ = P\text{–}(M)_2 + Ca^{2+} \tag{3}$$

If two M^+ are involved in the rate-limiting step, the rate of disappearance of Ca^+ from the surface will be given by Eqn 4 where

$$\frac{-d(Ca)_p}{dt} = k_1 \cdot (Ca)_p \cdot (M^+)_s^2 \tag{4}$$

$(Ca)_p$ is the concentration of adsorbed Ca^{2+}, $(M^+)_s$ is the concentration of M^+ near the surface close enough to react, and k_1 is the rate constant for the displacement. Assuming that M^+ is diffusion controlled and therefore proportional to $t^{1/2}$ Eqn 4 becomes

$$\frac{-d(Ca)_p}{dt} = k_1 k_2^{\,2} \cdot (Ca)_p \cdot t \tag{5}$$

where k_2 refers to the rate constant for diffusion. Integration of Eqn 5 yields Eqn 6

$$-\log \phi = k' t^2 \tag{6}$$

where k' is the observed rate and ϕ is the fraction of initial Ca^+ adsorbed at time t. The observed rate is related to the rate constants for diffusion and displacement by Eqn 7.

$$k' = \frac{k_1 k_2^2}{2(2.30)} \tag{7}$$

Alternative rate laws based only on diffusion of 1 or 2 M^+, displacement by only 1 M^+, or neglect of diffusion did not fit the data as well. The relative values of k' were determined from the slopes of $-\log \phi$ vs t^2 plots (e.g. Fig. 4) and are presented in Table I.

TABLE I

RATES OF Ca^+ DISPLACEMENT FROM FRACTION Pm BY VARIOUS M^+

Experimental conditions were those as described in Methods. Final bulk concentrations of M^+ were 0.1 M.

Ion	$k' \times 10^3$ (min^{-2})
Li^+	1.56
Na^+	2.90
K^+	5.41
Rb^+	2.62
Cs^+	4.26
NH_4^+	5.40
Tetraethylammonium cation	4.64
$N_2H_5^+$	3.51

Qualitatively similar differences in the rates of displacement of Ca^{2+} from stearic acid [10] and phospholipid [11] monolayers have been reported; however, a kinetic treatment of the data has been lacking.

Simple diffusion theory [12] predicts that k_2 is related to the diffusion coefficient, D, by Eqn 8

$$\frac{\Gamma}{t^{\frac{1}{2}}} = k_2 = 2C_0(D/\pi)^{\frac{1}{2}} \tag{8}$$

TABLE II

RELATIVE RATE CONSTANTS FOR Ca^+ DISPLACEMENT FROM FRACTION Pm BY VARIOUS M^+

Ion	$D \times 10^5$ (cm^2/sec) *	k_1
Li^+	1.26	0.422
Na^+	1.48	0.667
K^+	1.84	1.000
Rb^+	1.87	0.477
Cs^+	1.86	0.779
NH_4^+	1.84	1.000

* See ref. 20.

where C_0 denotes the bulk concentration of M^+. Since C_0 is the same for all ions, the relative k_1 can be calculated from Eqn 7 and the diffusion coefficient. The calculated values are given in Table II. The variation in k_1 could reflect differences in the activation enthalpies, ΔH, and entropies ΔS, for the displacement according to the Eyring relation expressed in Eqn 9 [13].

$$k_1 = \frac{kT}{h} e^{-\Delta H/RT} . e^{\Delta S/R} \tag{9}$$

where k and h refer to Boltzmann's and Planck's constants, respectively, T denotes absolute temperature and R is the gas constant.

There is a correlation between the relative k_1 calculated according to Equation 7 and the equilibrium amount of displacement $(1-\phi_\infty)$, where ϕ_∞ refers to the value of ϕ at equilibrium. A comparison of the data yields the empirical relation

$$(1-\phi_\infty)^2 \propto k_1 \tag{10}$$

demonstrated by Fig. 3. This relationship could mean that the same factors that control the activation parameters for the displacement reaction also influence the equilibrium displacement. Rb^+ appears to be somewhat exceptional in this regard.

The observed rate, k', is expected to vary in a complex manner with changes in the bulk concentration, C_0, of M^+. The theory of adsorption to an interface from the bulk [6] predicts direct proportionality between the diffusional rate constant and the bulk concentration according to a variant of Eqn 8. In addition both D and the various activity coefficients would be influenced by ionic strength. The surface properties of the protein are expected to change with ionic strength, e.g. the surface potential would be reduced at high ionic strength. A reduction in the bulk concentration of Na^+ by one-half decreased the observed k' by approximately a factor of three. Fig. 4 demonstrates the applicability of the rate law (Eqn 6) at both bulk concentration of Na^+.

The rates of displacement by NH_4^+ and $N_2H_5^+$ are of special interest because

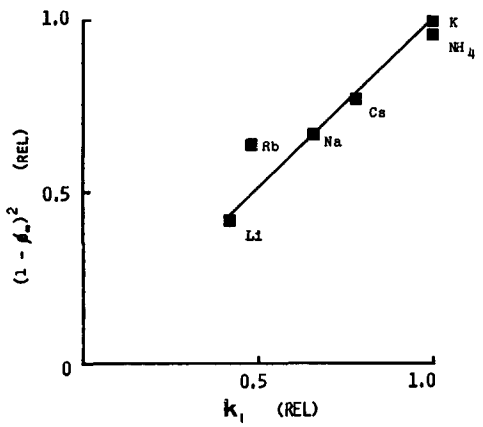

Fig. 3. Relation between the equilibrium displacement of Ca^{2+} from Fraction Pm by M^+ $(1-\phi_\infty)$ and the rate constant for displacement, k_1. The final concentration of M^+ was 0.1 M. The relative values of k_1 were calculated from Eqn 7 and the values of k' given in Table I.

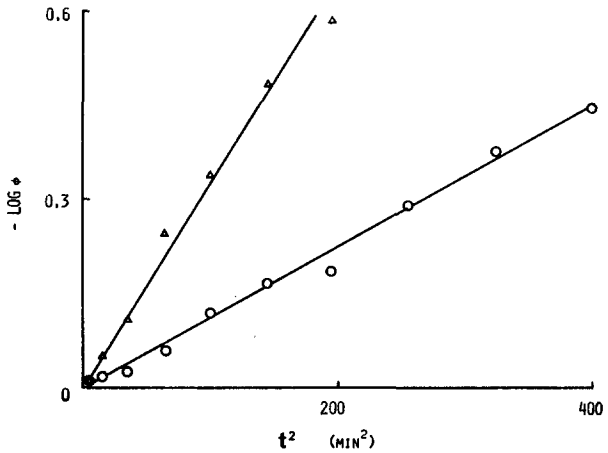

Fig. 4. Effect of bulk Na^+ concentration on the rate of Ca^{2+} displacement from Fraction Pm. Final Na^+ concentrations were 0.1 (△—△) and 0.05 (○—○) M. The data points represent the average of 5 experiments and the standard deviation was generally less than 10% of the mean.

these ions behave like K^+ and Na^+, respectively, with regard to their influence on the electrophysiology of certain nerves [14, 15]. The observed k' for NH_4^+ is identical to that for K^+ and $N_2H_5^+$ is closest to the rate of Na^+.

Displacement of Ca^+ by alkaline earth cations

The alkaline earth divalent cations displace Ca as well as the monovalents at approx. 1/20 the concentration and follow the rate law

$$-\log\phi = k't^{\frac{1}{2}} \tag{11}$$

derived analogously to that for the monovalents where

$$k' = \frac{2k_1k_2}{3(2.30)} \tag{12}$$

Representative data are shown in Figs 5 and 6. This large difference in reactivity between the alkaline earth and alkali metal cations may be the result of the dependence of $k'(M^+)$ on D and of $k'(M^{2+})$ on $D^{1/2}$ since $D^{1/2} \gg D$. Also the entropy of activation ΔS for k_1 should be more favorable for displacement by one ion compared with two.

Within the alkaline earth series the rates and values of equilibrium displacement for the ions follow the order Mg^{2+}, $Sr^{2+} < Ba^{2+}$. The diffusion coefficients and estimated diffusional rate constants are nearly identical for all the ions and differences in k' probably reflect the activation parameters for k_1. Al^{3+} and divalent transition metals, such as Mn^{2+} and Ni^{2+}, had rates comparable to those for the alkaline earth series.

Structural and chemical features of Ca^{2+} sites

In attempting to examine the kinetics of chelating agents, such as ATP, EDTA, and EGTA, on Ca^{2+} desorption it was noted that the agents were almost ineffective at final concentrations of 1 mM (data not shown). There are two possible explanations

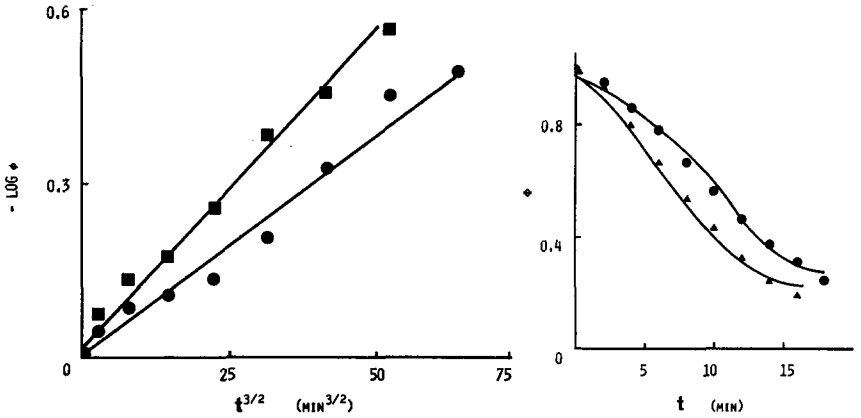

Fig. 5. Kinetic rate plots for Ca^{2+} displacement from Fraction Pm by Ba^{2+} (■—■) and Mg^{2+} (●—●) according to Eqn 11. Data for Sr^{2+} (not shown) was nearly identical to that for Mg^{2+}. Final bulk concentrations of divalent ions were $3 \cdot 10^{-3}$ M.

Fig. 6. Time course of the displacement of Ca^{2+} from Fraction Pm by Mg^{2+} (●—●) and Ba^{2+} (▲—▲). Solid lines represent experimental data and the symbols refer to points calculated from the data in Fig. 5 and Eqn 11.

for this unexpected finding: the vicinity of the bound Ca^{2+} was either too hydrophobic or electrostatically unfavorable because of dipole orientations and an excess of CO_2^- groups. It is conceivable that the region of the Ca^{2+} sites may be hydrophobic, since Ca^{2+} readily interacts with dipoles of medium strength, e.g. alcohols. The fluorescence quantum efficiency of chlorotetracycline complexed with Ca^{2+} in the inner mitochondrial membrane indicated that it is bound to a region of reduced polarity compared with an aqueous environment [16]. There is also evidence to indicate that at alkaline pH the electrical charge distribution within the interfacial protein is not favorable for anionic adsorption. It is known, for example, that ATP binds strongly to the protein at pH 4–5, but not at all at pH 7–8 [4]. Carboxyl groups are likely the primary anionic sites in the protein responsible for cationic binding at pH 7–8; however, Ca^{2+} could be additionally stabilized by the dipoles of carbonyl and hydroxyl substituents of tyrosine and serine residues. These ion–dipole interactions could account for the unexpectedly high reactivity of Mg^{2+}, since it is also well supported in environments of medium dielectric, whereas Sr^{2+} and Ba^{2+} are not.

If monovalent cations penetrate cell membranes by passage through polar regions extending through the membrane interior as suggested by some theories [17], then K^+ should interact better than Na^+ in several ways. In addition to the surface interactions described above, K^+ would bind better electrostatically to fixed carboxyl or phosphate groups by Coulomb's law because of its smaller hydrated radius. It could also more readily exchange an ion–dipole interaction with a water molecule for one with a carboxyl dipole projecting into a hydrophilic region. The net result of these interactions would be a preference for K^+ permeation of a cell membrane since the interactions that lead to translocation are consecutive and thus the rate constant that describes the overall process will be a product of the rate constants for diffusion, displacement of Ca^{2+}, etc.

It is appropriate to consider the possible configuration of the protein at the interface, since steric factors are obviously involved in the differential interactions of the various ions. Traditionally the protein structure at the air–water interface has been assumed to be in the pleated, β-conformation [18]; however, with the use of infrared spectroscopy and optical rotary dispersion, Bair and co-workers [19] have demonstrated that globular proteins still retain their α-helical configuration after interfacial spreading. As already alluded to, electron microscopic studies on surface films of the synaptic protein have also revealed that the globular configuration is retained [3]. It has also been shown that the presence of divalent cations, such as Ca^{2+} and Mg^{2+} contribute to the formation of lamellar arrays of the globular protein [3]; a finding which suggests that intermolecular as well as intramolecular interactions may be involved in Ca^{2+} interaction at the interface.

Specificity of Fraction Pm for Ca^{2+} binding

In an effort to determine whether Fraction Pm possessed any unique properties contributing to ionic interaction a number of other proteins were substituted. Included were bovine albumin, collagen, thyroglobulin, hemoglobin, myosin, myoglobin and a mixture of soluble proteins obtained from calves brains; and only slight $^{45}Ca^{2+}$ adsorption was demonstrable with any of these. Some interaction occurred with proteins extracted from the membranes of brain and muscle mitochondria, endoplasmic reticulum, and axonal membranes. Preliminary findings would suggest that cationic affinity is mainly a property of hydrophobic proteins derived from membranes. It has been shown that Fraction Pm is capable of forming surface micelles and trilamellar structures resembling natural membranes [3]; and it has been inferred that the formation of such structures may be a factor in the ATP-binding exhibited by Fraction Pm [4].

Effect of protein surface charge

Various theories have been proposed for ion selectivity at a charged interface; and the one most pertinent to the present study is that of Eisenman [1]. This theory states that cationic affinity for a fixed anionic site is a function of the difference in the electrostatic free energy of ionic interaction at nearest approach and the free energy loss by dehydration of the cations. Consequently, if the field intensity energy exceeds the hydration energies, the cations with the smallest ionic radius would be preferred; whereas at lower field strengths, the lesser hydrated cation exhibits the greater affinity. With intermediate electrical field strength nine other cationic sequences are possible. The theory is based on data obtained with glass electrodes or ion-exchange resin with oxyanionic sites, and to what extent it is applicable to protein systems is undetermined. Although oxyanions chiefly comprise the anionic sites of protein, they are heterogeneous and capable of charge fluctuations.

The magnitude of the surface charge can be assessed by studying the change in the surface potential, ΔV, of the protein films as a function of pH, which is described by the Eqn 13

$$\Delta V = 4\pi n\mu_0 + \psi_0 \tag{13}$$

where n is the number of net surface dipoles, μ_0 the dipole moment, and ψ_0 refers to the Guoy, or electrostatic potential [8]. At low pH, ΔV is 400 mV, where the CO_2^-

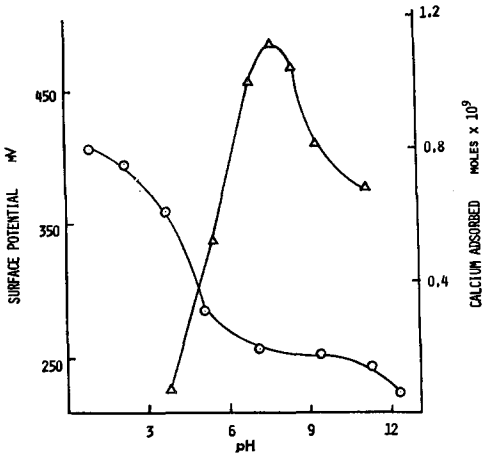

Fig. 7. Variation of surface potential and Ca^{2+} adsorption to Fraction Pm as a function of pH. Surface potential (○) of Fraction Pm was measured at a surface pressure of 20 dynes/cm and is expressed in mV. Ca^{2+} adsorption (△) is expressed in moles $\times 10^9$.

contribution is minimal (Fig. 7). The pH optimum for Ca^{2+} adsorption is about 8.0. The adsorption decreasing precipitously as the pH decreases (Fig. 7). Since the dipole contribution to ΔV is independent of pH [8], the negative electrostatic contribution to the potential can be calculated from the difference in the ΔV at pH 4.5 (the isoelectric point) and 250 mV. This value is about 80 mV. Ca^{2+} adsorption occurs despite the large positive potential by virtue of the relative position of the dipoles and ionic complexes within the interface. By keeping the ionic strength constant, as in the present study, ΔV was kept constant. The primary consideration, therefore, in Ca^{2+} displacement is not the potential but the energy barrier as well as steric factors arising from configurational changes in the protein during cationic exchange.

An important consideration in the present study is the extent to which the functional and biochemical properties of the synaptic protein was modified by treatment with dodecylsulfate. As discussed elsewhere [3] dodecylsulfate does destroy (Na^+,K^+)-activated ATPase but some Mg^{2+}-catalyzed ATPase and 5′-nucleotidase activity remains. The extent of destruction to other enzymes systems of synaptic membranes is not known, but they undoubtedly are affected by the treatment. Furthermore, structural alterations of the protein have resulted which could modify its normal functional and binding characteristics. On the other hand, the protein retains the ability to reform vesicular and lamellar structures resembling those of natural membranes and to bind ATP and other substances normally associated with synaptic membranes [3,4]. Nevertheless, the biological significance of the present findings will depend on similar experiments involving the use of reasonably intact synaptic membranes.

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