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ELECTRICAL EXCITABILITY OF ARTIFICIAL ENZYME MEMBRANES

I. ION-EXCHANGE PROPERTIES OF SYNTHETIC PROTEINIC FILMS

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This paper deals with the physico-chemical properties of artificial membranes. The membranes are produced with different protein molecules which offer amphoteric sites with weakly ionizable groups. The adsorption of phosphate and sodium ions in different artificial proteinic membranes is studied as a function of both pH and concentration of the external solution. The influence of the sign and density of fixed charges as the nature and concentration of mobile ions is studied by measuring the potential difference between both membrane compartments.

1. Introduction

Ion movement across membranes has been described by numerous models. Membrane charges, especially, have been taken into account, either fixed as in the model of Teorell [1], or mobile as in that of Rosenberg and Wilbrandt [2]. The study of membranes considered as ion exchangers was put forward by Teorell, Helfferich and others.

The incorporation of enzyme molecules into an ion-exchange matrix results in new effects and controls on ion movement which have been little studied. Blumenthal et al. [3] used papain sandwiched between two ion-exchange sheets for this purpose. Naparstek et al. [4] bound the enzyme in the matrix of the membrane itself by a co-cross-linking method using serum albumin as a support [5].

By taking into account theoretical ion-exchange properties of artificial albumin membranes, Zabusky and Deem [6] and Ree Chay [7] proposed a model dealing with proton diffusion and periodic behaviour of albumin/papain membranes.

The present paper is devoted to experimental results dealing with ion-exchange properties of artificial albumin and gelatin membranes. Their influence on membrane potential is studied.

2. Materials and methods

2.1. Membrane production

Proteinic films were produced by a previously described cross-linking method [8]. The films were prepared on a flat glass surface.

2.1.1. Albumin membranes

A cross-linking reaction was carried out for a period of 2 h in a solution containing 40 mg/ml bovine serum albumin and 4 mg/ml glutaraldehyde in 20 mM phosphate buffer at pH 6.8. The solution was spread onto a glass surface. After drying in a current of air, a 40 cm^2 membrane was obtained.

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2.1.2. Gelatin membranes

A solution of 50 mg/ml ossein gelatin or pig skin gelatin in 20 mM phosphate buffer at pH 6.8 was spread onto a flat glass surface. After drying for 3 h in a current of air, a film was obtained. The film was then cross-linked with 10 mg/ml glutaraldehyde solution in 20 mM phosphate buffer at pH 6.8 for 3 min.

2.2. Measurement of membranal ion concentrations

The membranal ion concentrations were meaured using an isotope-exchange method. Disks of 2.2 cm diameter were cut out from the proteinic films. The disks were suspended in phosphate buffer solutions labelled with $H_2{}^{32}PO_4^-$, $^{24}Na^+$ for 24 h at 25°C, surface dried between filter paper, and then counted by liquid scintillation techniques.

2.3. Measurement of potential difference

If there is a salt concentration difference between both sides of an ion-exchange membrane, a potential gradient between the solutions occurs without any external electrical stimulation. The membrane was placed in a diffusion cell where it separated two 25-ml compartments. The diffusion surface area was 0.5 cm^2. The solutions in both compartments were continuously agitated. The pH values were regulated on each side of the cell with pH stats.

The potential differences were measured using a vibrating-reed electrometer (Cary 401) with calomel reference electrodes.

3. Experimental results

The artificial membranes are produced with three different proteins; bovine serum albumin, considered to be an acid protein (isoelectric point close to 5), ossein gelatin which has also an isoelectric point close to 5 and pig skin gelatin whose isoelectric point is approx. 7.5.

3.1. Membranal ion concentrations

The artificial membranes can be considered as ion exchangers. In equilibrium with an external solution, the distribution of ions between the membrane and the external solution might be governed by a Donnan equilibrium operating at the interface between the phases.

Disks were equilibrated at 25°C for 24 h in phosphate buffer solutions. During equilibration, the vessel was shaken gently and the pH kept constant.

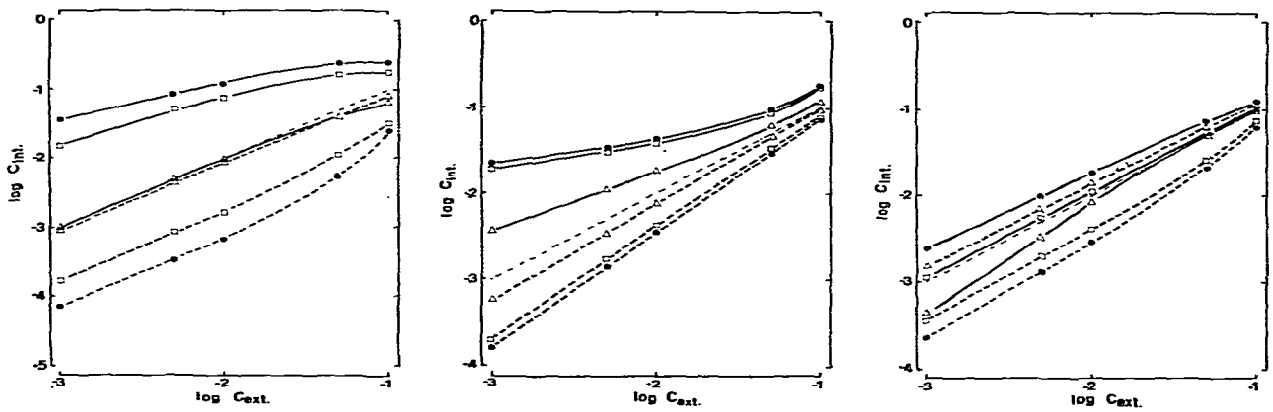

Fig. 1. Membrane ion concentrations as a function of the external concentration of Na^+ (unbroken lines) and $H_2PO_4^-$ (dashed lines) at pH 5 (△), 7 (□) and 9 (●). The theoretical slope for uncharged membrane is shown as (·—·—·). The inactive protein used was bovine serum albumin (a), ossein gelatin (b) and pig skin gelatin (c).

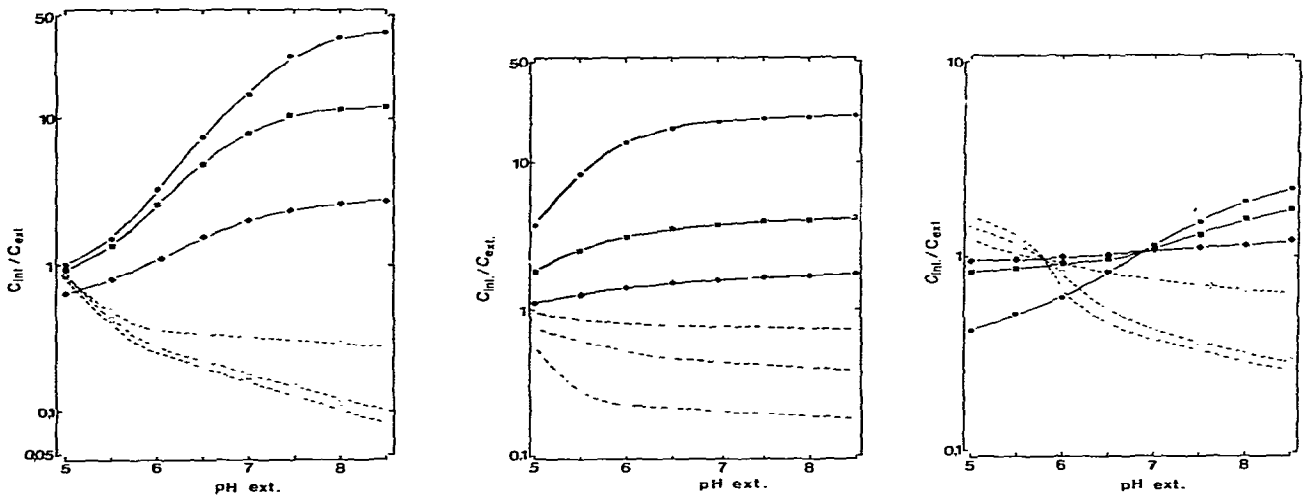

Fig. 2. Membrane ion concentration as a funtion of buffer solution pH for external concentration of Na^+ of 10^{-3} M (●), 10^{-2} M (■), 10^{-1} M (◆) and of $H_2PO_4^-$ of 10^{-3} M (○), 10^{-2} M (□) and 10^{-1} M (◇). The protein used was bovine serum albumin (a), ossein gelatin (b) and pig skin gelatin (c).

Fig. 1 shows the results obtained for the intramembranal concentrations in Na^+ and $H_2PO_4^-$ as a function of the external concentration at different pH values.

Bovine serum albumin and ossein gelatin membranes exhibit cation-exchange properties for pH values higher than 5. Pig skin gelatin membranes show anion-exchange properties for pH values lower than 7. In all cases, the lower the external concentration, the higher the Donnan exclusion. These results agree with the Donnan exclusion theory. The proteinaceous membranes offer amphoteric sites with weakly ionizable groups. We have studied the influence of external pH on internal ion concentration. Fig. 2 shows a plot of internal concentration divided by external concentration vs. external pH. With the three kinds of membranes, the counterion absorption, like the co-ion exclusion, increases with the fixed-charge density.

3.2. Membrane potential

Fixed-charge density is one of the leading factors affecting co-ion exclusion and, hence, mem-

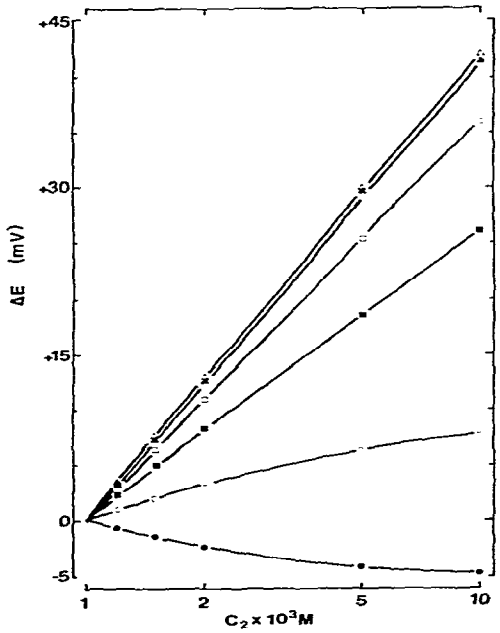

Fig. 3. Steady potential difference vs. salt concentration in compartment 2. The concentration in compartment 1 is constant and equal to 10^{-3} M for pH 5 (●), 5.5 (○), 6 (■), 6.5 (□), 7 (▲) and 8 (△). Bovine serum albumin membrane.

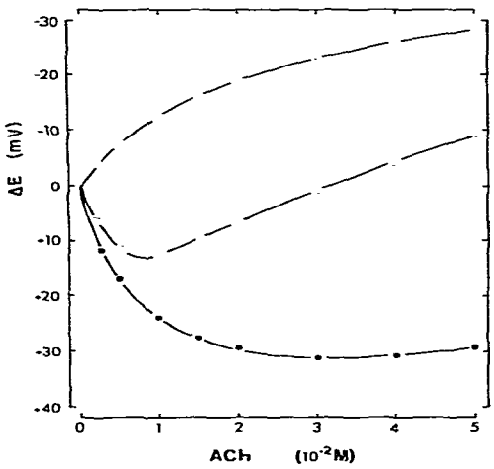

Fig. 4. Steady potential difference as a function of acetylcholine chloride (ACH) concentration injected into one compartment for bovine serum albumin (●), ossein gelatin (□) and pig skin gelatin (○) membranes.

brane potential. With artificial proteinaceous membranes, it is possible to increase or decrease the fixed charge density by varying the external pH.

Measurement of potential was performed with bovine serum albumin membrane and sodium phosphate buffer solutions at different pH values: membrane thickness, 5×10^{-3} cm; concentration of phosphate buffer in the first compartment, $C_1 = 10^{-3}$ M; concentration in the second compartment varied from $C_2 = 10^{-3}$ to 10^{-2} M.

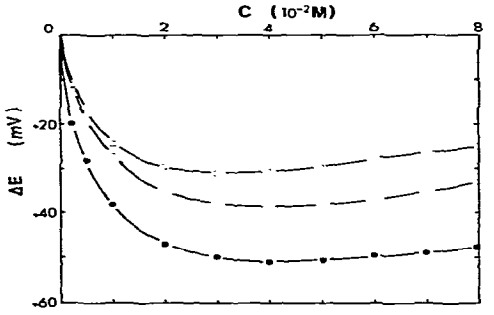

Fig. 5. Steady potential difference as a function of salt concentration injected onto one side of a bovine serum albumin membrane at pH 7.5. Injected salts were acetylcholine chloride (□), acetylcholine iodide (○) and NaCl (●).

Steady potentials are plotted in fig. 3 as a function of C_2 for pH 5–8.

As the external pH value is increased above 5, the sign of the potential difference changes.

The measured potential difference depends not only on bulk concentrations, but also on the fixed-charge sign and density. At pH 7.5, bovine serum albumin and ossein gelatin membranes are negatively charged. For pig skin gelatin membranes whose isoelectric pH is approx. 7.5, there is neutralization between $-COO^-$ and $-NH_3^+$ charges.

When bovine serum albumin, ossein gelatin or pig skin gelatin membranes separate two compartments containing identical 10^{-3} M phosphate buffer solutions at pH 7.5, it is possible, by injecting a salt only into one compartment, to measure a potential difference. Results are shown in fig. 4 as a function of acetylcholine chloride concentration injected into one side. Measured potentials for lower acetylcholine concentrations are of opposite signs for pig skin gelatin membrane compared to bovine serum albumin or ossein gelatin membranes. As salt concentration increases, the sign of the ossein gelatin membrane potential changes, recalling the phenomenon described by Teorell [1] as 'concentration effect'.

The steady membrane potential is also a function of the nature of the ionic species injected onto one side of the membrane.

Fig. 5 shows the potential difference measured across bovine serum albumin membranes in phosphate buffer at pH 7.5 as a function of salt concentrations on one side of the membrane. The difference between the curves can be easily interpreted by anion and cation mobility differences for each salt. The mobility difference between Na^+ and Cl^- is larger than that between ACh^+ and Cl^- due to the difference between the two cations. For ACh^+, Cl^- and ACh^+, I^- the difference between the two anions can explain the difference in behaviour.

4. Discussion

The artificial membranes consist of cross-linked proteins which offer amphoteric sites with weakly

ionizable groups. Within these membranes are distributed sufficient cations and anions to maintain electroneutrality. In equilibrium with an external solution, the distribution of the solute between the membrane and the external solution is governed by a Donnan equilibrium operating at the interface between the phases [9]: the higher the external salt concentration, the lower the co-ion exclusion; the higher the fixed-charge density, the greater the Donnan exclusion.

It is interesting to note that the artificial proteinaceous membranes are a useful aid in studying the effect of changing the fixed-charge density. The nature of the protein used is also important, since at pH 9, at the same ion external concentration, the internal/external Na^+ concentration ratio is 37, 22 and 2.5 for bovine serum albumin, ossein gelatin and pig skin gelatin membranes, respectively.

The data obtained for steady membrane potentials can be analysed by separating the major factors: the variation of ΔE is a function: (i) of the salt concentration gradient; (ii) of the fixed-charge density, and hence of the nature of the protein, and of the pH of the external solutions; (iii) of the nature of mobile ions.

5. Conclusion

The binding of an enzyme molecule into an ion-exchange matrix allows straightforward modelling when studying the reciprocal interaction between enzyme reaction and a polyelectrolyte environment [10].

A systematic analysis of the factors affecting ion movement across an enzyme membrane requires the integration of both fixed charges and ionic species resulting from the enzymatic reaction.

This paper deals with the physico-chemical properties of artificial membranes. The membranes are produced with protein molecules which offer amphoteric sites with weakly ionizable groups. The effect of permanent membrane charges has been studied by the adsorption of phosphate and sodium in different artificial proteic membranes. By modifying the pH of the solution, which modifies the fixed-charge sign and density, the ion adsorption has been studied. Qualitatively, the data obtained are in good agreement with the Donnan exclusion theory.

The membrane properties were also studied by measurement of the potential difference between both membrane compartments. The influence of the sign and density of fixed charges, and the influence of the nature and concentration of mobile ions can be studied. In all cases, artificial protein membranes behave as weak ion exchangers with variable fixed-charge density.

The next step of the work is the introduction, into an artificial protein membrane, of an enzyme activity affecting local ion concentrations and fixed-charge density.

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