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ELECTRICAL PROPERTIES OF SQUID AXON MEMBRANE

II. EFFECT OF PARTIAL DEGRADATION BY PHOSPHOLIPASE A AND PRONASE ON ELECTRICAL CHARACTERISTICS

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SUMMARY

Passive electrical characteristics of perfused squid axon membrane are investigated. In a previous publication, we reported that the capacitance of intact squid axon membrane is partly frequency dependent. We extended the same measurement to perfused axons. We found that the electrical characteristics of perfused axon membrane are essentially the same as those of intact axons. In this work, we investigated the effects of phospholipase A and pronase on the membrane capacitance. Phospholipase A is known to block the sodium activation and pronase to eliminate the sodium inactivation. Phospholipase A is found to increase the frequency dependent as well as the frequency independent capacitances. Our tentative conclusion is that this enzyme perturbs the lipid structure and decreases its thickness. Pronase is found to increase the frequency dependent capacitance slightly while the capacitance of the lipid layer remains unaltered. Although voltage clamp data indicate that the pronase disrupts the excitatory mechanism extensively, this enzyme has relatively little effect on the overall membrane capacitance.

INTRODUCTION

In the previous publication, Takashima and Schwan [1] reported that the membrane capacitance and conductance of squid axon are partly frequency dependent between 100 Hz and 50 kHz. They attribute the frequency independent part of the membrane capacitance to lipid molecules and the frequency dependent part to some other polar molecules. In the classical treatments, membrane capacity has been treated as frequency independent and has been considered as a quantity which represents the inactive bulk of biological membranes [2]. The presence of a frequency-dependent

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capacitance in excitable membranes indicate that biological membranes are not necessarily a rigid dielectric wall but are more like a liquid crystalline state. Hence, molecules in biological membranes are likely to have a certain degree of rotational freedom. If an electrical field is applied, polar molecules will respond to the field and undergo a restricted orientational polarization. The orientational polarizability of polar molecules in a highly viscous membrane manifests itself as a dispersion of capacity in a low frequency region [3].

At present, two types of molecular motions can be considered for the possible origin of the frequency dependent capacitance. The one is the bending motion of polar heads of phospholipids [4] and the other, rotation of membrane proteins [5]. The bending of side chains of large molecules usually have a characteristic frequency in microwave regions. The relaxation frequency of the dispersion of axon membrane capacitance is approximately 10 kHz and is definitely too low for this mechanism. On the other hand, the characteristic frequency of spherical or ellipsoidal protein molecules in aqueous solutions have a relaxation frequency between 100 kHz and 1 mHz [6]. The difference between these values and the observed relaxation frequency can, however, be resolved by the following consideration. The relaxation frequency of dipolar rotation is inversely proportional to the viscosity of the medium [3]. The viscosity of aqueous solution is usually of the order of 1 centipoise and if one assumes the viscosity of membranes to be 10–50 times higher than that of water, the characteristic frequency of protein molecules will be shifted to 10 kHz region. Although there may be yet other sources for the frequency dependent membrane capacity, the molecular orientation of membrane proteins may be the simplest explanation of the observed results. Although we are still far from the conclusive evidence to support this hypothesis, this seems to be one of the most probable explanations among other mechanisms.

The present work is a continuation of the previous one and we investigated, by use of certain enzymes, the effect of perturbing the lipid and protein parts on the membrane electrical characteristics. We used phospholipase A to disrupt the lipid bilayer and used pronase to perturb the protein part. The effects of these enzymes on the excitability of axon membranes have been studied by Abbot et al. [7] and by Armstrong et al. [8]. According to these authors, phospholipase A is known to destroy the sodium influx and pronase eliminates the sodium inactivation. The possible effects of these enzymes on the membrane capacitance and conductance can be interpreted in view of these previous results.

EXPERIMENTS

Giant axons of squid *Loligo pealeii* were used for all experiments. The giant axons were dissected for the length of 4–5 cm and mounted in an axon chamber. Thermostated artificial sea water was circulated and the temperature was 5–6 °C.

The method of internal perfusion is described by Tasaki [9]. Two canullae with diameters 300 μm and 100–120 μm were used, the larger canulla for the outlet and the smaller one for inlet of perfusion solution. The perfusion solution was a mixture of 0.6 M KF with 12 % of glycerol with pH adjusted to 7.3. Pronase samples were from Sigma Chemical Company and used at the concentration of 0.05 mg/ml to 0.15 mg/ml depending on the purpose of experiments. Phospholipase A was pre-

pared from lyophilized *Pyltron Piscivoras* venom purchased from Sigma and dissolved in the perfusion solution and the final concentration of the enzyme is 1 mg/ml.

Electrodes for impedance measurements and for voltage clamp experiments were inserted through the larger canulla. This arrangement enables us to continue the flow of either enzyme and/or perfusion solutions during the measurements. However, this arrangement requires a small electrode for voltage clamp experiments. In order to reduce the size of the electrode assembly, the voltage electrode, which is normally enclosed in a glass capillary is replaced by a Teflon-coated 25 μm Pt · Ir wire. The Teflon-coated wire was mounted on the current electrode, a bare 75 μm Pt · Ir wire. The current electrode was also used for the measurements of membrane capacitance and conductance. The current electrode was coated with platinum black by the method described by Moore [10]. The membrane admittance was measured by a Wayne-Kerr B 221 admittance bridge for a frequency range from 100 Hz to 50 kHz. The measured capacitance and conductance were corrected for the series resistance due to axoplasm, Schwann cell layers and sea water. The value of series resistance was determined by the method already described in the previous paper [1]. The value of series resistance is of the order of 5–7 ohms cm^2. The composition of artificial sea water was a mixture of 1 M solutions with the following proportion: 423 ml of NaCl, 9 ml of KCl, 9.27 ml of $CaCl_2$, 22.94 ml of $MgCl_2$, 25.5 ml of $MgSO_4$ and pH was adjusted with Tris buffer to 8.0.

RESULTS

As discussed in the previous paper, measured capacitance and conductance of membranes immersed in an electrolyte solution do not necessarily represent the real membrane admittance. The measured capacitance and conductance actually are functions of series resistance and frequency in addition to the membrane capacitance and conductance as shown below.

$$C = \frac{C_m R_m^2/(R_0+R_m)^2}{1+\left[\frac{\omega C_m R_m R_0}{R_0+R_m}\right]^2} \approx \frac{C_m}{1+(\omega C_m R_0)^2} \qquad (R_m \gg R_0) \quad (1)$$

$$G = \frac{1/(R_0+R_m)+\omega^2 C_m^2 R_m^2 R_0/(R_0+R_m)^2}{1+\left[\frac{\omega C_m R_m R_0}{R_0+R_m}\right]^2} \approx \frac{1/R_m+\omega^2 C_m^2 R_0}{1+(\omega C_m R_0)^2} \qquad (R_m \gg R_0) \quad (2)$$

where C_m and R_m are membrane capacity and resistivity, R_0 series resistance and $\omega = 2\pi f$ (f, frequency). Therefore, the true membrane capacitance and conductance must be calculated by use of Eqns 1 and 2 and the value of series resistance R_0. The values of series resistance was determined using the impedance loci. The details of this procedure are given in the previous publication [1].

Fig. 1 illustrates the measured capacitance and calculated membrane capacitance of an internally perfused axon.

The frequency profile of perfused axons is essentially the same as intact axons and is characterized by a frequency dependent inductive and capacitive admittances. The decrease of the membrane capacitance at low frequencies indicates the presence of a large inductive component. Likewise, the decrease of membrane capacity from

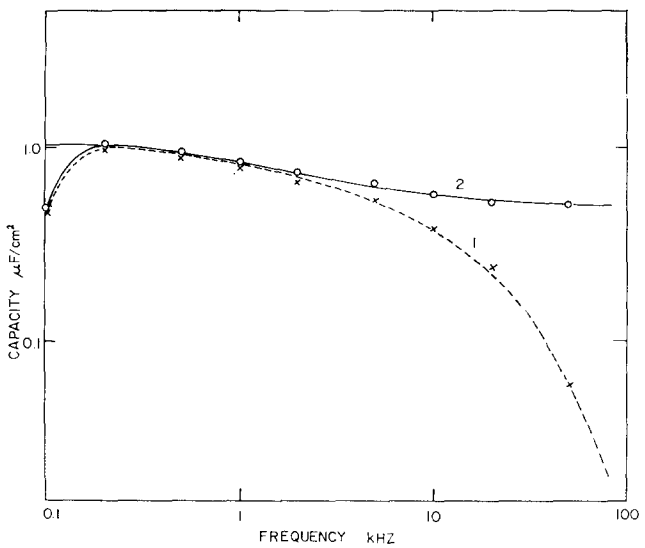

Fig. 1. Frequency profile of membrane capacity of perfused axon. Curve 1, measured capacity before correction for series resistance and curve 2, after correction. Temperature, 5 °C.

1 μF/cm^2 to 0.6 μF/cm^2 above 1 kHz indicates that the membrane capacity is partially frequency dependent [1]. In order to demonstrate this more clearly, the Cole-Cole plot [11] of the same membrane is shown in Fig. 2. In this plot, C' is the membrane capacitance and C'' is calculated by the following equation:

$$C'' = (G - G_0)/2\pi f \tag{3}$$

where G_0 is the low frequency conductance and G is the conductance at a given frequency. As shown in this figure, the intersections of the arc with the abscissa indicate the low and high frequency limiting values of the capacitance dispersion. According to Fig. 2, the intersections are found at 1.05 μF/cm^2 and 0.5 μF/cm^2,

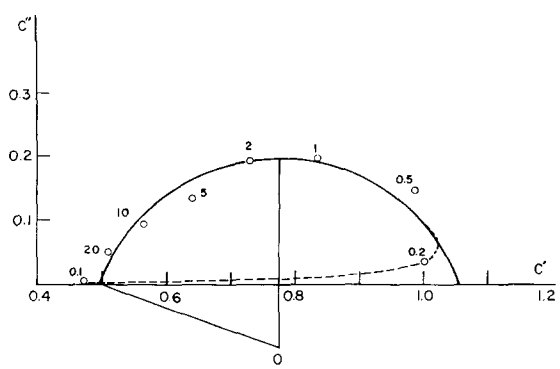

Fig. 2. Cole-Cole plot of perfused axon. The calculation is based on the data shown in Fig. 1. The abscissa is the real part and the ordinate, the imaginary part.

which are designated as C_0 and C_∞, respectively. The high frequency capacity (C_∞) 0.5–0.6 μF/cm^2 is very close to the membrane capacity of artificial bilayer membranes, i.e., 0.4–0.7 μF/cm^2.

Fig. 3 illustrates the permittivity of axon membrane superimposed with that of oxidizid cholesterol membrane [12]. It is well known that lipid bilayer membranes have a frequency-independent membrane capacitance [12–14]. As shown in this figure, the permittivity of oxidized cholesterol membrane is uniformly 4.6 in this frequency range while the permittivity of axon membrane varies from 8.2 to 4.2. It must be pointed out that the high frequency permittivity of axon membrane is indeed very close to that of oxidized cholesterol membrane. The capacity of phospholipid bilayers, on the other hand, is about 0.45 μF/cm^2 [15]. This value gives rise to a permittivity about 4.3 which is even closer to C_∞ of squid axon membrane. These observations suggest that the high frequency limiting capacity C_∞ is due to lipid molecules. The next logical step is to identify these polar molecules which manifest themselves as the frequency dependent permittivity.

The approach used in this experiment is to use certain enzymes to perturb the lipid part and protein part separately and study the effect of these perturbations on the electrical characteristics of the membranes. In this study, two enzymes are used, the one: phospholipase A and the other: pronase. Phospholipase A is an enzyme which converts lecithin to lysolecithin and pronase is a mixture of proteolytic enzymes. It is well known that these enzymes do not attack the membrane structure externally and therefore, they must be used internally.

a) Phospholipase A

During the internal perfusion of axons with phospholipase A solution, the

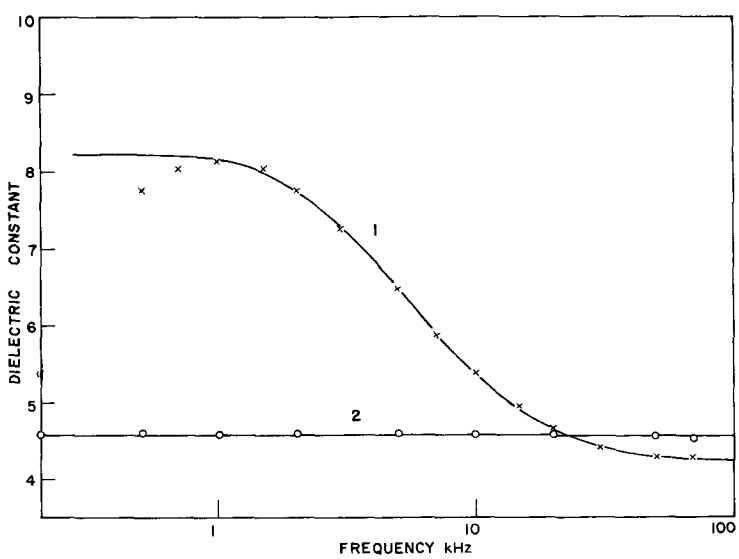

Fig. 3. Permittivities of squid axon and oxidized cholesterol membranes. Curve 1, squid axon and curve 2, oxidized cholesterol. The thickness of both membranes was assumed to be 80 Å.

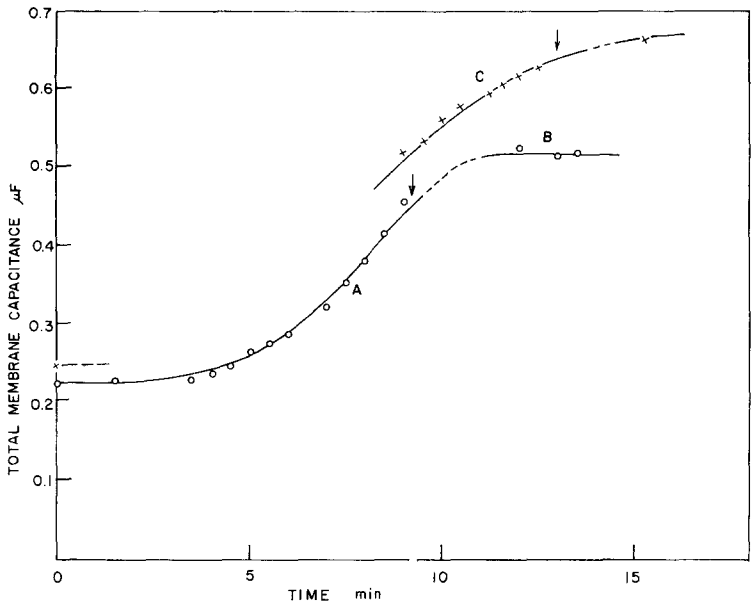

Fig. 4. The time course of the change in the membrane capacitance during the treatment with phospholipase A at the concentration of 1 mg/ml. The arrows indicate the removal of enzyme solution. Curve C was obtained with another axon.

action potential was continuously monitored and the enzyme digestion of the membrane was terminated when the height of action potential was reduced to about 70–80 mV. The duration of action potential is somewhat prolonged during the enzyme digestion from 1 to 1.6 ms. The change in the membrane capacitance is intermittently measured at 500 Hz where the membrane capacity is relatively independent of frequency and 1 $\mu F/cm^2$ before the enzyme digestion of the membrane. Fig. 4 shows the change in the membrane capacitance at 500 Hz as a function of time.

After a certain lag period, the membrane capacitance begins to increase as the decomposition of the membrane progresses. The capacitance reaches a higher limiting value asymptotically, i.e., 2.5–3.5 $\mu F/cm^2$. It is interesting to find that there is an upper limiting value and if the enzyme digestion is continued beyond this limit, the action potential deteriorates quickly and disappears. It may be significant that the transition from the lower to upper limiting membrane capacitance is smooth and continuous. In no case did we observe a sudden transition indicating cooperative type phase change of the membrane structure. The three points shown after the arrow were obtained after the enzyme solution was replaced by an ordinary perfusion. The enzyme degradation of membrane structure can be arrested by this replacement and the viability of the membrane can be maintained for a sufficiently long period of time.

Using these partially degraded membranes, the frequency profile of membrane capacity and conductivity were investigated. Fig. 5 shows an example of successful experiments.

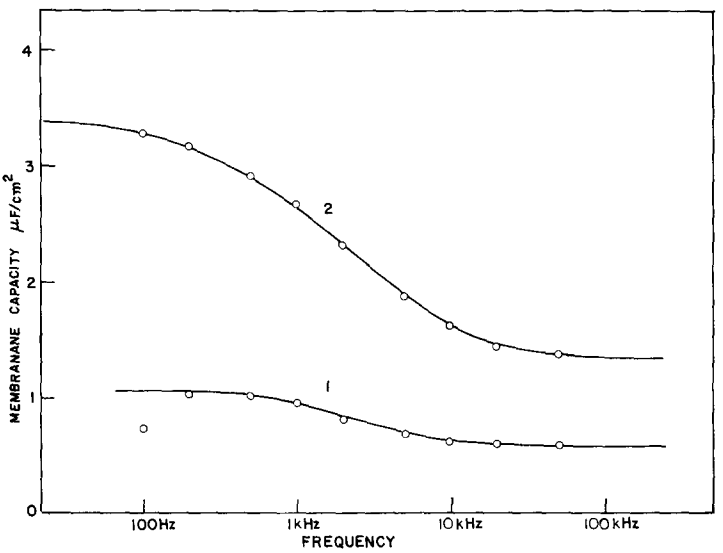

Fig. 5. Frequency profile of the membrane capacity of a phospholipase A treated axon. Curve 1, before enzyme treatment and curve 2, after the treatment. Both curves after the correction for series resistance. The sample is the same as curve C in Fig. 4.

A quick examination of these curves reveals two features. The one is the elevation of the high frequency limiting value (C_∞) and the other is the increase in the amplitude of the frequency dependent membrane capacity (C_0–C_∞). It is interesting to note that the increase in the high frequency capacitance is almost consistently two-fold after a prolonged but carefully controlled enzyme digestion. If the enzyme degradation was continued beyond this limit, the membrane apparently disintegrates with a sudden decrease in the membrane capacitance and an abrupt increase in the membrane conductance. Also, action potential deteriorates quickly and disappears at the same time.

The increase in C_∞ can be interpreted as the decrease in the average membrane thickness. It is also possible that because of the perturbation of the lipid part of the membrane and possible disruption of the interaction between proteins and lipids, the water content in the membrane has increased, hence the increase in the permittivity of the membrane. Although the second interpretation cannot be totally eliminated, it alone does not explain the consistent two-fold increase in C_∞ and also does not explain the presence of an upper limiting value of the membrane capacity.

The other aspect of these results is the increase in the frequency dependent membrane capacitance. Namely, the frequency dependent capacity (C_0–C_∞) of normal membrane is about 0.4–0.5 μF/cm^2. This value increases to 2–3 μF/cm^2 after phospholipase degradation. Moreover, the characteristic frequency (f_c) of the capacitance dispersion shifts toward a lower frequency region. This is a clear indication that the degradation of lipid molecules by this enzyme gives rise to a new frequency dependent capacitance. This is either due to an increase in the ro-

tational freedom of polar molecules or an increase in fixed charges which give rise to an α-dispersion.

A final comment about the results shown above is the behavior of the inductive reactance [16, 17]. As shown by curve 1 in Fig. 5, the inductive component manifests itself as a decrease in the membrane capacitance at low frequencies. After degradation of lipids, as shown by curve 2 in Fig. 5, the inductive component disappears nearly completely. This behavior should be compared to the inductive behavior of pronase degradated membrane (Fig. 6, vide infra).

b) Pronase Treatment

The other enzyme used in this experiment is pronase which is a mixture of various proteolitic enzymes and attacks proteins non-specifically. The experimental procedure was the same as for phospholipase A. It is known that pronase treatment prolongs the action potential without affecting the height. Therefore, the increase in the duration of action potential was continuously monitored during pronase digestion. Most impedance measurements were carried out when the duration of action potential reached 50 ms or 1 s. The effect of pronase digestion on the excitability of squid axon membrane was investigated by Armstrong et al. by using a voltage clamp technique. In their study, the potassium current was blocked by replacing the internal K^+ with Cs^{2+}. They observed, under these conditions, that the sodium inactivation was nearly completely eliminated and it resulted in the prolonged sodium influx. In our experiments, the potassium efflux as well as sodium influx and its inactivation were investigated. The voltage clamp diagrams are shown in Fig. 6 with various levels of depolarizations.

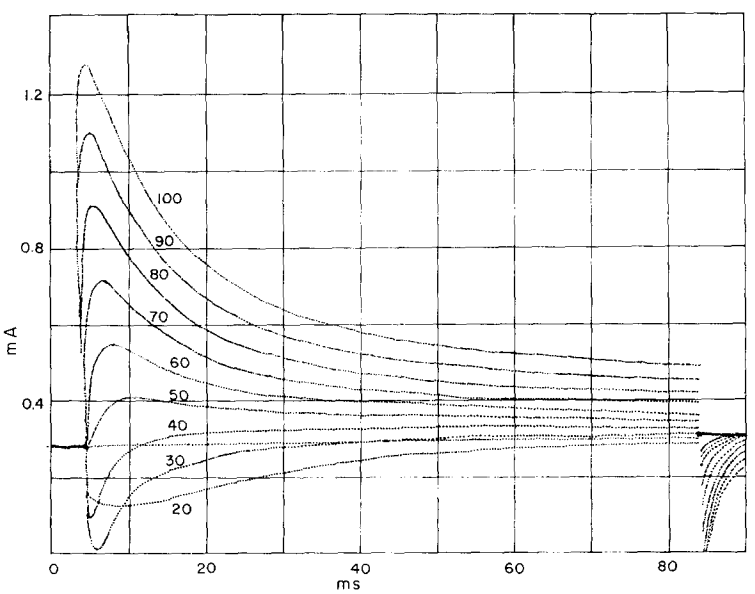

Fig. 6. Voltage clamp diagram of pronase-treated axon. Time scale, 10 ms and the numbers indicate the magnitude of depolarizations. The duration of action potential is about 2 s.

Curves in Fig. 6 show that pronase digestion prolongs the sodium influx as observed by Armstrong et al. The elimination of sodium inactivation is particularly clear at small depolarizations. As the magnitude of depolarization increases, a large transient outward current is observed which terminates after about 40 ms. After the transient outward current is terminated, the total current diminishes gradually and only a small outward current continues with a diminishing magnitude. The longest action potential we observed is 10 s and under these conditions, the voltage clamp diagram is characterized by a transient outward current with a very small initial inward current.

As shown above, the pronase digestion causes an extensive alteration in the excitatory mechanism by affecting sodium as well as potassium currents. It was our aim to study the membrane capacitance and conductance under these conditions to investigate whether the change in the excitatory behavoir is accompanied by an extensive alteration of the membrane structure.

Fig. 7 illustrates the frequency profile of the capacitance of pronase digested axon membrane. First of all, pronase digestion does not alter C_∞. This indicates, as anticipated, pronase does not change the high frequency capacitance which is perhaps due to lipid molecules. Secondly, the change in the amplitude of the frequency dependent capacitance is relatively small. Namely, the amplitude of the capacity dispersion increases only by a factor of 1.3–1.5. This is in contrast to the drastic increase caused by phospholipase A. Another difference between the effects of pronase and phospholipase A is the effect of these enzymes on the inductive component. While phospholipase-A eliminated the inductive component, pronase leaves the inductance almost unaffected. This is a strong indication that the disruption of the structure of membrane proteins hardly affects the magnitude of

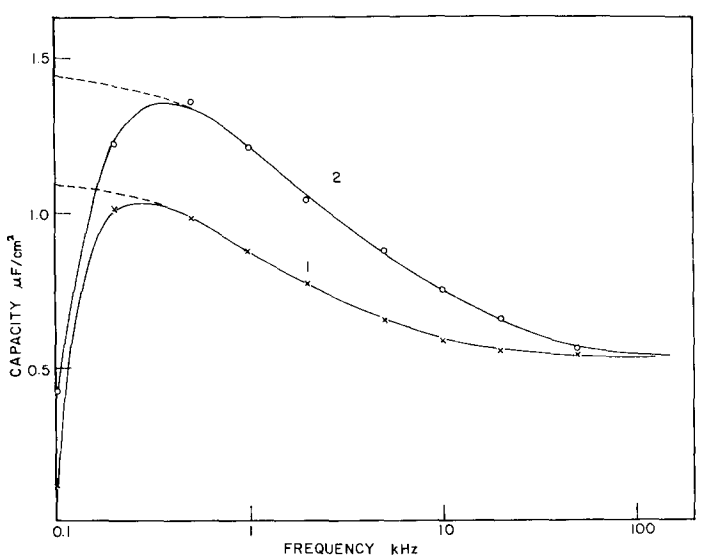

Fig. 7. Frequency profile of axon membrane capacitance after the treatment with pronase. The pronase concentration is 0.1 mg/ml. Curve 1, before and curve 2 after the enzyme treatment. Both curves the plotted after correction for series resistance.

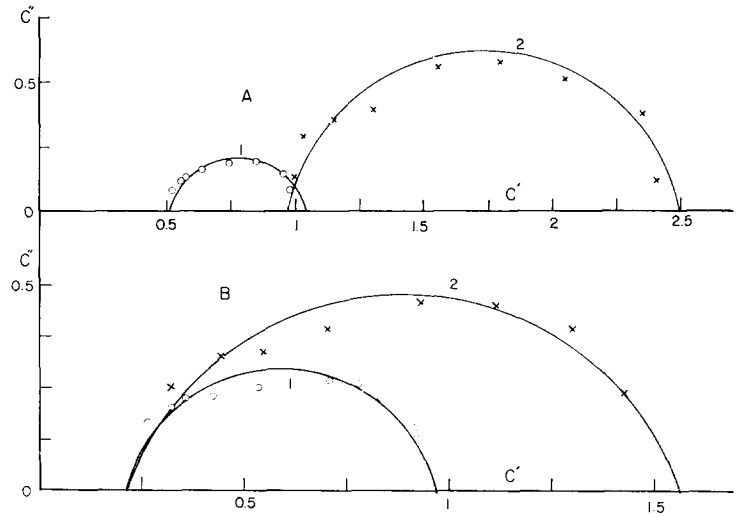

Fig. 8. a, Cole-Cole plots of squid axon membranes before (curve 1) and after (curve 2) the treatment with phospholipase A. Calculations are based on the sample shown by curve A and B in Fig. 4; b, Cole-Cole plots of squid axon membrane before (curve 1) and after (curve 2) treatment with pronase. Calculations are based on the data in Fig. 7. Inductive reactance is ignored in these diagrams.

inductive component.

Finally, the Cole-Cole plots of axon membranes digested with pronase and phospholipase are shown in Fig. 8. These plots establish the low and high frequency limits of membrane capacitance (C_0 and C_∞) before and after the treatments with these enzymes.

DISCUSSION

1) Significance of the frequency dependent membrane capacitance

For many years, the membrane capacitance has been considered less important compared to the membrane conductance. The observation by Cole and Curtis [18, 19] that the membrane capacitance did not change during the action potential while the membrane conductance underwent an enormous increase, suggested that the membrane capacitance represents only an inactive part of the membrane. Biological membranes have been considered a rigid dielectric wall with a small number of active sites and the membrane capacitance has been treated, in mathematical models, as frequency independent.

The observation that membrane capacitance is partly frequency dependent indicates that at least certain part of nerve membrane is not a rigid wall. Thus, macromolecules which are embedded in the matrix of lipid molecules may have a certain degree of rotational freedom. The implication of the frequency dependent membrane capacitance will be discussed in the context of the gating current which has been reported by several authors [22, 23].

If a square pulse is applied to dielectric materials, the total current can be given, with the use of the superposition theorem, by the following equation:

$$I(t) = C_\infty \frac{dV}{dt} + (C_0 - C_\infty) \int \frac{dV(t-u)}{dt} \cdot \varphi(u) du + \Sigma I_r \tag{4}$$

where C_0 is the total membrane capacitance and C_∞ is the capacitance which is instantaneously charged after the application of the pulse. $\varphi(u)$ is a decay function and ΣI_r is the sum of ohmic and/or non-ohmic ionic currents. This equation is similar to the Hodgkin-Huxley equation [20] except for the second term on the right hand side. This is a consequence of the assumption that the capacitive current is time dependent. We assume that the electrode geometry is plane parallel for the sake of simplicity and the current density is given by:

$$i(t) = kV(t) + \frac{1}{4\pi} \frac{dD(t)}{dt} \tag{5}$$

where k is conductivity and $D(t)$ is displacement. Substituting this equation in Eqn 4 and after rearrangement, we obtain an integral equation:

$$D(t) = C_\infty V(t) + (C_0 - C_\infty) \int \frac{dV(t-u)}{dt} \varphi(u) du \tag{6}$$

This equation can easily be applied for a periodic field $V = V_0 e^{j\omega t}$ with the definition $D = \varepsilon V$. Then,

$$\varepsilon = \varepsilon_\infty + (\varepsilon_0 - \varepsilon_\infty) \int_0^\infty \varphi(u) e^{-j\omega u} du \tag{7}$$

It is well known that the Fourier Transform of this equation leads to the Debye equation [21], i.e., a dispersion of frequency dependent permittivity with one relaxation time. Hence, the time dependent capacitive current gives rise to a frequency dependent capacitance as described above. It must be recalled that the articles by Armstrong and Bezanilla [22, 23] indicate that the gating current is essentially a time dependent displacement current which is related to the frequency dependent capacity by the Fourier Transform. In view of this, the frequency dependent membrane capacitance observed by us is similar to the gating current observed by Armstrong and Bezanilla. Armstrong and Bezanilla relate the time dependent capacitive current directly to the opening and closing of gates in the nerve membrane. Our observation also indicates the presence of a time dependent capacitive component and some fraction of this capacitive component may indeed be related directly or indirectly to the gate current.

2) Effects of Phospholipase-A

Previous studies by Abbot at al [7] indicate that this enzyme blocks the sodium current without affecting the potassium current. Also a marked increase in the leakage current is noted as the sodium current is progressively inhibited. This indicates an extensive disruption of the membrane structure and is in contrast to the pronase treatment where the excitatory mechanism is drastically altered without an appreciable increase in the leakage current.

Our impedance study indicates that the partial degradation of the membrane by phospholipase A causes an increase in the high frequency capacitance (C_∞),

by perhaps disrupting lipid layers and decreasing the thickness. This result can be used as the support for the hypothesis that C_∞ is the capacitance due to lipid molecules. The increase in the leakage current can be related to the disruption of the lipid bilayer or to the perturbation of the interaction between proteins and lipids. It was already mentioned that the treatment with phospholipase A increases the amplitude of the frequency dependent capacitance. It is now widely believed that there are two types of proteins in biological membranes, the one is loosely bound and the other, tightly bound protein molecules. It may be reasonable to assume that the loosely bound exogeneous proteins have a considerable orientational freedom and the small frequency dependent capacitance in intact membranes may be due to this kind of protein. The tightly bound endogeneous proteins are normally rigidly held in the matrix of membranes. Those proteins, however, become relatively free to rotate when phospholipase A disrupts the lipid-lipid and lipid-protein interactions. This in turn causes an increase in the frequency dependent capacitance. The above hypothesis is, however, highly speculative because of the many uncertainties and this interpretation should be considered as such.

3) Effects of pronase

Armstrong et al. (8) observed that pronase eliminates the sodium inactivation without affecting the sodium activation. In their experiments, membranes were treated with pronase solution at a concentration of 1 mg/ml and the internal potassium ion was replaced by cesium ions. In our experiments, the pronase concentration was at most 0.15 mg/ml and the enzyme treatment lasted for a much longer time than theirs. The results we obtained indicate that pronase disrupts almost all parts of excitatory mechanism, namely sodium activation and inactivation and potassium efflux. After a long treatment with pronase, the duration of action potential reaches as long as 10 s. Under these conditions, only a very small inward current and a relatively large outward transient current which lasts for about 100 ms were observed. In spite of these drastic alterations in the excitatory behavior of axon membrane, the membrane capacitance is relatively unaffected by the pronase treatment. First of all, C_∞ is nearly unaffected. Since pronase decomposes only proteins, this result indicates that C_∞ has no relation to protein molecules. The frequency dependent capacitance, on the other hand, increases its amplitude by a factor of 1.5 approximately. This change is much smaller than the increase resulted after the treatment with phospholipase A. It must also be pointed out that even after a long digestion by pronase, the leakage current is only minutely increased. All these results indicate that the membrane structure is generally intact even after an extensive treatment with this enzyme. This result is also in contrast to that with phospholipase A.

Finally, it may be of considerable significance to note that the inductive reactance was only slightly affected by the pronase treatment while phospholipase A nearly completely eliminated it. This suggests that the inductance is closely associated with the lipid part rather than the protein part.

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REFERENCES

1 Takashima, S. and Schwan, H. P. (1974) J. Memb. Biol. 17, 51–68
2 Cole, K. S. (1972) Membranes, Ions and Impulses, University of California Press, Berkeley, Calif.
3 Takashima, S. (1962) J. Polymer Science 56, 257–265
4 Goldman, D. E. (1964) Biophysical Journal 4, 167–188
5 Singer, S. J. and Nicolson, G. L. (1972) Science 175, 720–731
6 Takashima, S. (1969) Physical Principles and Techniques of Protein Chemistry, (Leach, S. J., ed.), Ch. 6, Academic Press, New York
7 Abbott, N. J., Deguchi, T., Frazier, D. T., Murayama, K., Narahashi, T., Ottolenghi, A. and Wang, C. M. (1972) J. Physiol. 220, 73–86, 1972
8 Armstrong, C. M., Bezanilla, F. and Rojas, E. (1973) J. Gen. Physiol. 62, 375–391
9 Tasaki, I. (1968) Nerve Excitation, p. 24–26, Thomas, Springfield, Illinois
10 Moore, J. W. (1963) Physical Techniques in Biological Research VI, (Nastuk, W. L., ed.), Ch. 5, Academic Press, New York
11 Cole, K. S. and Cole, R. H. (1971) J. Chem. Phys. 9, 341–351
12 Takashima, S. and Schwan, H. P. (1974) Liquid Crystals and Ordered Fluids, (Johnson, J. F. and Porter, R. S., eds), Plenum Press, New York
13 Hanai, T., Haydon, D. A. and Taylor, J. (1964) Proc. Roy. Soc. Lond. A281, 377–391
14 Schwan, H. P. and Thompson, T. E. (1966) Proc. Annu. Meeting Biophys. Soc.
15 White, S. H. and Thompson, T. E. (1973) Biochim. Biophys. Acta. 323, 7–22
16 Cole, K. S. (1971) J. Gen. Physiol. 25, 29–51
17 Cole, K. S. and Marmont, G. (1942) Fed. Proc. 1, 15–16
18 Cole, K. S. and Curtis, H. J. (1938) Nature 142, 209
19 Cole, K. S. and Curtis, H. J. (1939) J. Gen. Physiol. 22, 37–64
20 Hodgkin, A. L. and Huxley, A. F. (1952) J. Physiol. 117, 500–544
21 Debye, P. (1929) Polar Molecules, Dover, New York
22 Bezanilla, F. and Armstrong, C. M. (1974) Science 183, 753–754
23 Bezanilla, and Armstrong, C. M. (1973) Nature 242, 459–461