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LIGHT SCATTERING AND EXCITATION IN LOBSTER GIANT AXON

EFFECTS OF ION SUBSTITUTION

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Summary

Changes in the light scattering signal from single giant axons of lobster were observed during the propagation of the action potential in order to correlate membrane excitability with possible structural changes reflected in the optical properties of the axolemma. Substitution of guanidine and aminoguanidine for sodium resulted in a decreased action potential amplitude to 69 and 50% of control values, respectively. The amplitude of the light signal was, however, not significantly changed by these substitutions and is, therefore, reported to be independent of the transmembrane potential and current. The venom of the scorpion *Leiurus quinquestriatus* caused a marked prolongation of the action potential and the light scattering signal without significantly altering their amplitudes. A two-state model of the early (sodium) activation channel is suggested, in which the light scattering signal is correlated with a possible difference in the scattering efficiency between the states of the channel.

Introduction

Changes in light scattering have been used to obtain information about the underlying conformational mechanisms involved in the generation of the action potential of excitable membranes [1—5]. Changes in the light scattering signal from the squid giant axon, measured at 90° to the incident light beam, have been reported by Cohen et al. [4]. They suggested that the changes in light scattering, during the action potential, are primarily potential-dependent and secondarily current-dependent.

In our study, we investigated the effects of ion substitution and a scorpion venom on the 90° light scattering during excitation of the lobster giant axon. Ion substitution studies [6—8] have demonstrated that axons remain excitable when guanidine or aminoguanidine replaces the sodium in the bathing solution.

The presence of these ions causes a reduction of the action potential amplitude, a decrease in the peak inward current [6], and an increase in the action potential duration [8]. The reduction of action potential amplitude during these ion substitutions should result in a significant decrease of the light scattering amplitude according to the potential-dependent model of Cohen et al. [4]. If, however, the amplitude of the light scattering from the axon does not correlate with the action potential amplitude, then a different interpretation relating light scattering to axonal excitation is necessary. In addition, if light scattering reflects the open state of the sodium channel, its duration should increase when closure of this channel is delayed. To test the latter idea, we used the venom of the scorpion *Leiurus quinquestriatus* which was shown [9] to prolong the duration of the action potential by delaying the inactivation of the sodium conductance.

To explain the changes we observed in the light scattering signal, we propose that the principal light scattering element in the membrane is the early activation (sodium) channel structure. We further suggest that changes in the light scattering occur because the open and closed states of the channel structure are characterized by a different scattering efficiency. This model predicts that the light scattering signal amplitude should be independent of ion currents and transmembrane voltages. Preliminary accounts of this work have been reported elsewhere [10,11].

Materials and Methods

Giant axons from the ventral nerve cord of the lobster (*Homarus americanus*) were dissected by removing a length of nerve cord from the cerebral to the last thoracic ganglion [12]. A 15-mm segment of cord was excised and stretched over two glass rods 7 mm apart, mounted in the experimental chamber. A single axon (108 ± 22 μm) was freed of extraneous tissue for a length of 8 mm. The axon was penetrated with two glass microelectrodes (3 M KCl; 5—15 MΩ) one at each end. One electrode was used for current injection, the other for recording transmembrane potentials. Action potentials were evoked by supramaximal square pulses (100 μs duration) with an inter-pulse interval of 81 ms (WPI Series 800), and recorded through a unity-gain amplifier (WPI M4A). Axons with resting potentials polarized less than —65 mV in normal lobster saline were not used. No flow occurred in the chamber during measurements. The temperature was 10 ± 1°C except where noted.

The optical system we used (Fig. 1) is similar to those reported earlier [4,13]. A tungsten-halogen lamp (type FCR) was used as the light source. The light beam passed through a heat absorbing glass (Schott KG-4, thickness 3 mm) and was focused on a diaphragm. The light was then focused on the axon through a glass window (0.23-mm thick) and 4 mm of solution. The final diameter of the focused light beam was approximately 300 μm. Light from a rectangular area (280 × 100 μm) of the axon surface, scattered into a 1.5 steradian solid angle, was collected by a microscope objective (Zeiss UD 40) and focused on a photodiode (United Detector Technology PIN 40 A). The photodiode current was processed by a current-to-voltage converter (Burr Brown 3522J) and by an AC-coupled amplifier (Tektronix 7A22). 2000—4000 events were averaged

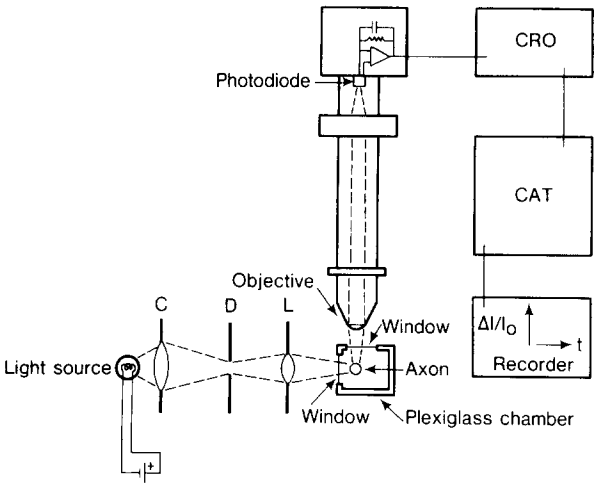

Fig. 1. Schematic diagram of the light scattering apparatus. The angle between the incident and scattered light beams was 90°. A heat absorbing glass (not shown) was inserted into the incident light beam between the condensing lens (C), focal length 25 mm and the diaphragm (D). The lens (L), focal length 22 mm, produced a convergent beam focused on to the axon. The scattered light was collected by a microscope objective (Zeiss UD 40; focal length 6.9 mm). Distance from the axon to the objective was approximately 7 mm. Distance from the axon to the light source was 28 cm. The feedback resistor and capacitor in the operational amplifier circuit were 22 MΩ and 1 pF.

(Nicolet Model 1072). The time constant of the optical system was 60 μs.

The action potential was evoked at one end of the axon (Fig. 2). An active region on the membrane (shaded area) of length L then propagates along the axon, past the optical recording site, to the voltage electrode. As the action potential passes the optical recording site, the shape of the measured light signal is a function of w, the length of axon seen by the optical system. The measured scattering signal is related to the true light scattering signal by Eqn. 1.

$$\begin{pmatrix}\text{measured}\\ \text{light scattering signal}\end{pmatrix} = b \begin{pmatrix}\text{true}\\ \text{light scattering signal}\end{pmatrix} \begin{pmatrix}\text{instrument}\\ \text{factor}\end{pmatrix} \quad (1)$$

The quantity b is a constant dependent on the sensitivity of the recording system. If affects the amplitude but not the time-dependence of the measured signal. The "instrument factor" is a function of the optical geometry of the recording system and it affects the time-course of the measured optical signal. In order to faithfully obtain the true time-course of the light signal, the instrument factor must be deconvoluted from the measured light signal. When $w << L$, the measured light scattering signal waveform approaches that of the true light scattering signal. In our study, $w/L \simeq 0.01$. Thus, the instrument factor has a negligible effect on the shape of the measured light signal waveform.

The change in light scattering (ΔI), measured as the maximum deflection above the resting scattering level (I_o), was normalized to I_o and results are given as $\Delta I/I_o$, and is referred to as the light scattering signal. The normalization procedure is necessary since small changes in optical alignment and battery condition result in significant changes in ΔI and I_o. The areas under the electrophysiological and light signals were measured with a planimeter. The duration of both

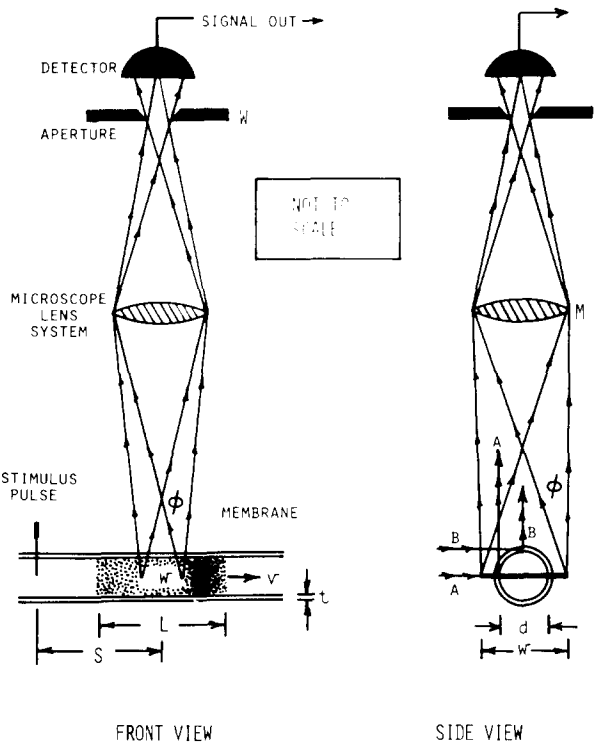

Fig. 2. Optical system geometry. Front view. The microscope lens system with magnification M and acceptance angle ϕ focuses the aperture with width W onto the axon as shown. Light from a rectangular section of the axon length W ($w = M \cdot W$) is accepted by the detector. The membrane has diameter d and thickness t. The active region of the membrane is indicated by the shaded area of length L, located a distance S from its point of origin (stimulus pulse). The velocity of propagation is v. Note that $w/L \simeq 0.01$ (see text). Side view. A lens directs indicent light onto the axon. Light rays A and B, scattered 90° by the membrane, are collected by the microscope and sent to the detector. The observed length w is slightly longer than axon diameter to minimize any residual vibrations from external sources.

signals was determined by extending tangents to the signal, at maximum slopes, of the leading and trailing legs to the baseline. The baseline was the resting potential level for the action potential and the optical baseline for the light scattering signal. This definition of signal duration was compared to the technique which uses the full width of the signal at half maximum amplitude. Although the magnitude of the duration by the latter method is shorter, the average values calculated by either method showed similar relationships. Signal amplitudes were measured from the respective baselines to the peak of the signals.

Electrical coupling between the optical and electrophysiological signals was minimized by shielding and grounding and checked according to Cohen et al. [4]. We observed no coupling in our system. The stimulus pulse was eliminated as a potential source of artifacts when no optical signal was detected from stimulated, dead axons or from viable axons stimulated with hyperpolarizing or subthreshold currents.

The composition of solutions used in this study is given in Table I. Normal lobster saline [12] was used for dissection and storage of axons. A low-potas-

TABLE I

COMPOSITION OF PERFUSION SOLUTIONS

Normal lobster saline (NLS) was used for dissection and storage of the preparation. Control lobster saline (CLS) was used during control measurements. Guanidine saline (GUAN) was a test solution in which guanidine replaced sodium in CLS. Aminoguanidine saline (AGN) was a test solution in which aminoguanidine replaced sodium in CLS. The AGND solution was a 1 : 1 mixture of AGN and CLS. A low sodium solution (LNA) was used to reduce the action potential size without affecting permeabilities. Osmolarity of all solutions was 935 ± 15 mosM at pH 7.35 ± 0.05. All concentrations are given in mM.

Solution	Na^+	K^+	Cl^-	Ca^{2+}	SO_4^{2-}	Mg^{2+}	Guanidine	Aminoguanidine	Tris-chloride	Tris-base	NO_3^-
NLS	465	10	534	25	4	8			1		
CLS	483	1	535	25					1		
GUAN		1	567	25			515		1		
AGN		1	52	25				543	1	<1	543
AGND	241.5	1	293.5	25				271.5	1	0.5	271.5
LNA	120.8	1	486.7	25					314.9	30.1	

sium control solution (CLS) * was used for control measurements of light scattering. Sodium chloride was replaced by an osmotically equivalent amount of guanidine · HCl, or by aminoguanidine nitrate (Table I). A low (25%) sodium solution (LNA) was prepared by substituting an osmotically equivalent amount of Tris for the sodium in control solution. The osmolarity of all solutions was 935 ± 15 mosM (Fiske Osmometer; Model G66) and the pH was adjusted to 7.35 ± 0.05.

For venom experiments, a stock solution of *L. quinquestriatus* venom (Sigma) was prepared in control solution. Just prior to use, the stock solution was diluted to a final concentration of 1 μg/ml. The venom was left in the chamber only until the onset of prolongation of the action potential.

Results

Light scattering in control saline

A small transient increase in the light scattering amplitude was observed during the action potential in control solution (Fig. 3 upper trace). The increase in $\Delta I/I_0$ averaged over 2000 action potentials was $5.4 \cdot 10^{-5}$. The time-course of the light signal was similar to that of the action potential (Fig. 3 lower trace). Although the light scattering signal appears to begin before the action potential, this is a recording artifact resulting from the distance between the recording sites of the two signals (3.5 mm). When the propagation time [14] over this distance is taken into account, the signals appear to begin simultaneously, within limits of experimental error.

The duration and amplitude of the light signal and the action potential were temperature-sensitive. Lowering the bath temperature (15 to 5°C) caused a 3-fold prolongation of the duration of both signals. The light scattering signal amplitude increased 4-fold over this temperature range (Fig. 4). A semi-log plot of

* See Table I for abbreviations.

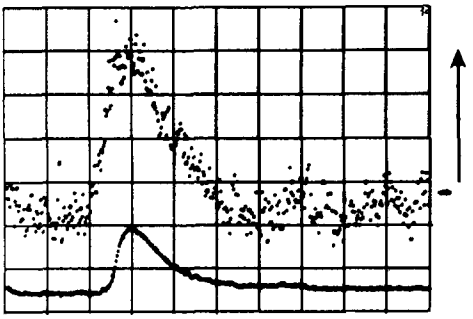

Fig. 3. Typical changes in the light scattering signal amplitude measured during the propagation of the action potential in CLS. Upper trace, light scattering signal averaged from 2000 action potentials. The length of the vertical arrow represents a mean increase in light scattering $\Delta I/I_0 = 4.2 \cdot 10^{-5}$ for one action potential. Lower trace, mean action potential averaged from about 150 sweeps and recorded at the end of the scattering measurement. The action potential amplitude was 98 mV, and the resting potential was —88 mV. One horizontal division is 1.5 ms for both traces. Temperature was 10 ± 1°C.

the scattering signal amplitude versus temperature shows that the amplitude varies exponentially with temperature. The action potential amplitude increased slightly as the temperature was lowered from 15 to 17°C, but then decreased as the temperature was further lowered to 2°C (Fig. 4). There was no correlation between the light scattering and action potential amplitudes nor between the light signal amplitude and the resting potential.

Light scattering in altered sodium solutions

Representative light scattering signal and action potential traces, obtained from axons superfused with control and guanidine solutions, are shown in Fig. 5. Replacement of sodium by guanidine or aminoguanidine prolongs both signals (Table II). As the permeability of the axon to the ion is decreased (sodi-

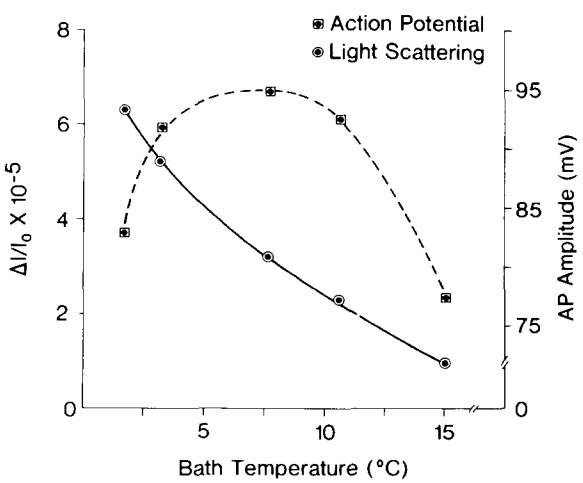

Fig. 4. The effects of temperature on the light scattering signal (solid line) and the action potential (AP) (dashed line) amplitudes in control solution. Data were obtained from one axon. Estimated errors in light scattering signal and action potential amplitudes are ±4% and 2%, respectively. Scattering was averaged from 2074 to 8461 action potentials.

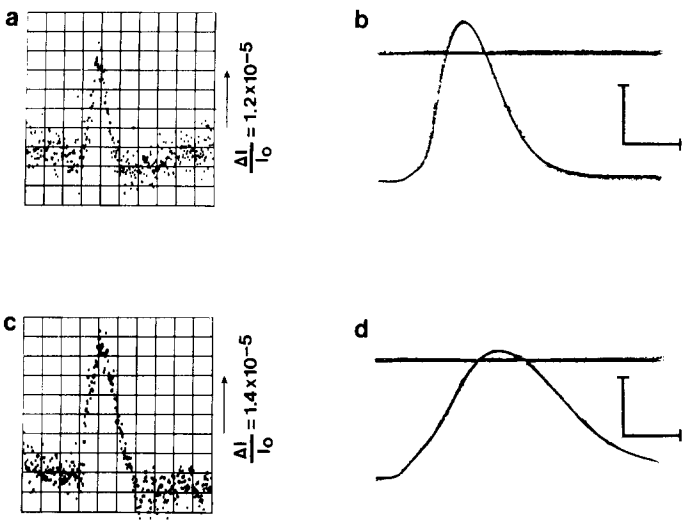

Fig. 5. Effects of guanidine substitution (GUAN) for sodium in the external bathing medium on the light scattering signal and the action potential of lobster axon. Panel (a) shows the light scattering signal in CLS. The signal was averaged from 2000 events. Panel (b) shows a single action potential in CLS. The resting potential was —80 mV. Panel (c) shows the light scattering signal in GUAN (mean of 2000 events). Panel (d) shows a single action potential in GUAN. The resting potential was —84 mV. Data from one axon. Calibration marks in panels (b) and (d) represent 40 mV (vertical), and 1.0 ms (horizontal). One horizontal division represents 1.5 ms in panels (a) and (c) and the arrow shows the direction of increase in scattered light intensity. Arrow length represents the mean light scattering signal intensity for one action potential.

um to guanidine to aminoguanidine), the duration of both signals is prolonged by a factor of four, approximately. A similar behavior was also observed when Tris was partially substituted for sodium (Table II). For the control solution, the duration of the light signal was 2.4 ± 0.1 ms and the duration of the action potential was 3.0 ± 0.2 ms. The difference in signal durations (0.6 ms) was significant ($P < 0.01$). When guanidine replaced sodium, the light signal and action potential durations increased to 4.4 ± 0.3 ms and 5.8 ± 0.6 ms, respectively, while the difference in duration between the two signals became 1.4 ms

TABLE II

EFFECTS OF ION SUBSTITUTION OF THE LIGHT SCATTERING SIGNAL AND ACTION POTENTIAL DURATIONS

The durations of the light scattering signal and action potential in control solution (CLS) were 2.4 ms and 3.0 ms respectively. Values in the table (X ± S.E.) are normalized to the duration of the respective signal in control solution. Temperature was maintained at 10 ± 1°C. Figures in parentheses here and in subsequent tables indicate the number of axons averaged for each measurement. See Table I for abbreviations.

	Solution				
	CLS	LNA	GUAN	AGND	AGN
Light scattering signal	1	1.6 ± 0.2	1.8 ± 0.2	1.8 ± 0.2	4.6 ± 0.6
Action potential	1	1.3 ± 0.1	1.9 ± 0.3	1.9 ± 0.3	3.3 ± 0.6
	(31)	(9)	(17)	(18)	(10)

($P < 0.05$). Partial sodium replacement by aminoguanidine (AGND), resulted in an increase in light signal and action potential durations to 4.4 ± 0.3 ms and 5.6 ± 0.4 ms with a duration difference of 1.2 ms ($P < 0.05$). Complete replacement of sodium by aminoguanidine (AGN) and partial replacement of sodium by Tris also resulted in the prolongation of both signals (Table II), but the differences between the durations were not significant. Resting potentials in control (CLS), guanidine (GUAN), aminoguanidine (AGN) and diluted guanidine (AGND) solutions were −93 ± 2, −84 ± 3, −86 ± 3 and −95 ± 2 mV, respectively.

Signal amplitudes

The amplitude of the light scattering signal in control solution was $(4.8 \pm 0.4) \cdot 10^{-5}$, ($X$ ± S.E.) averaged from 31 axons. This amplitude was not affected by substitution of guanidine or aminoguanidine for sodium (Table III). In contrast, the amplitude of the action potential was significantly reduced when the sodium concentration of the bathing medium was lowered. In low sodium (LNA), guanidine (GUAN), and aminoguanidine (AGN) solutions, the action potential amplitude decreased to 82, 69 and 50% of control values, respectively. No change in action potential or light scattering amplitude was observed when the axon was immersed in the 50% sodium : aminoguanidine (AGND) solution. The reduction in the action potential amplitude, in low sodium solutions (LNA and AGND), is in agreement with predictions from the Nernst equation. For example, removal of 75% (LNA) or 50% (AGND) of the sodium from the perfusion medium, results in a calculated change in the sodium equilibrium potential of 35 and 17 mV, respectively. This change in sodium equilibrium potential should be reflected in a proportional decrease in the overshoot potential. The measured reduction (1—2 mV) in AGND solution when compared to the predicted decrease may be explained by the low but finite permeability of the membrane to aminoguanidine. The measured overshoot reduction of 25 mV in the low sodium solution (LNA) is consistent with the calculated change. When low sodium solution was introduced into the chamber, there was a 3—5 mV hyperpolarization accompanied by a reduction in action potential amplitude. These two effects combined to give the reduced action potential amplitude (82% of control).

TABLE III

EFFECTS OF ION SUBSTITUTION ON THE LIGHT SCATTERING SIGNAL AND ACTION POTENTIAL AMPLITUDES

Amplitudes are given as the fraction of the respective signal amplitude in control solution. Values are ($\bar{X}$ ± S.E.). Number of axon as in Table II.

	Solution				
	CLS	LNA	GUAN	AGND	AGN
Light scattering signal	1	1.1 ± 0.1	1.0 ± 0.2	1.0 ± 0.2	1.0 ± 0.2
Action potential	1	0.82 ± 0.02	0.69 ± 0.08	0.99 ± 0.07	0.5 ± 0.1

TABLE IV

EFFECTS OF LOW SODIUM SOLUTION ON THE UPSTROKE AND DOWNSTROKE KINETICS OF THE LIGHT SCATTERING SIGNAL AND ACTION POTENTIAL

Values are given as the fraction of their respective control values. Data from 9 axons. Values ($\bar{X} \pm$ S.E.).

	Upstroke	Downstroke
Light scattering signal	0.5 ± 0.1	0.93 ± 0.06
Action potential	0.43 ± 0.06	0.70 ± 0.03

Rates of signal change

Reduction of external sodium reduced the rate of depolarization as well as the upward slope of the light signal to approximately 50% of control (Table IV). The rate of repolarization in low sodium (LNA) was slowed to 70% of the control value. However, LNA had no significant effect on the downward phase of the light signal. Substitution with guanidine, reduced the upward slope of the light signal and action potential to 70% ± 30% and 18% ± 4% of control, respectively.

Scattered light energy

The total light energy scattered into the detector was measured from the area under the light signal. An increase in the scattered energy was observed for both low sodium (LNA) and sodium-substituted solutions (Table V). Removal of 75% of the external sodium (LNA) resulted in an increase of scattered energy to 220% of control ($P < 0.05$). Under these conditions the action potential area did not increase. The light signal area increased to 170% ($P < 0.01$) when guanidine (GUAN) completely replaced sodium. This increase in area was accompanied by an increase in the action potential area to 130% of control ($P < 0.02$). In the 50% aminoguanidine-sodium solution (AGND) the area of the light signal increased to 160% ($P < 0.05$) and after complete sodium replacement, by aminoguanidine (AGN), the area increased to 300% of control ($P < 0.01$). In AGND, the area under the action potential increased to 190% ($P < 0.01$) and in AGN to 230% ($P < 0.01$) of control.

Effects of scorpion venom

Addition of scorpion venom (1 μg/ml) to control solution (CLS), for periods of 3 to 10 min, prolonged the duration of the action potential by decreasing

TABLE V

EFFECTS OF ION SUBSTITUTION ON THE LIGHT SCATTERING SIGNAL AND ACTION POTENTIAL AREAS

Areas are given as the fraction of the area in control solution. Values are ($X \pm$ S.E.). Number of axons as in Table II.

	Solution				
	CLS	LNA	GUAN	AGND	AGN
Light scattering signal	1	2.2 ± 0.5	1.7 ± 0.4	1.6 ± 0.4	3 ± 1
Action potential	1	1.07 ± 0.06	1.3 ± 0.2	1.9 ± 0.2	2.3 ± 0.6

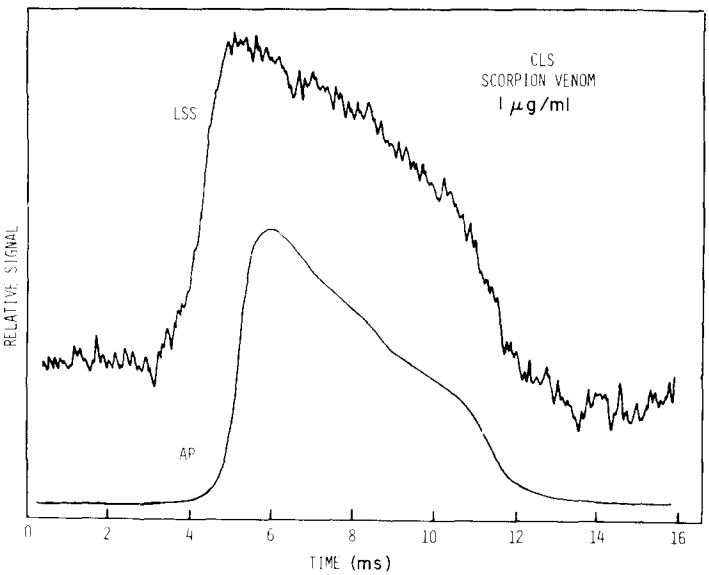

Fig. 6. The effects of scorpion venom (*Leiurus quinestriatus*) on the light scattering signal and action potential (AP) in control solution. Venom concentration 1 μg/ml. Scattering signal amplitude is $\Delta I/I_0 = 5.1 \cdot 10^{-5}$ averaged from 2048 action potentials. Action potential amplitude is 112 mV. Resting potential is —107 mV. Data from one axon.

the rate of repolarization (Fig. 6). The rate of repolarization depended on venom concentration and stimulation frequency. Slow stimulation rates (0.2 Hz) evoked action potentials lasting several seconds. Faster rates of stimulation (12.3 Hz) were used during optical measurements since the action potential duration remained constant at this rate and only one action potential was evoked by each stimulus. The duration of the action potential and light signal were increased 3- to 4-fold. (Note that repolarization has not been completed at the end of the trace in Fig. 6). Complete repolarization required over 50 ms under these conditions. Although these increases were due principally to a delay in repolarization, there was a small decrease (25%) in the rate of upstrokes of both signals. The amplitudes of the signals did not change significantly.

Discussion

Changes in optical properties (birefringence, [1,13]; fluorescence, [13,15]; scattering, [4,5,30] of membranes during excitation, may be explained by two primary processes: (i) conformational or structural properties of macromolecules involved in the excitatory process [16,17] or (ii) effects not directly related to excitation including ion and water accumulation in the periaxonal space [5] and thinning of the membrane due to attractive electrical forces [1]. The identification of the light signal with the latter processes is based, in part, on the potential- and current-dependent properties of the light scattering signal during voltage-clamp conditions [4,5]. The data presented in this study indicate that under conditions of ion substitution, the light scattering amplitude is independent of the action potential amplitude. Therefore, it is highly likely that the

light scattering signal reflects conformational changes rather than electrical processes associated with membrane excitation.

Our results are consistent with a model of light scattering from the axon in which: (1) the principal light scattering unit is the early activation (sodium) channel structure, (2) this channel exists in the open or closed state only, and (3) the open channel structure has a different light scattering efficiency than the closed channel.

The identification of the light scattering element with the early activation channel is derived from the close coincidence of the time-course of the light scattering signal with the initiation of the action potential. The light scattering signal begins virtually simultaneously with the action potential (Fig. 3 and ref. 4). Moreover, the light signal reaches considerable size before the activation of the potassium channel and while the sodium conductance predominates the excitable event.

The concept of a bistable ion channel behavior has been reported previously [18,19]. These authors suggested that the potassium channel, and probably the sodium channel as well, are capable of undergoing a direct nongraded transition from the open to the closed state. In addition, the squid axon membrane [20] was demonstrated to settle into either of two states, each characterized by an effective E.M.F. and conductance. Finally, Blumenthal et al. [21] have proposed a two-state channel consisting of a protomer lattice.

The light scattering intensity, at 90° to the incident beam, increases during excitation (Fig. 3) compared to its resting scattering level. Therefore, the scattering efficiency of the open channel is concluded to be higher than that of the closed channel. Thus, when a channel opens, its contribution to the total light scattering is constant for as long as it remains open. The amplitude of the scattering signal is then a function of the number of open channels. Additional evidence for this conclusion is derived from the behavior of the light scattering signal during venom application. The venom delays the inactivation of the sodium conductance [9] and thus may be said to delay the closing of the sodium channel. In agreement with our prediction, the signal duration increases during venom application, while no change occurs in its amplitude (Fig. 6).

Our data (Table II and Fig. 5) show that when guanidine or aminoguanidine (permeability relative to sodium 0.13 and 0.6, respectively [6]) is substituted for sodium, the durations of both the action potential and the scattering signal increase up to 4-times of the control values. As less permeant ions enter the channel, more hydrogen bonds could form with the channel constituents. This would require more broken hydrogen bonds, when the ion leaves the channel, possibly resulting in an increase in the time for the ion to exit from the channel [6]. This slowed transit time through the channel could account for the increases in the durations of the action potential and light scattering signals in solutions containing guanidine and aminoguanidine (Table II).

The light scattering which we observed (Fig. 3) resembles the early component of the scattering reported by Cohen et al. [4] but not the later, longer-lasting component which they attributed to the volume change in the periaxonal space [22]. The late component is unresolved in our study because of our stimulation frequency.

Cohen et al. [5] have proposed that the 90° scattering observed during exci-

tation, has a potential as well as a current-dependent component. Our ion substitution experiments permit a test of this hypothesis. Hille [6] demonstrated that the substitution of guanidine or aminoguanidine for sodium, in the node of Ranvier of frog nerve, reduces the early current amplitude to less than 10% of the control sodium current (calculated from Hille [6] and Figs. 5 and 6). Although it is not completely rigorous to generalize from frog voltage-clamp data to propagated lobster action potentials, it is nevertheless reasonable to expect significant reductions in current amplitudes through the membrane to occur during cation substitution. If, during excitation, the scattering is primarily current-dependent, the reduction of inward current through the channel (by substitution of guanidine or aminoguanidine) should have a marked effect on the amplitude of the light scattering signal. In our experiments ion-substitution was not accompanied by changes in the amplitude of the scattering signal (Table III).

Further evidence, for the independence of the light scattering amplitude from the early inward current during excitation, can be obtained from the slope data of Table IV. Removal of sodium or its substitution resulted in a 43 and 18% reduction in the rate of depolarization, respectively, indicating that the inward current was significantly reduced [23]. The current reduction, in the low sodium (LNA) and guanidine solutions, was not however accompanied by a concurrent reduction in the light scattering signal amplitude. This behavior strongly suggests that the light scattering signal amplitude is independent of membrane currents. If, however, the observed changes in the light scattering signal are due primarily to changes in the conformation of the channel, then the amplitude of the scattering signal should be independent of the current in the open channels. The intensity of scattered light would, therefore, depend on the number of open channels and the time they spend in that configuration. Our data are consistent with this interpretation.

Ion substitution can also be used to test the voltage-dependence of the light scattering signal amplitude during the propagated action potential. Substitution of guanidine or aminoguanidine for sodium, resulted in an action potential amplitude reduction of up to 50% of the control value. In addition, removal of three-fourths of the external sodium, resulted in an action potential amplitude reduction to 82% of the control value. The data of Table III show, however, that the light scattering signal amplitude is not significantly reduced under these conditions. From these observations, we conclude that the 90° light scattering intensity is not substantially potential- or current-dependent. Our results do not completely rule out a combined potential- or current-dependent effect. It is possible, as suggested by Cohen et al. [4], that a current-dependent decrease in light scattering would sum with a potential-dependent increase to yield a constant signal amplitude. It seems unlikely, however, that these two effects would exactly cancel one another under the different conditions we employed. Cohen [22] has set an upper limit to the light scattering intensity ($\Delta I/I_o$) which may be expected from the (squid) axon. However, in his discussion, he does not take into account possible variations in scattering efficiencies and differences in number of scatterers per unit membrane (σ_m). When these factors are taken into account, the fractional change in scattering intensity may be formulated as follows:

$$\frac{\Delta I}{I_0} = \left(\frac{\Delta\sigma_m}{\sigma_m} + \frac{\Delta\epsilon_m}{\epsilon_m}\right)\left(1 + \frac{1}{Kf}\right)^{-1} \quad (2)$$

Where ϵ_m denotes the scattering efficiency of the membrane units, f constitutes the membrane to axoplasm volume ratio and K is a variable relating the product of the scattering efficiency and scatterer population in the membrane to that in the axoplasm. Since both ϵ and σ can vary by orders of magnitude (from 0 to 100%) during excitation, the upper limit for $\Delta I/I_0$ can be much larger than the 10^{-4} value set by Cohen [22].

The fact that we observe scattering implies the existence of scattering units in the membrane. The scatterers may be the channels, extended molecular structures that form the channel complexes, or crystalline domains composed of several channel complexes. The participation of such complexes in the scattering process requires that the channel structure be of sufficiently large dimensions. The size of the channel complex was estimated from tetrodotoxin binding studies [24] to have an approximate molecular weight of $2 \cdot 10^5$. The complex size is thus large enough to scatter. The possible scattering processes may resemble Rayleigh scattering or scattering from two-dimensional Mie particles which are particles that have dimensions comparable to the wavelength of light in the plane of the membrane, but have Rayleigh particle dimensions perpendicular to the plane of the membrane. The perpendicular dimension of these particles is limited by the thickness of the membrane ($\approx$100Å). Ray A (Fig. 2) "sees" such a domain as a Mie particle; ray B "sees" the domain edge-on as a Rayleigh particle. The measured scattering intensity at the edges of the axon rules out Rayleigh particles as the primary scatterers. However, two dimensional Mie particles would give the observed scattering signal. Rays incident parallel would give the observed scattering signal. Rays incident parallel to the membrane surface would scatter less than the rays incident perpendicular to the membrane surface. The two-dimensional Mie particles could be the channel complexes composed of the channel and its associated molecules. They could also be crystalline domains of channel complexes as suggested theoretically by Kilkson [25], and implied ultrastructurally by Gemne [26]. The crystallites, according to our model, could exist in two states corresponding to the open or closed state of the channels. The difference in the scattering efficiencies of the two states could lead to the observed changes in the light scattering signal amplitude. Cooperativity in membrane permeability changes was suggested by Changeux et al. [27]. The concept of cooperativity was extended to crystalline domain transitions by Kilkson [25,28]. Therefore, we suggest that the crystallite transition, rather than the limited channel structure per se, may be responsible for the light scattering signal. Thus, the small number of channels per unit area, obtained from toxin blockage of excitability [29] and the size of the channel complex [24] does not rule out the amplitude of the light scattering signal we observed.

Our model is consistent with the ion substitution data we report here. Initiation of the action potential, in all solutions, is predicted to open all the available conductance channels in the membrane. Since the same number of channels is open in each solution, the light scattering amplitude, being proportional to this number, remains constant even though the action potential amplitude is reduced because of a smaller current flux across the membrane.

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