Raman Spectroscopy of the "Potential Sensor" of Potential-Dependent Channels

G. V. Maksimov, A. A. Churin, V. Z. Paschenko and A. B. Rubin

Moscow State University, Department of Biophysics, 119899 Moscow, USSR

Abstract. Raman spectroscopy (RS) study shows that the "potential sensor" responds to changes in intramembrane potential by conformational changes. The mechanism of regulation of the channel "gate" by the carotenoid potential sensor is discussed.

Key words: Nerve — β-Carotene — Excitation — Raman spectroscopy — "Potential sensors"

Introduction

Membranes in excited state have a negative surface potential produced by the surface charges. Changes in this potential have been suggested to modify the operation of the "gate" mechanism of ion channels sensitive to the membrane electric field. In many cases, the effects produced by changes in the extracellular Ca^{2+} concentration are similar to those induced by changes in the membrane potential. Presence of high concentrations of Ca ions in the membrane environment of a nerve fibre produces a local rise of the potential which is sensed by the "potential sensor" of the channel (Hodgkin and Huxley 1952).

A problem of current interest, which has received much attention in recent years, is identification of the molecular "analogue" of the "potential sensor" in an excited membrane. In this regard, of interest are investigations that have demonstrated the presence of minor lipids, C_{40}-carotenoids(terpenoid compounds) in nerve fibers. They are known as constituent of the "antenna" complexes in the photosystems, but nothing is known about their role in excited membranes (Koyama et al. 1979). A Raman spectroscopic investigation has been reported, demonstrating excitation-induced changes in the position of the peaks and in the intensity of the C_{40}-carotenoids Raman spectrum (Szalontai et. al. 1977). In our previous investigations we could show that changes in the of the Raman spectrum pattern upon excitation are produced by the sodium

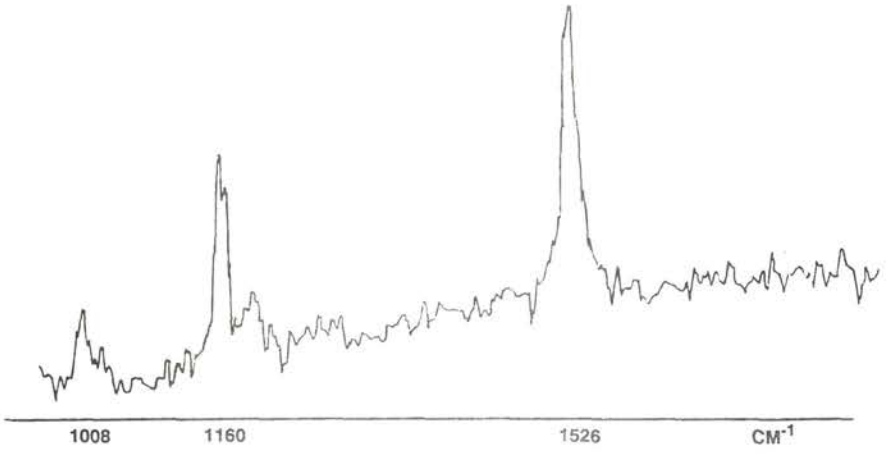

Fig. 1. The Raman spectrum of a frog nerve.

gradient set up in the excited membrane (Maksimov et. al. 1982, 1986).

The purpose of the present investigation was to examine whether the carotenoids present in the lipid phase of the membrane can serve as a "potential sensor", and to see if there is a relation between the conformation of the sensor and changes in the surface potential, acting as a regulation factor.

Material and Methods

Brown frog sciatic nerves were used in the investigation. A special sample holder allowed simultaneous recordings of the Raman spectrum and the action potential of the nerve preparation. The nerve was stimulated with square pulses, 450 500 mV high and 0.1 ms wide, with a repetition rate of 100 pulses per second (Maksimov et. al. 1982, 1986).

The monolayer liposomes were prepared by the method of Hauser (Hauser et. al. 1980). The lipid film contained 50 mg egg yolk lecithin and 0.5 mg β-carotene dispersion.

Pulse sources were a LG-67 argon ion laser and aLPM-11 He-Cd laser. Light filters and iris diaphragms were used to reduce the laser power to 20 mW.A DFS-24 monochromator was employed for scanning and isolating spectral bands. The monochromator had the following settings: spectral region 400 850 nm; slit 1:5.3; dispersion 0.45 nm/mm, half-band 1 cm $^{-1}$ at 550 nm; scan rate 0.018 nm/s; accuracy 4 8 cm $^{-1}$. Raman spectra were recorded on a FEY-79 photomultiplier operating in photon counting mode. A multichannel analyser NTA 1024 (Hungary) was employed for signal averaging.

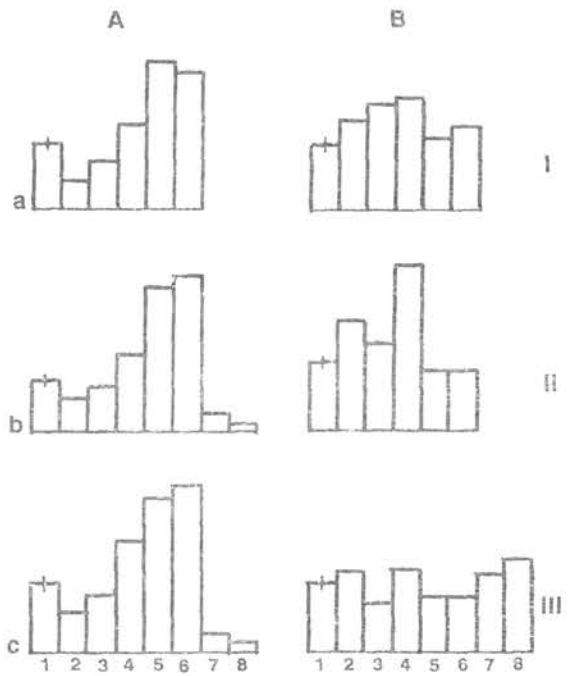

Fig. 2. Changes of the Raman spectrum bands intensities (*A*) and of their rations (*B*) under different experimental conditions: 1. resting conditions, 2. pulse stimulation with a pulse train at a pulse repetition frequency of 100 pulses/s during 20 min, 3. addition of TTX (10^{-5} mol/l), incubation time 20 min, 4. pulse stimulation (100 pulses/s, 20 min) in the presence of TTX in the incubation medium, 5. pH lowering to 6.4, reaction time 20min, 6. pulse stimulation (100 pulses/s, 20 min) in low pH medium (pH 6.4), 7. addition of EGTA (5×10^{-3} mol/l); 8. addition of $LaCl_3$ (4×10^{-3} mol/l).

I 1160/1008; II 1526/1008; III 1526/1160;
a 1008 cm^{-1}, *b* 1160 cm^{-1}; *c* 1526 cm^{-1}.

Results and Discussion

In the 900—1700 cm^{-1} region, the Raman spectrum of the frog sciatic nerve contains 1526, 1193, 1160, 1008 and 960 cm^{-1} bands (Fig. 1). The spectrum resembles in its major features that of C_{40}-carotenoids (Koyama et. al. 1979). The 1526 cm^{-1} band is associated with the $-C=C-$ vibrations of the polyene chain; the 1160 cm^{-1} band is due to $=C-C=$ vibrations; the 1008 cm^{-1} band reflects the vibrations of $C-CH_3$ methyl radicals (Szalontai et. al. 1977).

Rhythmic pulse excitation reduces the intensity of the major carotenoid bands with no noticeable shifts in the position of the peaks (Fig. 2). Rhythmic

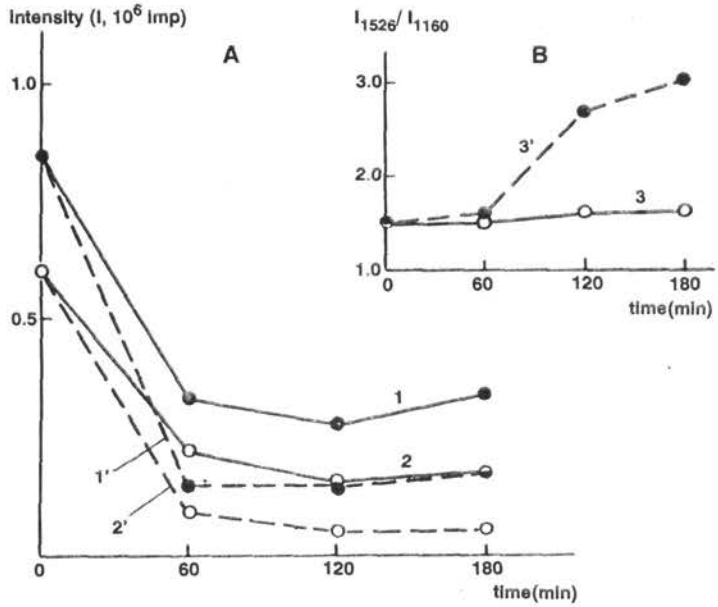

Fig. 3. Kinetics of changes in the major Raman spectral bands intensities (*A*) and their ratios (*B*) after addition of 5×10^{-3} mol/l EGTA (O——O) and $LaCl_3$ (O- - -O) into the incubation medium. 1,1': 1526 cm^{-1}; 2,2': 1160 cm^{-1}; 3,3': 1526/1160 cm^{-1}.

excitation of nerves is known to activate three potential-dependent channels: the sodium, the potassium, and the calcium channel (Hodgkin and Huxley 1952). The present investigation was aimed at studying the sodium channel; tetrodotoxin was used, known for its ability to block the sodium channel, as the probe of channel functioning (Hodgkin and Huxley 1952).

Tetrodotoxin (TTX) reduced the intensities of the 1008, 1160 and 1526 cm^{-1} bands of a resting nerve by 34, 13 and 20%, respectively. In the depolarized state during conduction of action potential, the blockage of the Na-channel by TTX had an opposite effect: the peak intensities were enhanced by 28, 39 and 60%, respectively. Hence, the Raman peaks of the carotenoids are enhanced when the channel is blocked, and are reduced when it is in operating conditions.

The surface charge of the exolemma and adjacent structures (myelin) was changed by varying the extracellular Ca^{2+} concentration by adding the chelator EGTA, or by acidifying the incubation medium, i.e. by the effect of competing $LaCl_3$ or H^+. The addition of EGTA caused the 1160 cm^{-1} and 1526 cm^{-1} bands to drop in intensity by 64 and 61%, respectively. The desorption of the

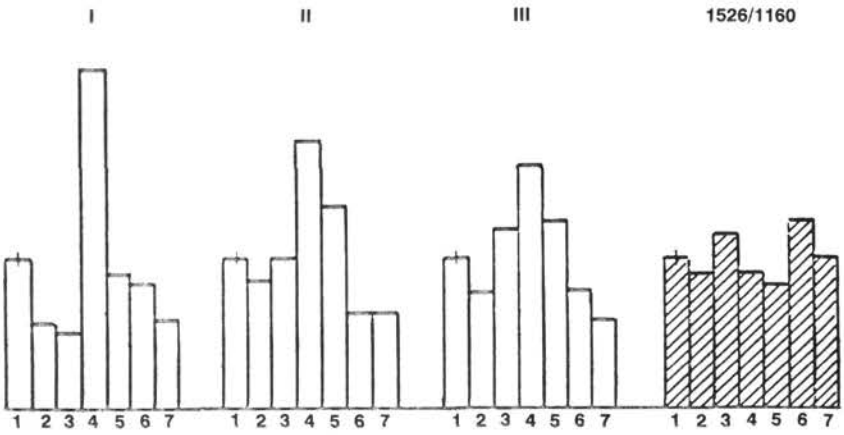

Fig. 4. Changes of Raman spectral bands intensities (empty columns) and their ratios (hatched columns) of β-carotene incorporated into liposomes.

I — 1008 cm^{-1}; II — 1160 cm^{-1}; III — 1526 cm^{-1}.

1. control, 2. in 30 mmol/l KCl, 3. in 30 mmol/l KCl in the presence of valinomycin (10^{-6} mol/l, 40 min), 4. in 30 mmol/l KCl in the presence of valinomycin (10^{-6} mol/l, 100 min), 5. in 20 mmol/l $CaCl_2$, 6. in 20 mmol/l $CaCl_2$ in the presence of A 23187 (10^{-6} mol/l, 40 min), 7. in 20 mmol/l $CaCl_2$ in the presence of A 23187 (10^{-6} mol/l, 100 min.).

membrane-bound Ca^{2+} by adding $LaCl_3$ resulted in an 85 and 87% drop of these peaks, respectively (Fig. 2). The substitution of the membrane bound Ca^{2+} by H^+, upon increasing the extracellular concentration of H^+ (by reducing pH from 7.4 to 6.4), resulted in a rise of the 1160 cm^{-1} and 1526 cm^{-1} peaks by 183 and 137%, respectively.

Fig. 3 shows the kinetics of the changes in the intensities of the 1160 and 1526 cm^{-1} bands of a nerve incubated with EGTA or $LaCl_3$. On reducing the Ca^{2+} concentration in the surrounding environment of the membrane-bound Ca^{2+}, the intensities of these two peaks steadily decreased in both cases toward the steady-state levels. Presented in Fig. 3*B* are the kinetics of changes of the I_{1526}/I_{1160} ratio (I is the intensity of the band peak) after the addition of EGTA and $LaCl_3$ to the incubation medium with $LaCl_3$ the I_{1526} ratio rose faster than with EGTA.

It is evident that excitation leads to increased I_{1160}/I_{1008}, I_{1526}/I_{1008} and I_{1526}/I_{1160} ratios. This suggests that the polyene chain of the carotenoid molecule undergoes compression upon excitation, and that the methyl radicals are displace closer to the centre line of the molecule (Koyama et. al. 1979; Szalontai

et. al. 1982). This is accompanied by a lowering of the intensities of the Raman spectral bands. The Na-channel opens as a consequence of a change in the intramembrane potential.

The carotenoid molecule is also compressed under the action of TTX, i.e. upon blockage of the Na-channel. TTX is known to leave the gate unaffected (Hodgkin and Huxley 1952). The changes of the carotenoid conformation in response to changes in the intramembrane potential were the same for both operating and blocked Na-channel. This suggests that the membrane-associated carotenoids act as a "potential sensor", i.e. as a sort of "an antenna" for the channel.

After desorption of the membrane-bound Ca^{2+}, the intensities of the bands decreased and the I_{1526}/I_{1160} ratio increased in parallel to changes in the Raman spectrum of carotenoids accompanying the rhythmic excitation of nerves. Probably, desorption causes a local change in intermembrane potential accompanied by an alteration of the carotenoid conformation. Substitution of the membrane-bound Ca^{2+} by H^+ is accompanied by an increase of the intensities of the 1160 cm^{-1} and 1526 cm^{-1} peaks with no effect on the carotenoid conformation. It is well known that the Na-channel is blocked at low pH by H^+ binding to the negatively charged groups (Hodgkin and Huxley 1952). However, in contrast to the TTX effect, an increase of the extracellular H^+ concentration is likely to inactivate the potential sensor.

The conformation of β-carotene incorporated into liposomes was studied as a model of potential dependent conformation changes in biological membranes.

To study potential-dependent changes of conformation of β-carotene incorporated in liposomes, K^+ or Ca^{2+} gradient was set up across the membrane of vesicles. Then, potassium or calcium ionophore was applied (valinomycin or A23187) and effects of gradient changes on the RS band intensities of β-carotene were determined. Fig. 4 shows the results of experiments with potential-dependent conformational changes of β-carotene.

Addition of potassium or calcium ions into the medium resulted in a decrease of the intensity of all bands; in the second case the amplitude remained either unchanged or it increased. Opposite effects were observed in the presence of ionophores. The amplitudes of all bands increased in the presence of valinomycin and decreased in the presence of A 23187.

To evaluate the conformational state of the β-carotene molecule the ratio of band amplitudes at 1526 cm^{-1} and 1160 cm^{-1} was calculated (Szalontai 1977). Fig. 4 shows that in the presence of ionophores this ratio increased in the first 40 min, followed by decrease to almost the initial levels.

The results obtained provide evidence for the occurrence of potential-dependent conformational changes of the β-carotene molecule incorporated

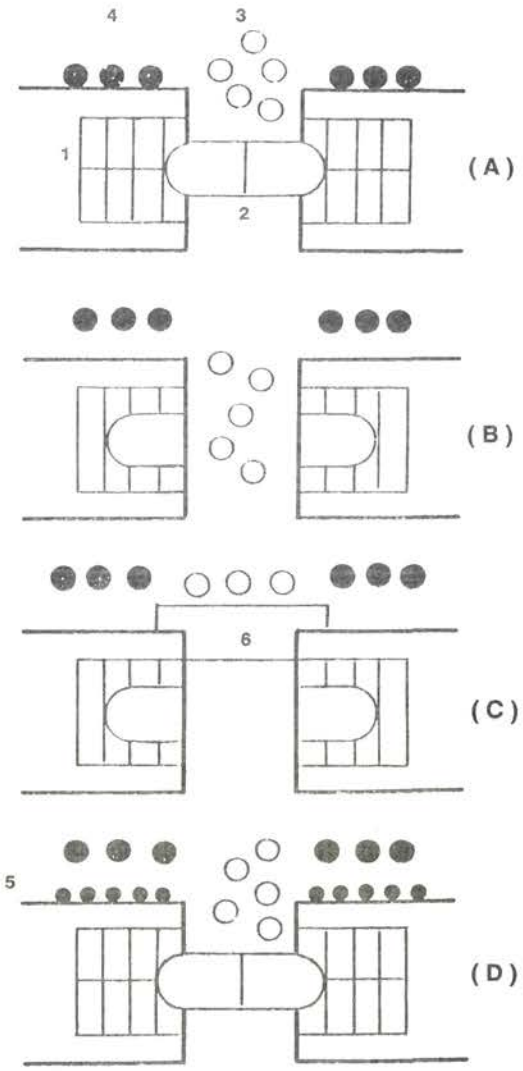

Fig. 5. Regulation of Na-channel activity by the potential sensor. (*A*) at rest; (*B*) excitation; (*C*) blockage by TTX (*D*) excitation in a low pH medium. 1. "potential sensor"; 2. "gate" mechanism; 3. Na^+ ions; 4. Ca^{2+} ions; 5. H^+ ions; 6. TTX

into lecithin liposomes. It should be noted that with the exception of the changes in the RS band intensities in the presence of valinomycin and A 23187, the 1526/1160 cm^{-1} band ratio changed in the same direction. One can suppose that changes in K^+ or Ca^{2+} membrane gradients result in compression of the polyene chain of the β-carotene molecule. As soon as there are no more changes in membrane potential, the molecule conformation returns to the initial state. There was no ionic specifity of the potential-dependent conformational change of β-carotene; during ion translocation across the bilayer membrane the carotene molecule undergoes structural changes resulting in the compression of the molecule. When the carotene molecule is a part of the potential-dependent channel, it probably can play the role of the "potential sensor".

Fig. 5 offers a diagrammatic presentation of the possible operation of the potential dependent Na-channel, assuming carotenoids as the potential sensor. In a closed channel (Fig. 5*A*), the gate shuts the entrance pore and partly penetrates into the hydrophobic region of the membrane. The polyene chains of carotenoids are also located in the hydrophobic region. Membrane depolarization is associated with the compression of the carotenoid molecules in response to the establishment of a negative local potential, or as a consequence of Ca^{2+} desorption. This enables the gate to immerse deeper into the hydrophobic phase of the bilayer and to open the channel for ion transport (Fig. 5*B*). Under the effect of TTX or low pH the channel is blocked. However, the effect of TTX is associated with a potential-dependent change in the carotenoid conformation, whereas this is not the case at low pH. It is reasonable to assume that upon increasing extracellular H^+ concentration the site of channel blockage is inside rather than outside of the channel. The conformation of the potential sensor remains unchanged in this case (Fig. 5*D*).

References

Hauser H., Guyer W., Pascher I. (1980): Polar group conformation of phosphatidylcholine: Effect of solvent and aggregation. Biochemistry **19,** 367—373

Hodgkin A. L., Huxley A. F. (1952): The dual effect of membrane potential on sodium conductance in giant axon of Loligo. J. Physiol. (London) **116,** 497—500

Koyma Y., Long J., Martin W. A., Carey P. R. (1979): The resonance Raman spectrum of carotenoids as an intrinsic probe for membrane potential. Biochim. Biophys. Acta **548,** 153—160

Maksimov G. V., Tilov B. O., Lichachov Ir. V., Churin A. A., Rubin A. B. (1982): The resonance Raman spectrum of nerve during excitation and block. Dokl. AN SSSR **262,** 1272—1275

Maksimov G. V., Churin A. A., Paschenko V. Z., Rubin A. B. (1986): The potential-dependent changes of resonance Raman spectrum of β-carotene. Biofizika **31,** N 3, 456—458 (in Russian)

Szalontai B., Bagynkas C., Horvath L. I. (1977): The resonance Raman spectrum of nerve. Biochem. Biophys. Res. Commun. **76,** 660—665

Final version accepted February 19, 1990