CHANGES IN THE RAMAN SPECTRUM OF FROG SCIATIC NERVE DURING ACTION POTENTIAL PROPAGATION

B. Szalontai, Cs. Bagyinka, and L.I. Horváth
Institute of Biophysics, Biological Research Center, Hungarian Academy of Sciences
H-6701 Szeged, P.O.B. 521, Hungary

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Summary: Raman spectra of frog sciatic nerves were recorded in different states of functioning. During excitation reversible changes were observed in the C_{40}-carotenoid peaks enhanced by the resonance Raman effect. This change can be explained by transient carbon-carbon bond equalization of the polyene chain. Possible biological consequences are also discussed.

Introduction: Several carotenoids are natural constituents of biomembranes. The carbon skeleton of C_{40}-carotenoids consists of 9-11 conjugated double bonds which give rise to an intense $\pi^{+} \leftarrow \pi$ transition in the blue region (at 476 nm) and, thus the vibrational modes of the carbon chain are strongly enhanced by the resonance Raman effect using blue excitation (1-6). The resonance Raman spectra of C_{40}-carotenoids are dominated by two intense bands at 1525 cm^{-1} and 1158 cm^{-1} which were assigned to ν(-C=C-) and ν(=C-C=)+δ(CH) vibrations, respectively (3-5). Recently it was suggested that carotenoids being membrane constituents can be utilized as *in situ* reporter molecules since (i) minute amounts yield well-resolved spectra through the resonance enhancement, and (ii) the enhancement is very sensitive to local changes in the microenvironment of the carotenoid molecules (5). The firt thorough investigations were done on erythrocyte ghosts (7), and it was suggested that membrane perturbations bring about dras-

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tic changes in the microenvironment of the reporter β-carotene. Frog sciatic nerve is also known to exhibit carotenoid Raman pattern (8), and we here report on the effect of biological excitation upon the Raman spectra of carotenoid molecules.

Experimental: Sciatic nerves were dissected from the frog Rana esculanta. During the preliminary experiments we found that the nerve fiber was very sensitive to the intense laser light. To prevent its damage we designed a special sample holder in which the center part of the fiber exposed to the laser beam was bathed in Ringer solution. This enabled us to detect Raman spectra during simultaneous electric stimulation as well. Raman spectra were taken at room temperature with a Cary 82 spectrometer using an argon ion laser at 514.5 nm and 200 mW. Variability in the condition of the animals may account for slight variations in optical responses. Nerves were stimulated by suare pulses of max. 1 V and 0.1 msec width at 200 Hz. Good quality action potential could be observed for 2-3 hours without scanning the laser beam on the nerve.

Results and Discussion: In the 900-1700 cm^{-1} region the Raman spectrum of the resting frog sciatic nerve was remarkably similar to that of carotenoids (5,10). In fact from the peak positions and the relative intensities we drew the conclusion that these are the resonance enhanced modes of C_{40}-carotenoids (5),(Fig.1). In accordance with previous data (5) we found that in the case of β-carotene the ν(-C=C-) was shifted from 1527 cm^{-1} to 1520 cm^{-1} depending on carotene-solvent interaction. Since the ν(-C=C-) mode was observed at 1520 cm^{-1} in the resting nerve we concluded that the nerve C_{40}-carotenoid(s) should interact with its molecular environment. (This comparison of nerve C_{40}-carotenoid with β-carotene is justified by the fact that the Raman spectra of C_{40}-carotenoids are almost indistingushible from each other (5).) Although neither the structure nor the role of carotenoid-binding in the membrane is as yet known it is interesting to mention that the membrane-bound carotenoid remained more or less intact even after prolonged exposure to laser light whereas β-carotene proved to be very unstable in

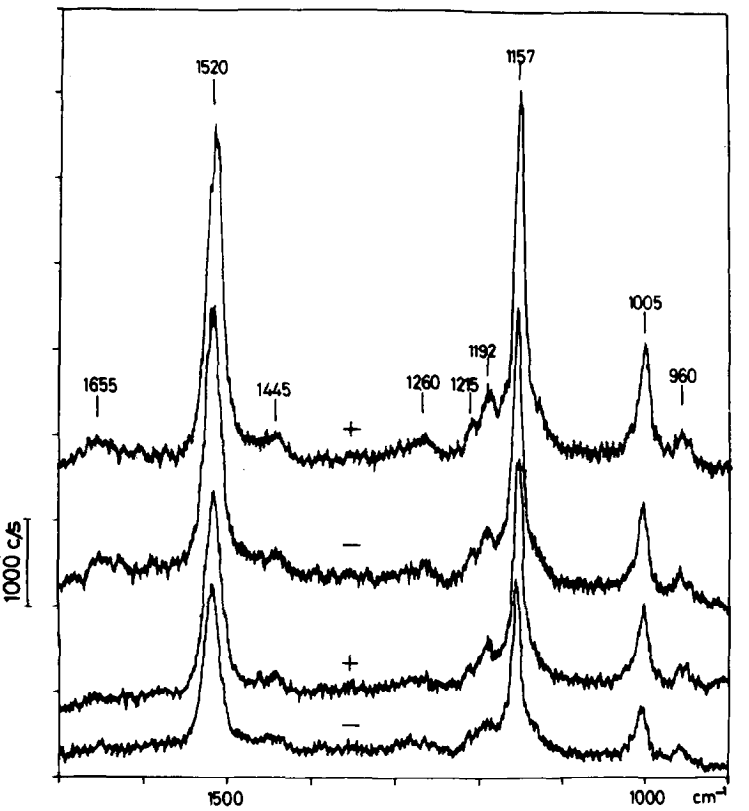

Fig. 1 Raman spectrum of frog sciatic nerve dominated by resonance enhanced C_{40}-carotenoid peaks. Instrumental setting: slit 4 cm^{-1}, scan speed 1 $cm^{-1}sec^{-1}$, pen period 1 sec. Signs + and - denote spectra taken in excited and resting state of the nerve, respectively. Note the overall deterioration of the spectra during the experiments.

lecithin multi-bilayers (L.I. Horváth to be published).

During the excitatory process the ν(-C=C-) mode exhibited characteristic changes:

(i) The ν(-C=C-) band was shifted from 1520 cm^{-1} to 1518 cm^{-1} as revealed by high resolution scans.

(ii)The I_{1520}/I_{1157} ratio was decreased (Fig.1). The spectra exhibited a slight (although apparent) deterioration and, thus, all I_{1520}/I_{1157} values were corrected for simulta-

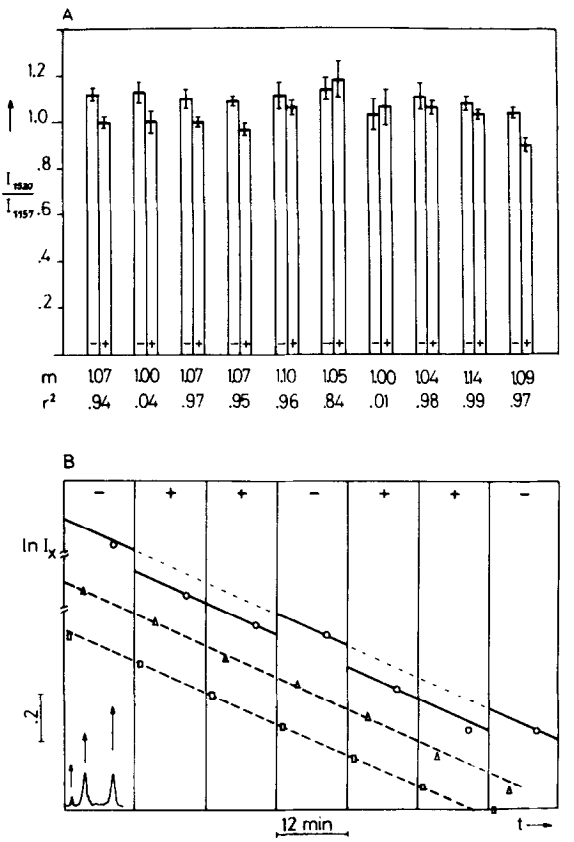

Fig. 2 a - Corrected I_{1520}/I_{1157} values of different nerve tissues: Each column-pair represents the average of 4-6 measurements on a nerve in stimulated (+), and resting (-) state.
Bars indicate the standard deviation. m is the correction factor for simultaneous peak amplitude measurement. r^2 is the correlation of m ; note that those nerves which exhibited no effect have poor correlation with varying deterioration.

b - The logarithm of uncorrected band intensities versus time: The intervals represent successive measuring cycles in the resting (-) and excited (+) state of the nerve. (I_{1520}: o-o-o, I_{1157}: Δ-Δ-Δ, I_{1005}: □-□-□)

neous peak amplitude measurement. This correction factor (m, Fig.2a) could be calculated from the observed deterioration rate and the scan speed. Finally all I_{1520}/I_{1157} values were collected in Fig.2a grouping data of each individual nerve

tissue in a column-pair. Since the I_{1520}/I_{1157} ratio characteristically depends on the state of functioning we clearly have something to explain. As a resonable assumption the question arose whether the biological excitation affected the deterioration rate of the spectra. Plotting the logarithm of uncorrected band intensities versus time (Fig.2b) this possibility could be ruled out since all bands exhibited a uniform deterioration rate not depending on the state of functioning. Note that the point of the 1520 cm^{-1} band fit to two paralel lines in Fig.2b, indicating that during excitation the intensity of the ν(-C=C-) band was lowered. This finding was also confirmed by comparing the ratios I_{1520}/I_{1005} and I_{1157}/I_{1005} in the excited and resting states. (The 1005 cm^{-1} band was suggested to be taken as an internal reference (5).) From these results we concluded that it was the ν(-C=C-) band alone which was responsible for the changing I_{1520}/I_{1157} ratio.

An explanation for the decreasing Raman intensity is provided by the fact that the observed I_{1520}/I_{1157} ratio (during excitation) is similar to that of longer polyene chains where the π-electrons are strongly delocalized (5). The observed shift of the ν(-C=C-) mode towards lower frequencies also indicates that the decreasing Raman intensity should be connected to changing bond order. Since other peaks are considerably less (if at all) affected changes in the $\pi^{+} \leftarrow \pi$ transition probabilities can be ruled out. However the polarizability and, thus, the Raman intensity also depends on the mixing term which describes the mixing of S_1 (first excited) state electronic wavefunctions under vibrational perturbation (9). S_1 state properties changing due to transient π-electron delocalization should manifest through changes in the resonance en-

hancement. In this case the enhancement of the ν(-C=C-) band may change selectively since the 1157 cm^{-1} and 1005 cm^{-1} bands are known to be insensitive to delocalization and chain lenth (5). On the basis of this argument we tentatively assign the transient reversible decrease of the ν(-C=C-) band to partial carbon-carbon bond equalization of the polyene trough.

Raman optical responses were observed during action potential propagation and the bond order of membrane-bound carotenoids were found to be changed. Carotenoids have been proposed to operate as electronic conuctors in biomembranes (10). In view of this, our finding on transient carbon-carbon bond equalization (i.e. rearrangement of π-electrons) may raise the possibility that the membrane-bound carotenoids are involved in the conduction of nerve impulses.

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