A Study of Demyelination of Nerve Fibers Using Dynamic Phase Contrast Microscopy

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Dynamic phase microscopy was used for evaluation of changes in myelinated axon segment in the paranodal region of nerve fibers during demyelination. Normally paranodal myelin sheath is characterized by regular oscillations of the optical path difference with frequences of 4.2 and 6.7 Hz. Demyelination decreased the amplitude and conduction velocity in nerve fibers and shifted the characteristic frequencies of optical path difference oscillations to 2.8, 3.2, and 11 Hz. These shifts of optical path difference frequencies probably resulted from disturbances in the state of charged phospholipids and a decrease in the level of bound Ca^{2+} during demyelination of nerve fiber.

Key Words: *axon; dynamic phase microscopy; demyelination*

Some pathological processed in the nervous system are associated with disintegration of myelin sheath (demyelination), which decreases the conduction velocity of action potentials (AP) and desynchronizes conduction of rhythmic excitation (RE). This condition is referred to as Landry—Guillain—Barre syndrome (acute idiopathic polyneuritis) in the peripheral nervous system or multiple sclerosis in the CNS [8,9,11]. Electrophysiologically, demyelination is manifested by changes in the conduction velocity and amplitude of AP, and by the ratio of the reproducible optimal and maximum rhythms [9]. At the cellular level, demyelination is manifested by a decrease in myelin content in the internodal region, appearance of additional K^+ channels in naked nerve fiber segments, and changes in activity of electrogenic Na pump. Accumulation of lysoforms of some phospholipids and disturbances in Ca^{2+} homeostasis in internal myelin layers (Schmidt—Lantermann clefts and mesaxon) affect the interaction between the axon and Schwann cell [6,8,11]. The character of this interaction in myelinated nerve fiber determines the dynamics of rapid and regular changes in its myelin sheath.

Our aim was to study the dynamics of fast and regular changes of myelin structure in the region of Ranvier node during nerve fiber demyelination.

MATERIALS AND METHODS

Experiments were performed on isolated myelinated nerve fibers from *Rana temporaria*. Demyelination of nerve fiber was caused by incubation in a medium containing lysophosphatidylcholine (LPC, 10^{-4} M) in dimethyl sulfoxide. This treatment initiates demyelination with signs characteristic of autoimmune demyelination [6,11]. Standard extracellular recordings of the threshold, excitation rate, and AP amplitude were carried out for evaluation of changes in electrical parameters of the nerve during demyelination [1,2].

For light microscopy the nerve fiber was placed into a special chamber containing the following solution (in mM): 111.2 NaCl, 1.88 KCl, 1.08 $CaCl_2$, (18-20°C, pH 7.2). A Tsitoskan laser-based computerized phase microscope was used (Fig. 1, *a*), which consisted of an interferential microscope modified by the method of Linnik with phase modulation of the refe-

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rence wave, a He-Ne laser (λ=633 nm) to illuminate the specimen with coherent light, and an image dissector (a coordinate-sensitive photodetector) [3,4,10]. The measurements of thin transparent biological specimens were performed in the reflected light. An Olympus objective (20×0.45) was used for recordings with following parameters: visual field 20×20 μ, measurement time in one point of the scan-line T_i=1 msec, sampling time of track-diagram 14.7 sec. The microscope formed the real-time images as digitized two-dimension phase distribution function h(X,Y) measured in length units (optical path difference, OPD). Dynamic phase microscopy is based on periodic measurements of the phase along an arbitrarily segment (scan-line) of the image [7]. The resulting numerical matrix obtained during repeated scanning contained the data about changes of the local phase altitude in the points of the scan line. The matrices were processed to reveal fluctuations of the local path difference (phase altitude) h(X,Y), which is proportional to projection of local refraction index n(X,Y,Z). At n(X,Y,Z)<<1, the phase altitude is a function of diffraction coefficient, so it does not relate to the real geometric thickness of the specimen.

Phase altitude h(X) was measured periodically along the scan-line (6.1 μ) chosen transversely to the fiber at X coordinate (Fig. 1, *b*, *c*). The data array was converted into ASCII codes presented in two-dimension rectangular matrices 40×400 processed off-line with standard software. The control measurements in OPD were carried out with silicon carrier.

Fluorescent measurements of membrane-bound calcium are based on the ability of antibiotic chlortetracycline (CTC) incorporated in the membrane to form a complex with Ca^{2+}, which increases the quantum yield of fluorescence [5]. Fluorescence was recorded from the same segment of isolated fiber with a Lyumam I-3 luminescent microscope (LOMO). Fluorescence of CTC was induced by a KGM 9×70 incandescent halogen lamp combined with FS-1-6 and SZS 21-2 filters. Recording was performed with photometric head and interference light filters with maximum transmission wavelengths of 490 and 550 nm. Diameter of the examined segment was 50 μ, when a ×10 objective was used.

RESULTS

In myelinated nerves LPC impairs AP conduction along the fibers (Fig. 2, *b*): 5 min after the start of demyelinating treatment conduction velocity and amplitude of AP decreased by 70 and 80%, respectively. These data confirm our assumption that LPC-induced demyelination is associated with myelin degradation and impairment of axon—Schwann cell interaction [8,11]. These disturbances were studied by dynamic phase microscopy highly sensitive to changes in the refraction index.

To study isolated myelinated nerve fiber, we choose a myelin region neighboring to the Ranvier node. Phase altitude and the shape of phase profile were used to assess the changes in OPD (Fig. 1, *c*). Incubation of nerve fiber in LPC solution during 40 min produced characteristic changes in the phase profile: it decreased its altitude from h_1=140 nm to h_2=110 nm and its width (measured at the 0.5 level) from 4.0 to 3.5 μ. Probably, the decrease in the phase profile parameters

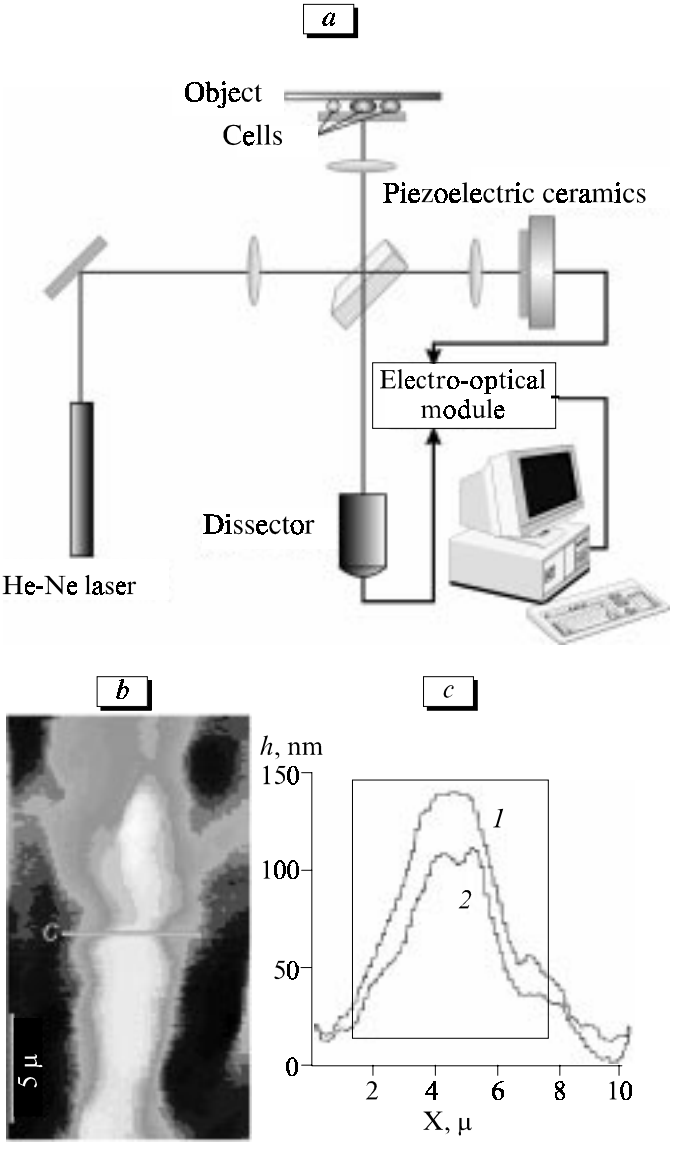

Fig. 1. Block-diagram of a Tsitoscan computerized phase microscope (*a*), phase image of a segment of nerve fiber (*b*), and changes in the profile of phase altitude *h* of a fiber during 40 min of the action of lysophosphatidylcholine (*c*; the frame shows the scan-line profiles). *C* and *X* designate a scan-line and its coordinate, respectively. *1*) control phase profile at the beginning of experiment; *2*) phase profile after incubation in lysophosphatidylcholine.

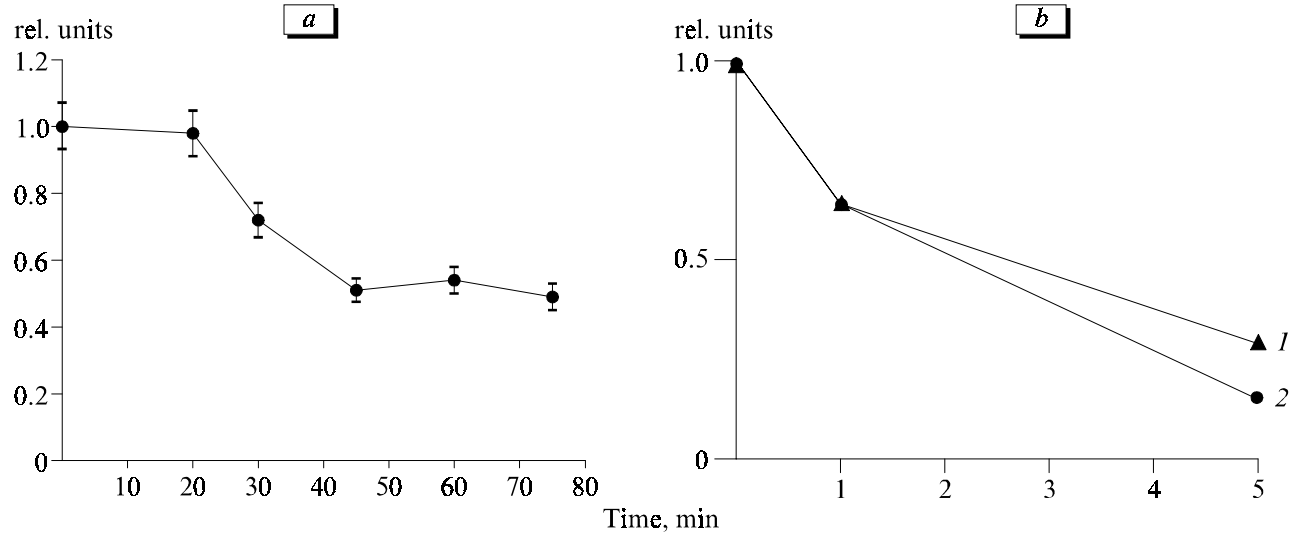

Fig. 2. Effect of lysophosphatidylcholine on the content of membrane-bound Ca^{2+} (*a*), conduction velocity (*b*, *1*), and amplitude of action potential (*b*, *2*) in myelinated nerve fibers.

during demyelination by 20% results from a decrease in myelin optical density.

Previously we observed no changes in the altitude of phase profile in the paranodal region during rhythmical excitation of myelinated nerve fiber, although the phase altitude of the nodal region was clearly changed under these conditions. Probably, under the normal conditions a decrease in optical density in the nodal region not accompanied by significant changes in its transverse size attests to changes in the refraction index of the axolemma (and/or Schwann cell) and are not related to myelin. By contrast, in a demyelinated fiber, a decrease in the phase profile altitude is accompanied by the changes of the transverse geometrical

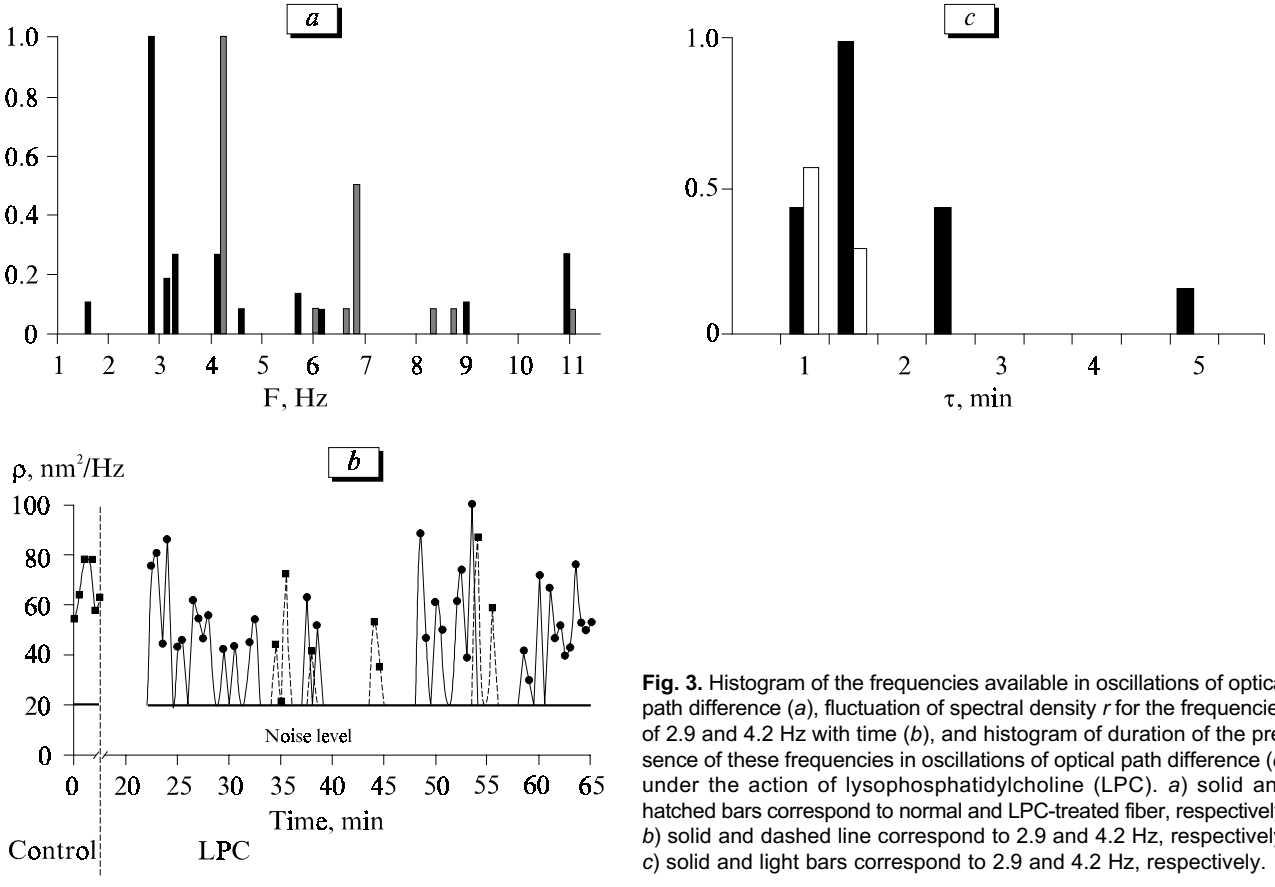

Fig. 3. Histogram of the frequencies available in oscillations of optical path difference (*a*), fluctuation of spectral density *r* for the frequencies of 2.9 and 4.2 Hz with time (*b*), and histogram of duration of the presence of these frequencies in oscillations of optical path difference (*c*) under the action of lysophosphatidylcholine (LPC). *a*) solid and hatched bars correspond to normal and LPC-treated fiber, respectively; *b*) solid and dashed line correspond to 2.9 and 4.2 Hz, respectively; *c*) solid and light bars correspond to 2.9 and 4.2 Hz, respectively.

size in the region where myelin borders to axolemma of Ranvier node, which attests to myelin contribution into the changes in OPD and phase profile.

For evaluation of possible mechanisms of these changes in myelin OPD during demyelination, we used the spectrums of phase profile fluctuations (Fig. 3, *a*). In the control spectrum of myelin phase altitude fluctuations, the maximum probability of fluctuations corresponds to oscillation in optical density at the frequencies of 4.2 and 6.6 Hz, the probability to reveal oscillations at other frequencies being pronouncedly lower. LPC significantly changed the spectrum of myelin phase altitude fluctuations: the decrease in probability of myelin optical density oscillations at 4.2 and 6.7 Hz was accompanied by appearance of new oscillations at 2.8, 3.2, and 11 Hz. Therefore, demyelination is accompanied by redistribution of frequencies of optical density fluctuations or myelin volume.

To reveal the details of this redistribution, we examined changes in the spectral densities at the frequencies characteristic of the normal and demyelination states (4.2 and 2.9 Hz, respectively, Fig. 3, *b*). The data indicate that spectral densities of both frequency components can oscillate with different periods during the action of LPC (Fig. 3, *c*). However, the maximum probability of 2.9-Hz oscillations (characteristic of damaged myelin) is observed for a longer time than that of 4.2-Hz oscillations.

The fact that similar changes in myelin OPD did not occur in the paranodal region of the nerve fiber attests to localization of the regular structural changes in myelin in the contact region of myelin, axolemma, and Schwann cell. The decrease in the frequency of phase altitude fluctuations attests to primary disturbances in the interaction between axon and myelin during demyelination. Indeed, while disturbing myelin integrity, demyelination changed the content of bound Ca^{2+} (Fig. 2, *a*), which decreased the quantum yield of fluorescence CTC. It seems that myelin degradation and desorption of bound Ca^{2+} promoted a decrease in oscillations of the intercellular processes, which control the volume and optical density of the myelin.

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