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SKEW ORIENTATION OF BIOLOGICAL SAMPLES *ANACYSTIS NIDULANS* CYANOBACTERIA AND THEIR FRAGMENTS IN A POLYMER MATRIX

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SUMMARY: *Anacystis nidulans* cyanobacteria and their fragments embedded in unstretched, uniaxial and skew (two axes of stretching forming an angle of 40^0) stretched poly(vinyl alcohol) films have been investigated. Polarized absorption spectra for uniaxial and skew stretching samples were measured. Both unoriented and oriented samples were photographed under fluorescence microscope. In skew samples a high degree of cell orientation was reached. Skew deformation of polymer matrix compared to one axis stretching provides better band resolution in polarized absorption spectra of *Anacystis nidulans* samples. The shapes of absorption components measured in respect to the first and second axis of stretching are different which gives the opportunity to investigate position of various group of chlorophyll molecules in membrane.

The biological tissue is a predominantly anisotropic material. In the case of biological membranes it is the liquid crystalline structure-oriented and to some extent fluid (1). The anisotropic structure of biological membranes is closely related to their functions. In photosynthetizing organisms like plants, algae and bacteria the pigments responsible for light absorption and excitation energy transfer to the photochemical reaction centers are attached in such a way to protein macromolecules embedded in bi-layers membrane that the process of energy transfer is very efficient. In photodynamic therapy the selectivity of incorporation of used dyes to leucemic cells membranes also depends on the membrane structure (2). For pure and applied photobiology it is important that the structure of natural biological membranes can be investigated

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using the polarized light spectroscopy to artificially oriented cells or their fragments (3-5).

The pigment-protein complexes, membrane fragments or whole bacteria can be artificially arranged using squeezed gel or several procedures such as introducing the organisms into anisotropic film, into the polymer film which become anisotropic as a result of mechanical deformation or by suspending them in nematic liquid crystals oriented by electric or magnetic field (6).

There has been a long history of investigation the stretching polymer films to produce uniaxial or biaxial states of orientation (7,8). However, little attention has been given on process in which the films anisotropy is created using skew (i.e. nonperpendicular) stretches. Lately, Wu et al. (9) have reported about several physical properties of skew stretched polymer films. They have found that symmetry axes of sequential skew stretched films trend to move towards second stretching direction as increasing second stretching degree. At a low second stretching degree the samples exhibit a biaxial behaviour. We have used the skew stretched poly(vinyl alcohol) films (PVA) to the orientation of elongated dye molecules (10), and biological samples (11). It has been shown that protonated and free base forms of stilbazolium merocyanine molecules exhibit different distribution of emission transition moments around the skew axes of PVA film. The absolute values of absorption and emission anisotropy of dye molecules in the skew samples are higher than for uniaxially stretched films. Additionaly, what is even more important in the case of biological systems with several chromophores, the angular distribution function of emission and absorption transition moments around the preferential orientation axis is narrower in the case of the skew films than in uniaxially stretched ones. The last feature gives an opportunity to resolve the contributions from various chromophores even with strongly overlapped spectra when they differ in the orientations in macromolecular frame.

In this paper two examples of biological preparations of whole and fragments cyanobacteria *Anacystis nidulans* embedded in unstretched, uniaxially and skew stretched PVA films have been investigated. The purpose of this note is not to discuss the structure of the samples but only to demonstrate the advantage of skew method in comparison with uniaxial film deformation.

MATERIALS AND METHODS

The methods of *Anacystis nidulans* cyanobacteria culturing and embedding in PVA films were previously described (12-14). The polarized absorption spectra were recorded on Perkin-Elmer 553 UV/VIS spectrophotometer. The unstretched, uniaxially and skew stretched samples were photographed using Nikon Measurescope UM2 (Japan) provided with Nikon-Super High Pressure Mercury Lamp with Power Supply Model HB-10101 AF Sony-Triniton Color Video Monitor and Sony Video Graphic Printer UP-850. The samples were illuminated with λ = 420-490 nm, photographed in light with λ > 590 nm.

RESULTS AND DISCUSSION

The two differ in shape types of samples give the opportunity to demonstrate the results of skew stretching for elongated filements (*Anacystis nidulans* whole cells, Fig. 1A-C) and shorter parts of filements (*Anacystis nidulans* fragments, Fig. 1D-F). As it was previously shown for *Tolypothrix tenuis* cyanobacteria in skew PVA films the film stretching of long filements caused predominantly the elongation of nonfluoresecent sheaths (11). Similar effect is shown for *Anacystis nidulans* cells (Fig. 1B and 1C) as an increase of dark distances between fluorescent cells almost without the changes in a shape of fluorescent pigmented cells. These dark spaces are almost twice longer in the skew than in uniaxially oriented samples. At the uniaxial stretching more fragments of filements are still unoriented than at the skew stretching. Average for 50 filements angle of inclination of their long axis from stretching direction is lower, in the case of *Anacystis nidulans* whole bacteria, calculated in respect to the second axis of the skew sample (equal $\langle\alpha_3\rangle = 14^0$), higher for uniaxial stretching ($\langle\alpha_1\rangle = 25^0$) and the lowest for the second axis of skew stretching ($\langle\alpha_2\rangle = 36^0$). This gives a differece in average $\langle\cos^2\alpha\rangle$ (having influence on dichroism of absorption) equal $\langle\cos^2\alpha_1\rangle = 0.82$, $\langle\cos^2\alpha_2\rangle = 0.66$ and $\langle\cos^2\alpha_3\rangle = 0.94$.

Figs. 1E-F show that shorter fragments of *Anacystis nidulans* filements exhibit higher degree of orientation ($\langle\alpha_1\rangle = 20^0$, $\langle\alpha_2\rangle = 28^0$ and $\langle\alpha_3\rangle = 10^0$) and similar elongation of sheaths. The deformation in both types of stretching is similar but the inclination from preferential orientation axis is smaller (in respect to the second axis) for skew then for uniaxial stretched films. The large bright regions are probably related to clusters of the cell fragments. It was previously shown (15) that chloroplasts can also be deformed in stretched PVA films. Small elongated

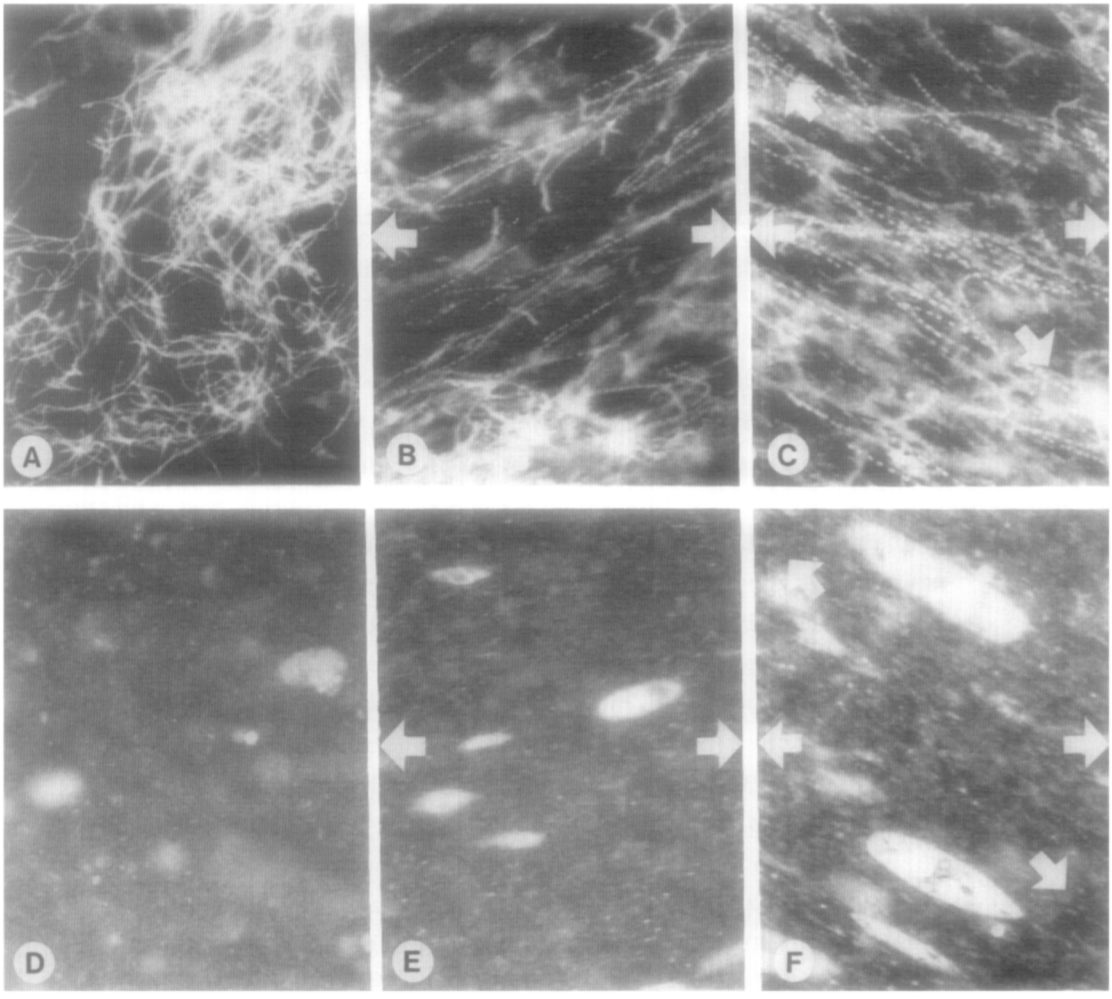

Fig. 1. Photographs of investigated whole (A,B,C) and fragments (D,E,F) cyanobacteria *Anacystis nidulans* in: unstretched (A,D), one axis stretched (B,E) and skew stretched (C,F) PVA films.
The stretching directions are marked on the photographs. Magnification x140.

biological samples are prediminantly oriented whereas larger and spherical or discoidal ones are usually deformed. In both cases the anisotropy of absorption and emission spectra has been observed. In deformed samples, however, the mutual orientation of chromophores can be changed as a result of film stretching. In Fig. 2 polarized absorption spectra of investigated samples *Anacystis nidulans* at uniaxial orientation and the absorption

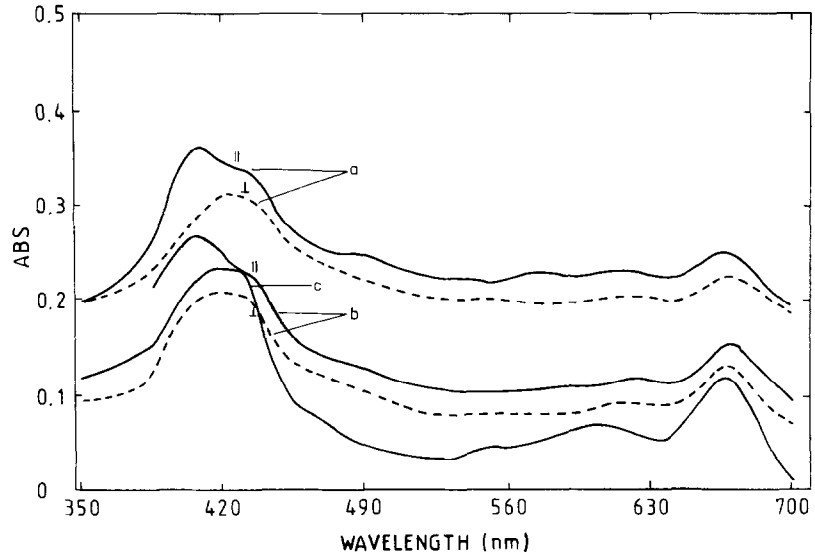

Fig. 2. The polarized absorption spectra of one axis stretched samples of: a/ *Anacystis nidulans* whole bacteria, b/ *Anacystis nidulans* fragments and c/ the absorption spectrum of unstretched sample of *Anacystis nidulans* fragments.

spectrum of unstretched sample of *Anacystis nidulans* fragments are shown. The polarized absorption spectra (Fig. 2) show that even at one axis deformation *Anacystis nidulans* fragments are oriented in a high enough degree to create the spectral anisotropy. Comparing the absorption anisotropies of investigated samples at uniaxial and skew film deformations in red and Soret bands of the *Anacystis nidulans* whole bacteria (Figs. 2 and 3) one can conclude that deformation provides higher degree (about 30 %) of absorption anisotropy for skew then for uniaxial stretching. A more interesting feature of skew stretching appears also from the comparison of Figs. 2 and 3. As it follows from Fig. 2, the difference in the shape of parallel and perpendicular components of absorption spectra is only observed in Soret band region. In polarized light spectroscopy Soret band region is especially complicated because of overlapping both B_x and B_y components (16) of various forms of pigments. As it follows from Fig. 3 the shapes of absorption components measured in respect to the first and second axis of skew stretching are considerably different.

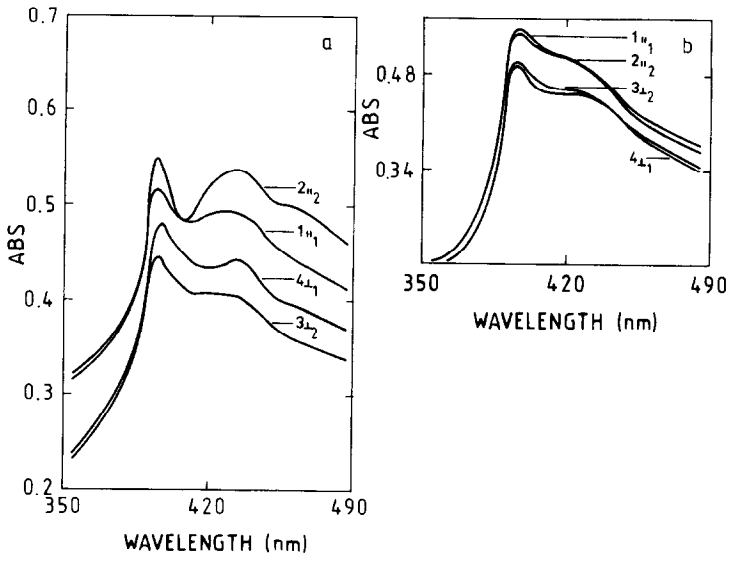

Fig. 3. The polarized absorption spectra of skew stretched samples of: a/ *Anacystis nidulans* whole bacteria, b/ *Anacystis nidulans* fragments. Curves 1 and 4 are parallel and perpendicular components obtained for the first axis of stretching, curves 2 and 3 to the second axis of stretching.

This gives an additional opportunity to investigate position of various group of chlorophyll molecules in membrane. This opportunity has been used in the investigation of of various complexes orientation at different light adaptation processes of cyanobacteria. It can also be used in many other biological or photobiological investigations where it is important to establish location of natural chromophore or artificially introduced dye in biological membrane.

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REFERENCES

1. Brown, G.H. and Wolken, J.J. (1979) Liquid Crystals and Biological Structures Academic Press New York-San Francisco-London.

2.Schlegel, R.A., Phelps, B.M., Waggoner, A., Ferada, L. and Williamson, P. (1980) Cell 20, 321-328.

3.Breton, J. & Vermeglio, A. (1982) Photosynthesis: Energy conversion by plants and bacteria Vol. 1 (Govindjee ed.) pp. 153-194 Academic Press, New York.

4.Frackowiak, D., Wrobel, D. and Dudkowiak, A. Trends in Photochemistry and Photobiology (in press).

5.Frackowiak,D. (1990) Appl. Fluorescence Technology 2, 11-14.

6.Thulstrup, E., Michl, J. and Eggers, J.H. (1977) J. Phys. Chem. 74, 3868-3874.

7.Uejo, H. and Hoshino, S. (1970) J. Appl. Polym. Sci. 14, 317-321.

8.Cakmak, M., White, J.L. and Spruiell, J.E. (1986) J. Polym. Eng. 6, 291-300.

9.Wu, J.P., White, J.L. and Cakmak, M. (1989) Colloid Polym. Sci. 267, 881-888.

10.Frackowiak, D. and Skibinski, A. (1991) Spectroscopy Lett. 24, 1317-1328.

11.Frackowiak, D., Zelent, B., Skibinski, A. and Leblanc, R.M. Photochem. Photobiol. submited for publication.

12.Govindjee and Rabinowitch, E. (1960b) Biophys. J. 1, 73-89.

13.Goedheer, J.C. (1969) Biochim. biophys. Acta 172, 252-265

14.Fiksinski, K. and Frackowiak, D. (1980) Spectroscopy Lett. 13, 873-883.

15.Lorrain, L., Frackowiak, D., Romanowski, M. and Leblanc, R.M. (1987) Photosynthetica 21, 43-50.

16.Frackowiak, D., Hotchandani, S. and Leblanc, R.M. (1983) Photochem. Photobiophys. 6, 339-350.