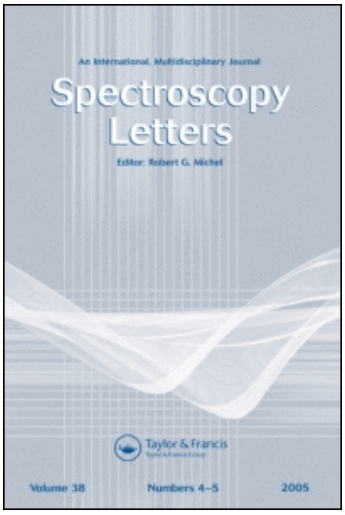

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The Texture of Stretched and Unstretched Polymer Films with and Without Embedded Biological Materials

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THE TEXTURE OF STRETCHED AND UNSTRETCHED POLYMER FILMS WITH AND WITHOUT EMBEDDED BIOLOGICAL MATERIALS

Key words: Atomic Force Microscopy, polymer film, polarized light spectroscopy, green photosynthetic bacteria.

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ABSTRACT

The stretched polymers with embedded biological samples are widely used as anisotropic matrix uniaxially orienting samples. The texture of a polymer film surface influences the properties of polarized emission and photothermal spectra. This textural properties for the unstretched and stretched polymer films with and without the embedded biological objects of various dimensions (pigment molecules, photosynthetic bacterial cells, and cell fragments) were established using AFM (Atomic Force Microscopy).

INTRODUCTION

The orientation of absorption and emission transition moments in molecular structures, as well as the orientation of pigment molecules in biological tissue can be established for a partially aligned sample by using polarized light [1, 2]. For such samples several type of spectra can be obtained. These include absorption [1-5], fluorescence [1-5], delayed luminescence, photothermal [6, 7], CD [8] and MCD. These spectral measurements yield information not only about the sample structure but also about processes such as excitation energy transfer [1,5], or thermal deactivation [6,7] occurring in the pools of differently oriented chromophores. Naturally occurring biological tissue very often contains partially oriented molecules [1, 2]. It is possible to investigate such systems using photoselection by polarized light [2] but sometimes it is necessary to improve the chromophores ordering by artificial orientation of the sample [1, 2].

There are several methods of sample alignment: electric or magnetic fields [1], squizzed gel [9],etc., but the most widely used method of uniaxial alignment is by embedding the sample in a stretched polymer film [1, 10].

The texture of a polymer surface has an influence on the light scattering and reflection properties of the surface boundary between the sample and air. The dimensions of the surface increase with a decrease in its smoothness. This surface smoothness also changes the heat transport between the sample and surrounding gases in the case of photoacoustic spectral measurements [6, 7].

Therefore in this study we decided to investigate, by means the Atomic Force Microscopy, the texture of unsteretched and stretched polyvinyl alcohol (PVA) films containing green photosynthetic bacterial cells, their cell fragments, and pigment molecules. These films were compared for textures with results for similar films without embedded particles. The effect of orientation caused by stretching is measured by polarized absorption spectra of the same samples.

MATERIAL AND METHODS

Prosthecochloris aestuarii (Chloropseudomonas ethylica) 2K strain was grown anaerobically in a culture medium described by Holt [11] with 1400 lux

illumination. Whole bacteria or their fragments (obtained by sonification for 15 min. at 4 °C at 36,000 g) were introduced into an aqueous solution of polyvinyl alcohol (PVA) mixed with resin (AG1-XB) in order to obtain non acidic solvent (pH from 7.0 to 7.4). The chlorophyll *a* (Chl *a*) was obtained and purified chromatographically [6] and it was also introduced into the PVA via a resin solution. The PVA films were produced and stretched as previously described [6, 10]. The reference film, without samples but with and without resin addition, were also prepared and stretched.

A room temperature atomic force microscope (AFM) (OMICRON) has been used to study the film topography [12, 13]. AFM images in constant force mode were taken for all samples in two different regions. The measurements were made in air at 10 ^{0}C. The samples were placed on a single tube piezoelectric translator and tips of silicon nitride (Si_3N_4) under cantilever were used. Maximal area available to scan was 4000 nm x 4000 nm and typical images consisted of 256 x 256 points/lines. The scan rate was within range of few Hz per line. The AFM resolution was 0.01 nm in the vertical direction and 0.1 nm in the surface plane. The lateral and vertical calibration was carried out by imaging the known atomic structure and height of the atomic steps of a mica standard.

The polarized absorption spectra were recorded using the Specord M40 spectrometer (Carl Zeiss Jena, Germany) equipped with polarizers.

RESULTS

Fig.1 shows the polarized absorption spectra of stretched samples with whole bacteria cells (a), cell fragments (b) and film with Chl *a* molecules (c). As it follows from these spectra stretching caused only slightly different anisotropy of absorption of various samples. The isolated pigment molecules interact strongly with polymer chains and therefore are ordered in a definite way: Chl *a* usually is oriented by line connecting I-III pyrrol rings (Q_y transition moment) around the film stretching axis. Elongated cell fragments are usually ordered in a higher

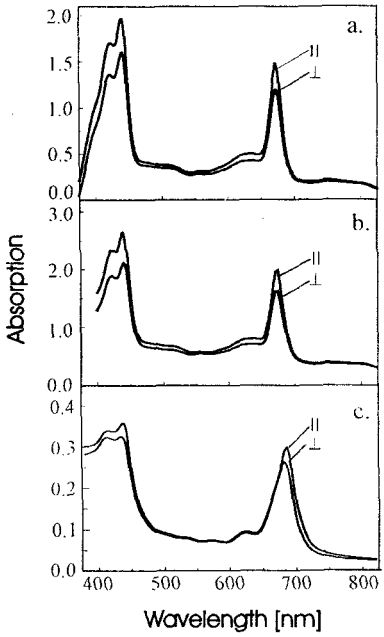

Fig. 1. Polarized absorption spectra of samples in stretched PVA film: a) whole bacteria cells, b) bacteria fragments, c) molecules of chlorophyll *a*

degree than almost spherical (but slightly deformed by stretching) whole green bacterial cells [10].

Fig. 2 shows the examples of AFM pictures of nonpigmented samples. The three dimensional pictures of all investigated samples were also taken (not shown). From these pictures the maximal vertical differences between various points on investigated surfaces were obtained. For samples shown in Figs. 2a and 2b, it was observed that film stretching causes strong changes in a film texture: film became more flat (the highest parts are located at 60 nm in elongated film whereas in unstretched film irregular high hills are reaching the 150 nm level).

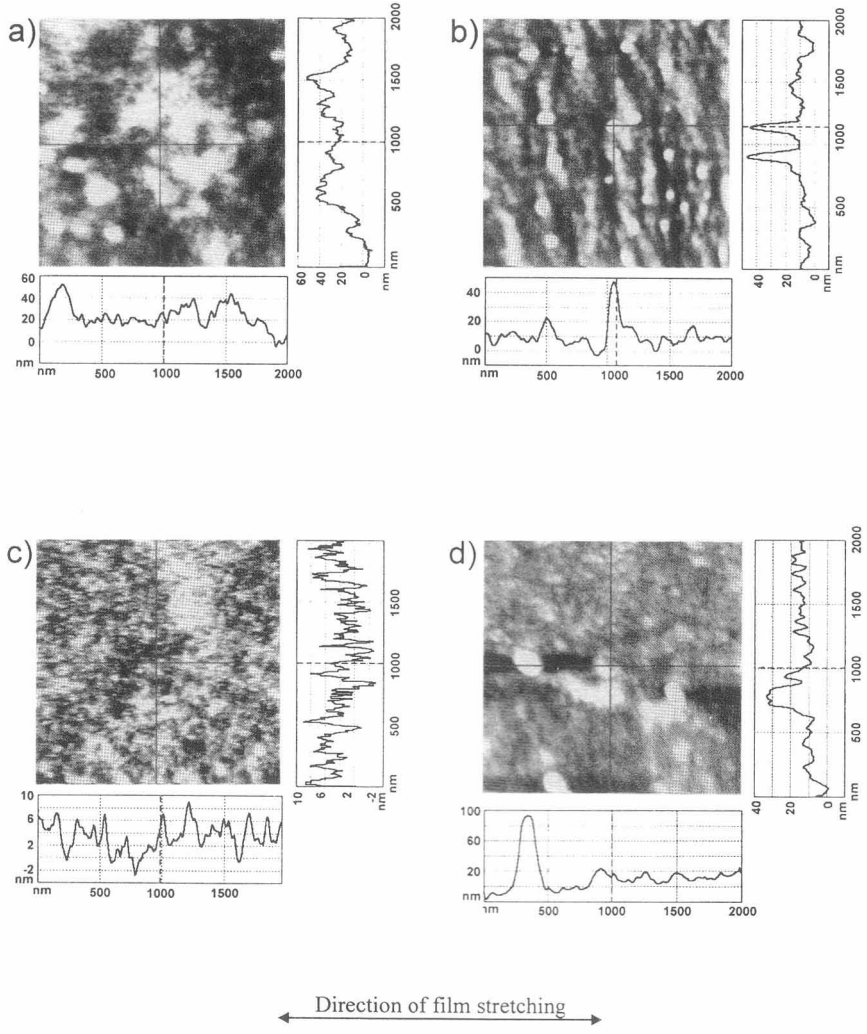

Fig. 2
Atomic Force Microscope picture of samples in unstretched (a, c) and stretched (b, d) PVA film without resin (a, b) and with resin addition (c, d). Direction of film streching is shown on the picture.

Additionally strips almost perpendicular to the direction of the film stretching are seen. This second effect was not expected due to observations from previous experiments [1-5, 10]. As seen in Fig. 1 all embedded samples are oriented along the stretching axis, therefore long polymer chains must be oriented in this direction. From Fig. 2 b it is clear that on the surface there are higher and lower strips formed with small peaks located at the higher strips. The same phenomenon is seen at higher magnification (not shown). The addition of resin (which is added in order to reach a pH suitable for native biological complexes) changes this property (Figs. 2c, 2d). In this case, the unstretched film is more flat (e.g., differences about 15 nm occur in the vertical direction) than the stretched film (with vertical differences of 100 nm); and regular strips perpendicular to the stretching direction are not seen.

From the comparison of Figs. 2a and 2b with Figs. 2c and 2d, it is clear that with the application of resin into the film containing embedded biological samples, the reference film must also be prepared with resin addition.

Figs. 3a, b present unstretched film with whole bacteria. The average diameter of the almost spherical bacterial cells used is about 1000 nm. In Fig. 2a the bacteria are seen located near the film surface where they caused deformation. The film surface was deformed over an area of about 300 nm^2 with a height of approximately 30 nm. The stretching caused the film to become a little more flatter to approximately 20 nm in the vertical direction. Bacteria were better hidden in some films, while others demonstrate rather irregular strips. In films containing fragments (not shown) the situation is similar, but pictures of the embedded objects show smaller and more elongated particles.

Quite different results are shown for film colored by Chl *a* (Figs. 3d, 3c). The isotropic film is much more flat (e.g., 30 nm vertical differences) than stretched (e.g., 80 nm differences); with a demonstration of strong regular strips on the surface.

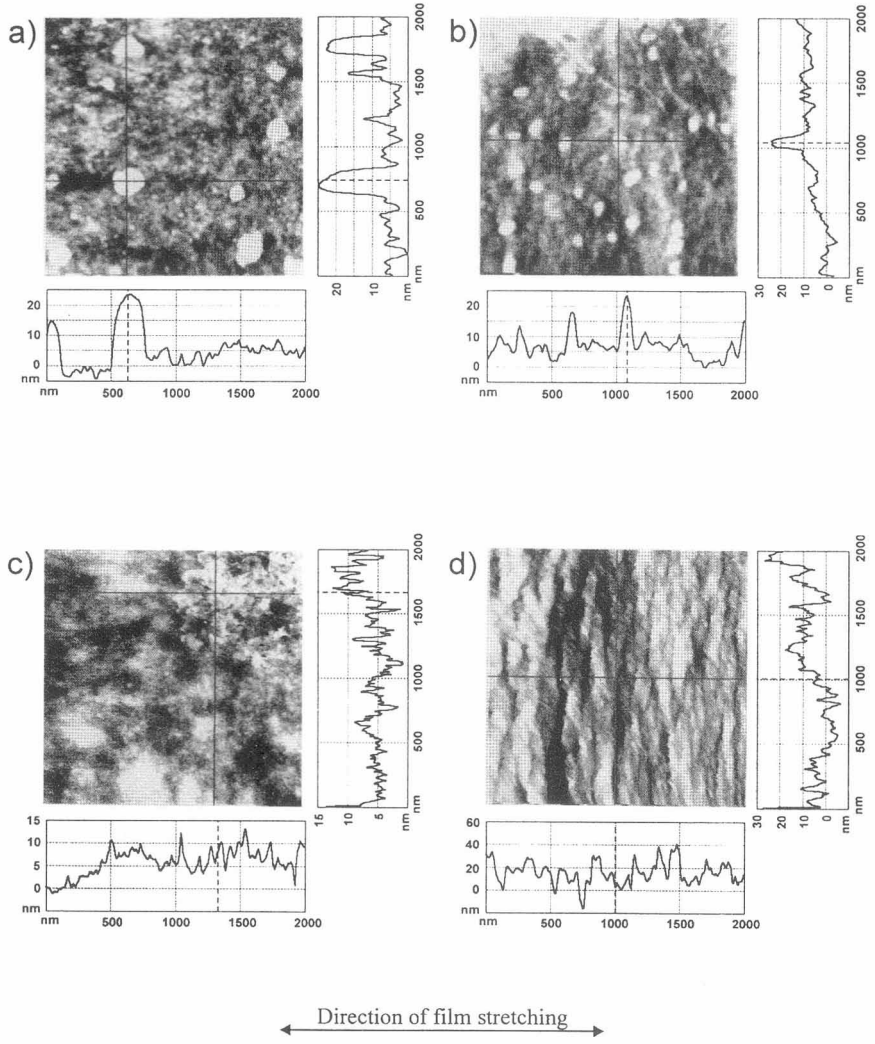

Fig. 3
Atomic Force Microscope picture of PVA film (a, c unstretched film, b, d stretched).
a, b - whole green bacteria cells;
c, d - film with chlorophyll *a*;

Conclusions

The texture of the surface of polymer films is changed as a result of resin addition, film stretching and by the embedding the large objects within the film. These effects can cause the change in measured photothermal spectra as well as in anisotropy of emission. These effects have to be taken into account, evaluated as experimental error, or eliminated by proper calibration of results.

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