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IMMOBILIZATION OF DNA FRAGMENTS BY LANGMUIR - BLODGETT TECHNIQUE

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ABSTRACT DNA fragments can be immobilized by Langmuir - Blodgett films of 1-hexadecylamine. The inclusion in these LB films of 5'-GTAAAACGACGGCCAGT-3' was proved by a radioactive technique. An X-ray diffraction study proved an overall lamellar ordering of the samples and suggested a structural organization in which layers of oligonucleotide molecules are sandwiched between two monolayers of 1-hexadecylamine. These monolayers are likely to turn their amino groups surfaces to the oligonucleotide molecules as to screen their anionic charges.

Keywords: Langmuir-Blodgett films, DNA fragments

INTRODUCTION

Langmuir-Blodgett (LB) films, able to immobilize proteins without damaging their native structure and therefore also their biological activity, were recently reported (1). This technique allows protein films to be produced and used as sensing elements in bioelectronic devices.

To our knowledge, no LB immobilization technique was so far reported for DNA and nucleic acids. This could open new perspectives for biosensing by DNA and for structural studies of nucleic acids as well.

In order to functionalize the LB film to make the deposition and immobilization of DNA possible we used surfactants able to grasp the anionic charges of the nucleic acid chains. We report in the present paper a method which allows oligonucleotides, and most likely also DNA, to be immobilized by LB films. The structural organization of this guest-host systems is suggested on the basis of an X-ray study of these films.

MATERIALS AND METHODS

The oligonucleotide 5'-GTAAAACGACGCCCAGT-3' was synthesized by a PCR -MATE (Applied Biosystems) . The LB films were deposited by Langmuir Trough 4 (Joyce Loebl, England) instrument. A teflon trough, smaller (100 ml) than its original one, was used. Films were deposited onto

silicon substrates by the horizontal lifting technique (2).

In each deposition step the monolayer of 1-hexadecylamine molecules was built up at the water (tridistillate) / air interface at a surface pressure π = 25 mN/m. Then an oligonucleotide buffer solution (5 10^{-2} mg/ml) was injected into the water under the monolayer. The resulting concentration of the oligonucleotide in this water subphase was 5 10^{-4} mg/ml. After the oligomer injection the hexadecylamine monolayer, before being transferred onto the substrate, was left aside in the trough for about one hour.

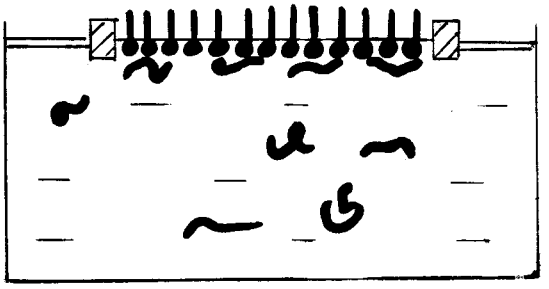

FIGURE 1 An assembling monolayer of 1-hexadecylamine at the water/air interface captures and binds oligonucleotides molecules solubilized in the water sub-phase.

The oligonucleotide was labelled by 32P-γATP at its positions 5' by the method described by Maniatis and coll, (3). This allowed the immobilization process to be followed by radioactivity measurements (4) after

each deposition step.

An X-ray diffraction analysis was carried out on samples prepared by 30 successive deposition steps. A small-angle X-ray diffractometer (5) with a linear position-sensitive detector in a swing geometry (6) was used. The angular resolution of the detector was 0.02^{o} which assures a space resolution of 0.5 A.

The incorporation of radioactive isotopes into the oligonucleotide molecules was estimated by counting the β particles by using a RACK beta counter (LKB, Sweden). The specific radioactivity of the oligonucleotide was thus estimated. The efficiency of the oligonucleotide transfer to the LB layers was calculated on the basis of this specific radioactivity in terms of surface density of the oligonucleotide on each monolayer.

RESULTS AND DISCUSSION

LB technique allows continuous and quasi-crystalline films to be deposited onto a solid substrate by keeping under control their thickness at a molecular level. This is possible by transferring, by a sequence of deposition steps, ordered monolayers from an air/water interface to the substrate. The aim of this investigation was to build up LB films with immobilized DNA fragments.

Radioactivity measurements proved that by the technique presented in this paper it is possible to immobilize DNA fragments within LB films. An oligonucleotide surface density of 170 ng/cm^2 within

each monolayer, was reached .

The surfactant we chose was 1-hexadecylamine. The compressed monolayer, built up by these surfactant molecules at the air/water interface, protudes and dips their hydrophilic amino groups inside the water. These groups capture and bind DNA fragments ,injected and solubilized under them (fig. 1). Then, when the LB layer is transferred onto the substrate, DNA fragments are also extracted and immobilized within the crystallizing film.

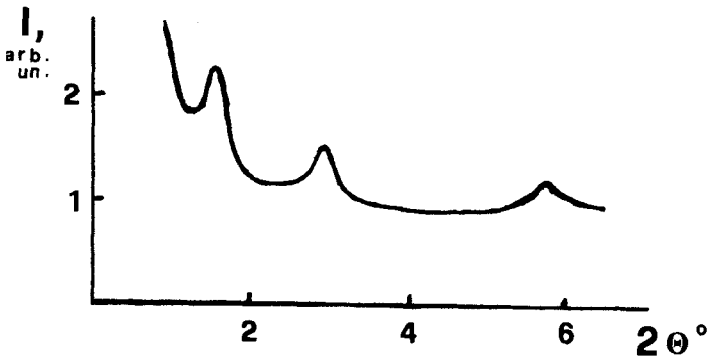

FIGURE 2 X-ray pattern of 1-hexadecylamine - oligonucleotide LB films

The X-ray diffraction pattern of samples prepared by this technique is reported in FIGURE 2. Its shape is characterized by three peaks, corresponding to the 1st, 2nd and 4th orders of reflections.

The presence of these reflections indicates that a well ordered lamellar structure was obtained. The angular position of these reflections corresponds to a spacing (period of reproducibility) D = 60.5 A.

This width of the repetitive unit suggests the

structural arrangement sketched in FIGURE 3. In fact the thickness of a 1-hexadecylamine bilayer can span values from 44.1 A, when the hydrocarbon chains are perpendicular to the substrate, to 35.0 A, when the hydrocarbon chains are tilted (7).

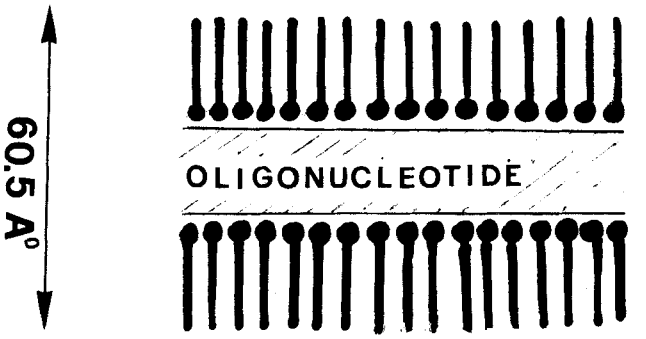

FIGURE 3 Structural organization of the molecules of 1-hexadecylamine and DNA in the elementary unit of the LB film

The X-ray diffraction curve of FIGURE 2 proves that the distribution of the oligonucleotides follows the repetitive layered pattern of the LB film. The elementary unit can therefore be made up of two monolayers of 1-hexadecylamine and one monolayer of DNA fragments.

FIGURE 3 sketches the most likely mutual orientation of the 1-hexadecylamine monolayers, being DNA an anionic

polyelectrolite. Screening effects ought to lead to this structural ordering. Within the multilayer organization of the film the anionic DNA fragments are thus sandwiched and screened between two amino group surfaces.

This configuration can be achieved only if the molecules are able to rearrange themselves inside the LB film during each deposition step. It was recently shown by T.Kato (8) that this is possible. During horizontal lifting deposition of fatty acid salts the 3 monolayers lastly deposited were in fact found to change the mutual orientation of their constituent molecules. This process takes place in order to accomplish a more stable structural organization.

CONCLUSIONS

DNA fragment molecules can be immobilized by an LB matrix of long chain amines. This was proved by radioactive measurements. X-ray diffraction studies provided evidence of a good lamellar ordering within these films. The X-ray diffraction patterns also suggest that the repetitive units of these LB films are likely to be made of one monolayer of DNA sandwiched by two monolayers of 1-hexadecylamine.

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REFERENCES

1. Yu.Lvov, V.Erokhin, S.Zaitzev, Sov.Biol.Membr., 7, 917 (1990).

2. I.Langmuir, V.Schaefer, J.Amer.Chem.Soc., 60, 1351 (1938).

3. T. Maniatis, E.E. Fritsch, S. Sambrook, Molecular Cloning. Cold Spring Harbor, 1982, p 127.

4. T. Maniatis, E.E. Fritsch, S. Sambrook, Molecular Cloning. Cold Spring Harbor, 1982,p 415..

5. L.Mogilevsky, A.Dembo, D.Svergun, L.Feigin, Sov. Crystallography, 29, 587 (1988).

6. L.A.Feigin, Yu.M.Lvov, V.I.Troitsky, Sov.Sci.Reviews, Physics Reviews, 11, 285 (1989).

7. V.Erokhin, Yu.Lvov, L.Mogilevsky, A.Zozulin, E.Ilyin, Thin Solid Films, 178, 153 (1989).

8. T.Kato, Jap.J.Appl.Phys.,27, 1358 (1988).