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MODULATED FRINGE PATTERN PHOTBLEACHING APPLIED TO LIPID-WATER CUBIC PHASES: STRUCTURAL INFORMATION

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Résumé - Nous avons effectué des mesures de diffusion latérale dans des phases cubiques, dans le but de corréler propriétés structurales et comportement dynamique. Nous avons utilisé la technique de photoblanchiment selon un réseau de franges, permettant de créer d'abord une inhomogénéité de fluorescence, et de suivre ensuite le retour vers l'homogénéité. La cinétique de ce retour permet de mesurer les diffusions dans le système. Nous observons différents types de diffusion que nous pouvons attribuer à différentes structures topologiques. Dans certaines situations, nous sommes capables de conclure à la coexistence de grands domaines continus et de petits domaines fermés, ce qui pourrait correspondre à la structure contenant à la fois un labyrinthe tridimensionnel ouvert et des éléments micellaires fermés, proposée précédemment sur la base d'expériences de diffraction des rayons X.

Abstract - We present lateral diffusion experiments performed on lipid-water cubic phases with the purpose of correlating dynamic and structural properties of the system. The experiments involve the modulated fringe pattern photobleaching technique : after flashing a bleaching beam, and thus generating a fluorescence heterogeneity, we observed the kinetics of fluorescence recovery. The analysis of the time course of the recovery yields information regarding the lateral diffusion of the fluorescent molecules in the system. We observed different types of diffusion, clearly correlated with the topological properties of the different structures, as established by the previous X-ray scattering studies. For one of the cubic phases (Q^{223}), the results of the fluorescent recovery study appear to be consistent with a structure containing both an open hydrocarbon labyrinth and a family of closed micellar elements, rather than with a model containing only closed micelles.

Introduction :

Lipids are known for their extraordinary polymorphism; lipid-water systems display a large number of phases whose structures are remarkably different from each other /1/. Several of the phases belong to one of six space groups of the cubic system /2/. The structure analysis of those cubic phases has been tackled using a variety of techniques - X-ray scattering, electron microscopy, field gradient N.M.R. - and models have been proposed for the structure of the six phases: at least for one phase, yet, the structure is still controversial /2,3/. We introduce here a novel approach to the problem, based upon diffusion measurements performed using modulated fringe pattern photobleaching experiments /4/.

Modulated fringe pattern photobleaching :

The modulated fringe pattern photobleaching technique consists of flashing a striped bleaching pattern (formed by interference fringes) on a sample containing a lipid phase doped with a fluorescent probe attached to a lipid molecule. Instead of monitoring the fluorescence recovery, we measure directly the contrast of the striped object as a function of time, by rapidly scanning the fringe pattern back and forth /4/. A signal is schematically represented in the figure :

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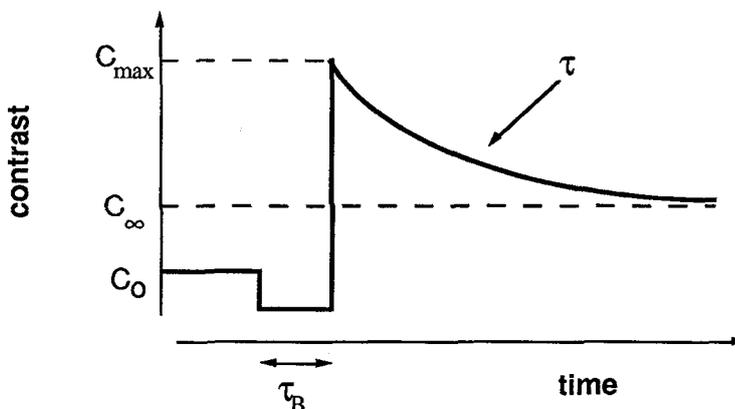


Fig. - The fluorescence contrast after photobleaching is schematically represented as a function of the time. C_0 stands for the contrast before the photobleaching flash. C_{\max} stands for the maximum contrast, just after the photobleaching flash, and C_∞ stands for the contrast after photobleaching after a long time (typically 10 s in our experiments). τ_B is the photobleaching flash duration, which is always short as compared to the time of the exponential decay τ ($\tau_B < \tau/10$).

The decay of the fluorescence contrast between bleached and non-bleached areas is multiexponential. Each time constant (τ) of the decay curve is related to the diffusion constant (D):

$$D = i^2 / 4 \pi^2 \tau$$

where i is the interfringe spacing. In our work, i was varied from 6 to 20 μm so that the smallest τ was typically of the order of 0,4-1 s; therefore the observed diffusion coefficients are in the range 1-25 $\mu\text{m}^2 \text{s}^{-1}$.

The plateau yields information regarding the non-diffusible molecules. The immobilized fraction can be evaluated by the ratio:

$$(C_\infty - C_0) / (C_{\max} - C_0)$$

The above ratio corresponds to the fluorophores which, in the time scale of the experiment (typically 10 s), and over a distance defined by i , do not diffuse.

An argon laser (Spectra Physics model 164-08) tuned to 488 nm was used as the excitation source for a fluorescent inverted microscope (Zeiss IM 35). An oil immersion objective lens was employed with a magnification of either x25 or x40.

Lipid phases :

The desired phases were obtained at concentration and temperature chosen according to the phase diagram (for each surfactant, the reference is given in the last column) :

Surfactant	% water	Temperature	Phase	Type	Reference
MO	26%	22-36°C	Q ²³⁰	II	/5/
MO	38%	22-36°C	Q ²²⁴	II	/5/
DTACl	15%	34°C	Q ²³⁰	I	/6/
DTACl	30%	23°C	H	I	/6/
DTACl	50%	23°C	Q ²²³	I	/6/
PLPC	60%	22°C	Q ²²³	I	/7/

Phase H consists of a 2D hexagonal array of rods; in structures of type I the rods, filled by the hydrocarbon medium and lined by the polar headgroups are embedded in a polar matrix. The cubic phases Q²³⁰ and Q²²⁴ are known to be bicontinuous and to consist of two 3D labyrinths, mutually intertwined and unconnected. The two labyrinths may be polar and separated by an apolar septum (structure of type II), or *vice-versa* (structure of type I). For the phase Q²²³ two structures have been proposed: one consists of a 3D labyrinth of type I enclosing globular particles also of type I /8/, the other contains two types of globular particles, also of type I /9,10/.

The topological properties of the various phases are such as to suggest different types of diffusion behaviour: in the labyrinths of the cubic phases, as well as in the rods and the matrix of the hexagonal phase, the lipid molecules are expected to diffuse quite easily, whereas the diffusion should be more difficult when the fluorescent probes are enclosed within discrete micelles.

Fluorescent labeling :

Different fluorescent probes were synthesized with the purpose of mimicking the polar/apolar properties of the lipid molecules of the system. All the probes bear a [7-nitrobenz-2-oxa-1,3-diazol-4-yl] (NDB) group as fluorophore. The concentration of fluorescent molecules was approximately 0.2% (w/w) .

Sample preparation :

The samples were prepared by mixing lipids and fluorescent molecules in an organic medium, evaporating the solvent and adding the appropriate amount of water. The samples were mounted in vacuum-tight holders, approximately 0.2 mm thick, provided with thin mica windows; the holders were designed for both the X-ray scattering and the fluorescence experiments. The structure of each of the samples was tested by X-ray scattering controls before performing the fringe pattern photobleaching experiment.

Results and interpretation :

In all the experiments we measured at least one diffusion constant.

For the phases Q^{230} , Q^{224} and H the fluorescent contrast always decreases to C_0 , indicating that the fluorescent molecules are all free to diffuse through the sample, in keeping with the presence of long-range labyrinths. Moreover, the numerical values of the diffusion constants (not reported here) agree quite closely with those observed in the N.M.R. experiments /11/.

In all the experiments performed on phase Q^{223} , C_∞ was invariably found to differ from C_0 . The ratio of immobile probe varies with the nature of the lipid and of the fluorescent molecule (the range is 15-65%). *Prima facie*, those observations are in better agreement with the labyrinth-and-micelles model /8/ than with the entirely micellar ones proposed either by Eriksson and coworkers /11/ on the basis of N.M.R. diffusion measurement, or by Charvolin and Sadoc /10/ using formal geometric arguments. A more quantitative comparison of the ratio of immobile probe with the volume fraction of the micelles in the labyrinth-and-micelle model /2/ is of limited interest since the probe molecules may well partition preferentially in one or the other of the structure elements.

Before drawing firm conclusions, though, it is essential to make sure that the limiting process is diffusion inside the crystallites rather than along structure defects. Experiments aimed at clarifying that issue are underway.

A more detailed manuscript describing the experiments reported above and those underway is in preparation.

ABBREVIATIONS :

MO	1-monooleoyl-rac-glycerol
DTACI	dodecyltrimethylammonium chloride
PLPC	palmitoyllysophosphatidyl choline

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