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Spectral and thermodynamic characterization of bacteriochlorophyll *c* and dipalmitoylphosphatidylcholine in the binary mixed monolayers

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The spectral and thermodynamic properties of bacteriochlorophyll *c* and dipalmitoylphosphatidylcholine in binary mixed monolayers have been investigated. The model system of biological membrane was built up of a mixture of phospholipids and pigments dissolved in chloroform, at several mole ratios. The isotherms of these samples were recorded and then the monomolecular layers on quartz plate were produced. For the Langmuir layer obtained on the air–water interface the thermodynamic parameters were calculated. Additionally, by means of the absorption spectroscopy methods in natural and polarized light, pigment–pigment and pigment–lipid interactions were studied. The information about the average surface per molecule, aggregation properties and the molecular orientation of pigments in Langmuir–Blodgett layers, was obtained.

1. Introduction

In vivo bacteriochlorophyll (BChl) *c* aggregates form the rod-elements of the chlorosomes of green photosynthetic bacteria and works as a light-harvesting antenna [1–3]. The interaction between the pigment molecules depends on their mutual orientation and molecular ordering with respect to their anisotropic microenvironment. The behaviour of various pigment forms is usually different. In biological cell membranes, pigments and pigments–protein complexes are oriented within lipid membranes in two ways: uniaxially and/or in the plane of the membrane.

The Langmuir or Langmuir–Blodgett (LB) layers [4–6], two-dimensional films, could be considered as a physical model of the anisotropic biomembrane. These films were used to investigate the behaviour and molecular orientation of BChl *c* in mixed pigment–phospholipid monolayers on the air–water interface as well as on solid substrate.

The photosynthetic pigments in LB films have also been of interest, as a part of molecular engineering, from the point of view of their use for industrial and medical applications [1,2,7]. Simple devices such as a photoelectric cell, converting the light energy into electric one, may consist of just one ordered pigment monolayer located between the hole and the electron injecting electrodes. In medical application, pigment molecules are introduced and oriented into an anisotropic cell membrane and the LB technique allows us to study the mutual pigments and pigment–environment interactions at the molecular level. The purpose of the present study was to gain a better understanding of the molecular orientations of BChl *c* influenced by the pigment–lipid interaction, in two-dimensional organized assemblies which could be also adopted for the study of *in vivo* chlorosome-like structure.

The pigment activity in medical or industrial applications depends on a molecular organization (in particular the orientation and aggregation) and intermolecular interactions in anisotropic environment. In the present work, BChl *c* (Fig. 1a) and phospolipid (dipalmitoylphosphatidylcholine (DPPC)) (Fig. 1b) in mixed Langmuir and LB films were characterised by the electronic absorption (of the natural and linearly polarized light) spectroscopic method. The stability and miscibility in a mixed molecular layer as well as thermodynamic parameters (such as the excess areas (ΔA^E), excess free energies (ΔG^E) and compression modulus ($1/C$)) were also determined. Finally, the model of molecular arrangement of BChl *c* in a monolayer was proposed on the basis of the results of the experiments and calculations.

2. Materials and methods

BChl *c* and its Mg-free derivative – bacteriopheophytin (BPhe) *c* were obtained from *Prosthecochloris aestuarii*. Extraction, purification and identification procedures were described in ref. [3]. Synthetic DPPC, in 99% purity, was purchased from Sigma (Aldrich). The individual or mixed monolayers of BChl *c* (or BPhe *c*) and DPPC were obtained by spreading the compounds or their mixtures at various molar fractions (MF) of pigments, from a chloroform solution onto the water subphase (Tris/HCl buffer system, pH 8.2) [8,9].

Abbreviations: BChl, bacteriochlorophyll; DPPC, dipalmitoylphosphatidylcholine; FWHM, full-width at half maximum; LB, Langmuir–Blodgett; MF, molar fractions.

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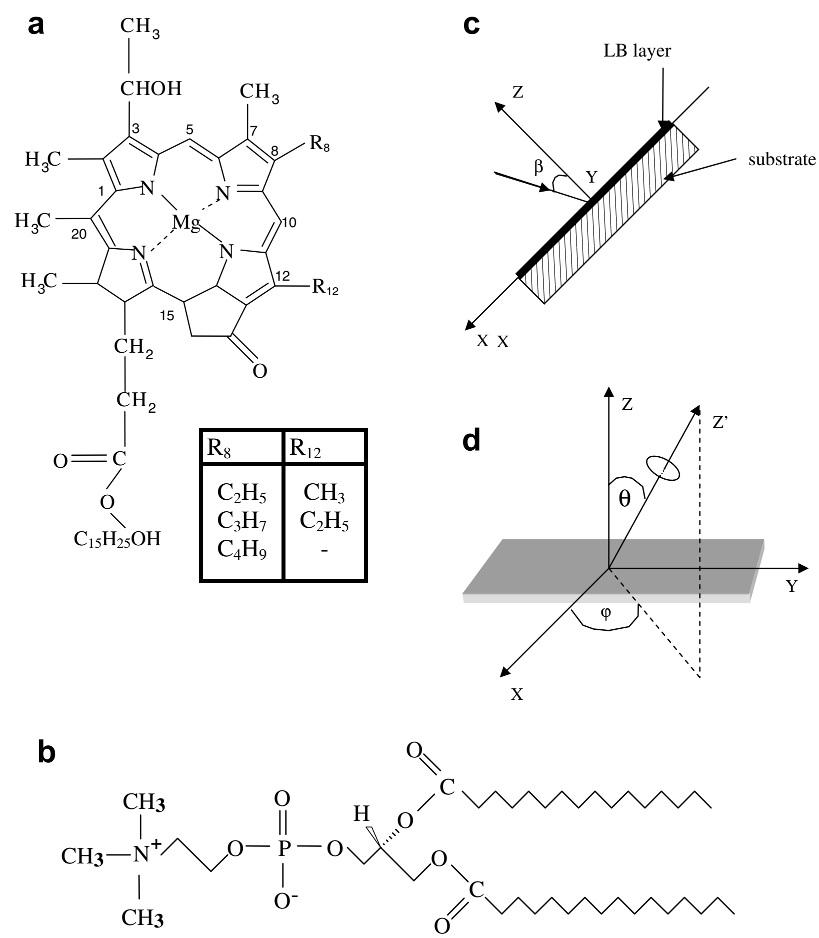

Fig. 1. Molecular formulae of BChl *c* (a), DPPC (b) and the direction of the polarized light incident on the substrate (c) as well as space coordinates (X,Y,Z) for expressing pigment orientation (d); Y-axis: dipping direction, Z-axis: normal to the substrate, Z'-axis: the central axis of the pigment represented by the circular plate.

The stock solutions, of 0.1 mM concentration of pure pigments and also DPPC, were used to make pigment–lipid mixtures.

The surface pressure–area ($\pi - A$) isotherms were recorded using a KSV-5000 Langmuir system (KSV, Finland). Further experimental details about preparation of the monolayer are given elsewhere [8,10]. All experiments were performed in dim light. Surface tension was measured by means of a platinum Wilhelmy plate. The rate of shift of the teflon barriers compressing a given substance in the bath for monolayer production has been established to be 3 mm/min. Stability tests were performed for Langmuir films checking the area per molecule variation to keep the pressure constant for 1 h. The films were found to be stable and each isotherm was repeated at least twice to ensure good reproducibility.

On the basis of the $\pi - A$ isotherms of the binary mixed monolayers, some thermodynamic parameters (such as ΔA^{E}, $A_{12}{}^{T}$, ΔG^{E}) were calculated from the following equations [11,12]:

$$\Delta A^{E} = A_{12} - A_{12}^{T} \quad (1)$$

$$A_{12}^{T} = A_1 x_1 + A_2 x_2 \quad (2)$$

$$\Delta G^{E} = \int_0^{\pi} \Delta A^{E} d\pi \quad (3)$$

where A_1, A_2 and A_{12} represent the molecular area of a monolayer of each component and their mixture, respectively, while x_1 and x_2 are the molar fractions of each component in the mixture.

In addition, the compression modulus of monolayers ($1/C$) is a useful parameter to understand the phase behaviour and the molecular interactions. It can be directly calculated using the isotherm data from the formula:

$$\frac{1}{C} = -A\frac{d\pi}{dA} \quad (4)$$

The LB layers were fabricated onto a quartz plate with a hydrophilic surface. The monolayers were transferred by the vertical dipping method (at the rate of 2 mm/min) onto a plate at a constant surface pressure. Successful deposition of one film layer onto the quartz took place only by raising of the substrate (Z-type of LB). Monolayers of BPhe *c* were transferred at fixed surface pressure of 18 mN/m. The transfer ratio (1.0–1.3) was satisfactionary only for first monolayer, hence for further analysis only one layer samples were taken into consideration.

Polarized absorption spectra of the films deposited onto quartz plates were measured by a Carry 4000 (Varian) spectrophotometer equipped with a Glan-Thompson polarizer and an angular sample holder. Two absorption components $A_{||}$ and $A_{\perp}$ were measured with the light electric vector parallel and perpendicular to the direction of the emerging LB layer, respectively. These components were measured in two geometrical arrangements, i.e. with the light beam (1) perpendicular to the sample surface ($\beta = 0°$) and (2) falling on the surface at the incidence angle (β) (Fig. 1c).

3. Results and discussion

3.1. Behaviour of Langmuir monolayers

The ($\pi - A$) isotherms of Langmuir monolayers at various MF of pigment on the buffer subphase are shown in Fig. 2a. The isotherms for BChl *c* (Fig. 2a) and its Mg-free derivative (BPhe *c*)