ADSORPTION OF DYES AND PROTEINS TO MONOLAYERS

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Summary

The reflection of light at the air/water interface is modified by formation of a monolayer. In the presence of dye molecules at the interface the reflection spectrum coincides with the absorption spectrum of the dye monolayer. Using the reflection technique the adsorption of ionic dyes from the aqueous subphase to mixed monolayers including head groups of opposite charge have been investigated. Electrostatic binding of the dye is enhanced at constant charge density if the charge distribution matches that of the adsorbate. Binding of cytochrome c to phospholipid monolayers is dominated by electrostatic interactions. The porphin chromophore of the adsorbed protein is oriented parallel to the monolayer plane.

1. Introduction

Organized monolayers at the air/water interface are adequate model systems for the investigation of interactions of small molecules and proteins with membranes. By systematic variation of the composition of mixed monolayers of different lipids the relative contributions of the different intermolecular interactions may be characterized.

Light reflection from the air/water interface is modified by formation of a monolayer. From the reflectivity change the thickness of transparent surface films may be determined [1]. Further, the reflection method provides a possibility to detect dye molecules located at the interface [2,3]. Molecules dissolved in the aqueous subphase do not contribute to the signal. By the reflectivity measurement the dye molecules are detected in their ground state in contrast to fluorescence techniques which provide time delayed information about the excited state of molecules. Using plane polarized light under oblique incidence the average orientation of the transition moments may be determined by a reflection measurement [3]. In this case again the reflectivity measurement is superior to the fluorescence techniques since energy transfer processes may delocalize the excited state in particular in two-dimensional systems. Such processes, however, cause uncertainties about the orientation of the primarily excited transition moments.

The reflection technique has been used to investigate the adsorption of small dye molecules and of proteins. Binding of proteins to lipid monolayers is mainly followed by measuring the change of monolayer area at constant surface pressure or the change of surface pressure at constant area as well as the change of the surface potential at constant area [4]. The optical method provides independent information, in particular on the orientation of chromophores that are part of the adsorbed proteins and leads to a more detailed understanding of processes at membrane interfaces. The following review does not contain experimental details that may be found in the original publications.

2. The reflection method

The optical properties and the thickness of thin transparent films on solid substrates are usually determined using ellipsometric techniques [5]. With this method the change in polarization of the light due to reflection is measured. The change in reflectivity, i.e. a measurement of the intensity, allows one also to determine the film thickness, provided that the refractive index of the film is known. A dense-packed monolayer of methyl stearate at the air/water interface causes an increase of the reflectivity by $\Delta R = 0.9 \times 10^{-4}$ [1]. Addition

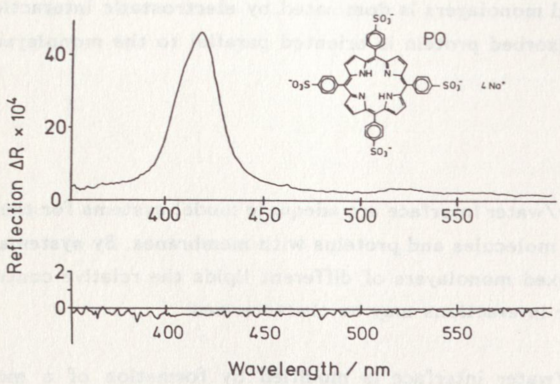

Fig. 1: Reflection spectra obtained from the monolayer-covered surface of solutions of the porphyrin PO. Top: monolayer of eicosylamine and methyl stearate, molar ratio 1:4, on top of 2×10^{-7} M PO in water; bottom: monolayer of arachidic acid on top of 10^{-5} M PO in water. Adsorption of PO to positively charged head groups; no contribution to the signal ΔR by porphyrin molecules in solution; surface pressure: 20 mN/m; 20° C.

of four more CH_2 groups to the hydrocarbon chain yields a further increase by $\Delta R = 0.55\times10^{-4}$. The variation of the thickness of a monomolecular film of gramicidin upon compression was optically monitored in this way [1].

The reflection method is particularly advantageous in the investigation of the adsorption of dye molecules from the aqueous subphase to monolayers on top of the solution since only the dye molecules located at the interface contribute to the optical signal. Fig. 1, top, shows th reflection spectrum ΔR of a mixed monolayer of eicosylamine and methyl stearate after adsorption of the porphyrin PO from the aqueous subphase to the protonated amino groups [2,6]. Clearly, the typical Soret band of the porphyrin with maximum at 430 nm can be seen. No porphyrin is adsorbed, however, to a monolayer of arachidic acid, see Fig. 1, bottom. The positively charged head groups of the mixed two component monolayer act as binding sites for the porphyrin anion.

3. Influence of the charge distribution in the lipid monolayer on the adsorption of polyvalent ionic dye molecules from the aqueous subphase

The water soluble porphyrin PO is not adsorbed to a monolayer of arachidic acid as a tetra-anion since the interface has an electrical potential due to partial dissociation of the carboxyl groups that repels the dye anions. This porphyrin could, however, be adsorbed although the local concentration of PO near the interface is very small, if binding sites like ammonium groups were incorporated in the head group region.

Weak adsorption of the porphyrin PO to a mixed monolayer of arachidic acid, eicosanol and eicosylamine was indeed observed [7]. Fig. 2, top, shows the reflection spectrum with the Soret band at 420 nm, characteristic for the porphyrin PO adsorbed to the monolayer. This monolayer, however, does not provide optimal conditions for the adsorption of PO since the binding sites (protonated eicosylamine) are statistically distributed in the matrix of arachidic acid and eicosanol. The interaction of the porphyrin tetra-anion with one or two binding sites could be too weak and therefore a small fraction only of binding sites being in the appropriate geometrical order may adsorb PO.

Four binding sites may be arranged in the geometry optimal for adsorption of the PO, when one molecule of the amphiphilic porphyrin AP (structure see Fig. 2, bottom) is incorporated in the matrix monolayer of arachidic acid and eicosanol instead of four molecules of eicosylamine. Fig. 2, bottom, shows the difference between the two reflection spectra of the mixed monolayer including the porphyrin AP obtained on top of a solution with PO and on top of a solution without PO, respectively. The Soret band of PO in this difference reflection spectrum is about four times higher than in Fig. 2, top. The replacement of 4 molecules of eicosylamine by 1 molecule of AP causes a four times larger surface density of adsorbed PO at practically the same interfacial potential [7]. It should be mentioned, that the two reflection spectra of Fig. 2 obtained at normal incidence of light may be directly compared

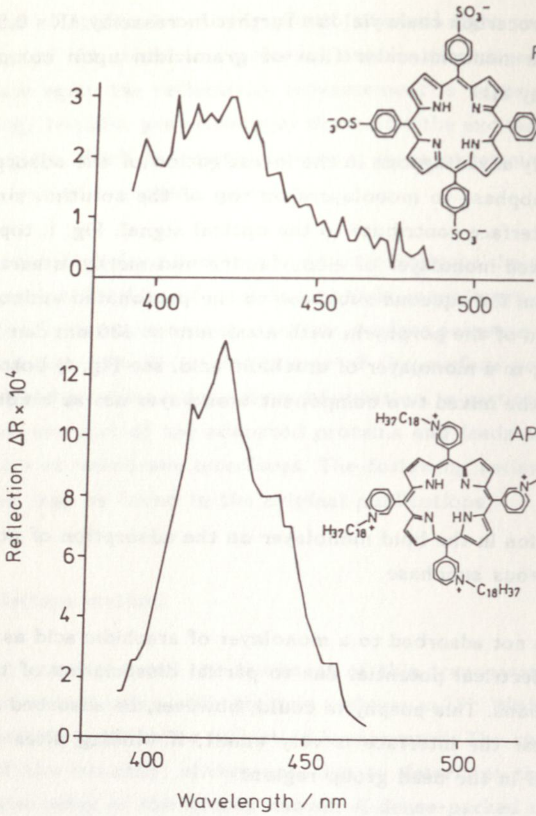

Fig. 2: Reflection spectra obtained from the surface of a monolayer-covered solution of the porphyrin PO. Top: monolayer of eicosylamine, arachidic acid and eicosanol, molar ratio 4:10:10, on top of 10^{-6} M PO; weak adsorption of PO to the positively charged binding sites. Bottom: monolayer of the porphyrin AP, arachidic acid and eicosanol, molar ratio 1:10:10; difference of the reflection spectra obtained on top of a 10^{-6} M PO and on top of water, respectively; enhanced adsorption of PO partly due to optimal geometry of binding sites.

since the average orientation of the PO is parallel to the monolayer plane in both cases.

The result of Fig. 2, however, may not be interpreted simply as a consequence of an improved electrostatic interactions between PO and AP as compared to that between PO and four molecules of protonated eicosylamine. Upon association of PO to AP additional attractive interaction between the π-electron systems of the two porphyrins become important. In any case, a surface with optimal geometrical arrangement of binding sites and therefore maximal electrostatic interaction should bind a large charged molecule stronger than a surface with the same density of binding sites that are, however, disordered.

The water soluble copper phthalocyanine tetrasulfonate CuPc (structure see Fig. 3) adsorbs to a mixed monolayer of eicosylamine and methyl stearate on top of the solution. The binding sites (protonated eicosylamine) may diffuse laterally even in a dense-packed monolayer and slowly reach positions that are optimal for electrostatic interaction with adsorbed CuPc molecules. A monolayer that is organized in this way may be fixed by transfer to a hydrophobic glass plate by the Langmuir-Blodgett technique. Still in contact with the aqueous solution, the adsorbed CuPc is removed from the organized monolayer by deprotonation of the ammonium head groups (by increase of pH). Subsequently, the CuPc may be readsorbed from a more dilute solution, now again at low pH = 5. After coating a protective monolayer on top of the glass plate with the adsorbed CuPc the absorption spectrum shown in Fig. 3 (organized), was measured. The bands characteristic for the adsorbed CuPc at 625 nm and 680 nm are clearly seen.

A monolayer of the same composition, however not organized by interaction with the CuPc, binds practically no CuPc under the same conditions from the dilute solution. This is evidenced by the absorption spectrum shown in Fig. 3 (statistical). The statistical distribution of binding sites does not provide for sufficiently strong interactions. On the other hand, the result demonstrates that a charge pattern complementary to that of the adsorbate may be permanently imprinted in a mixed monolayer of lipids with different head groups.

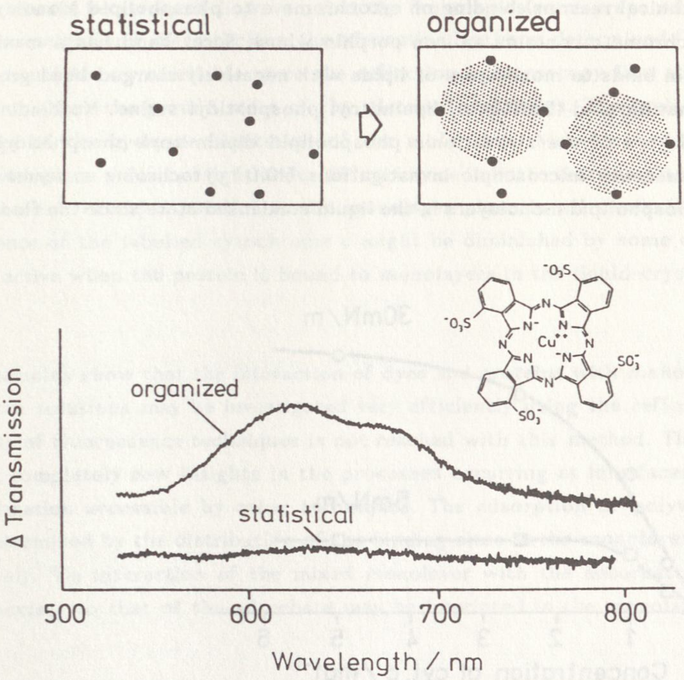

Fig. 3: Absorption spectra of glass plates after adsorption of the copper phthalocyanine tetrasulfonate CuPc to a monolayer whose binding sites were organized by interaction with the adsorbate and subsequent fixing of the charge pattern (organized) and to a monolayer of same composition, however statistical distribution of binding sites (statistical). The CuPc is clearly adsorbed to the organized surface and no adsorption of CuPc to the surface with statistical distribution is observed.

4. Binding of proteins to monolayers

The protein insulin binds to monolayers with positively charged head groups [8,9]. For example, at constant area the surface pressure of a monolayer of dimethyl dioctadecyl ammonium bromide (DOMA) increases from an initial value of 5 mN/m within 30 minutes to 20 mN/m. For the optical investigation insulin labelled with fluorescein was used. The time dependence of the optical signal after spreading and compression of the DOMA monolayer resembles very closely that of the surface pressure, if the initial surface pressure was 5 mN/m. A completely different behaviour is observed when the initial surface pressure was 40 mN/m. The surface pressure increases slightly in the beginning to 47 mN/m and levels off within 70 minutes at 44 mN/m. In contrast, the optical signal, i.e ΔR, at 505 nm (band of the fluorescein) increases continuously and does not reach a steady state value after 70 minutes. This result may indicate a slow reorganization of the adsorbed insulin. A more detailed evaluation is, however, not meaningful since the label (negatively charged fluorescein) influences the adsorption of insulin to the positively charged monolayer.

Since the reflection change upon adsorption of a protein could be measured in the visible range only (for technical reasons) binding of cytochrome c to phospholipid monolayers was investigated. Cytochrome c contains an iron porphin whose Soret band has a maximum at 410 nm. This protein binds to monolayers of lipids with negatively charged head groups like dimyristoyl phosphatidic acid (DMPA) or dipalmitoyl phosphatidyl serine. No binding is observed with monolayers of the zwitterionic phospholipid dipalmitoyl phosphatidyl cholin. According to fluorescence microscopic investigations [10,11] cytochrome c seems to bind preferentially to phospholipid monolayers in the liquid condensed state since the fluorescence

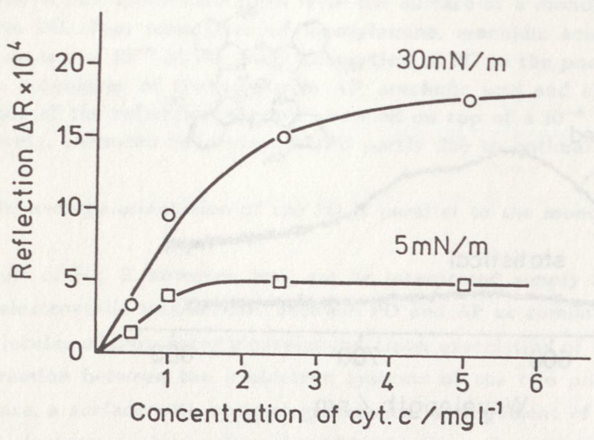

Fig. 4: Adsorption isotherms of cytochrome c bound to monolayers of dipalmitoyl phosphatidic acid. Plot of values of the reflection change ΔR at the maximum of the Soret band (410 nm) taken from the reflection spectra against the concentration of the protein in the aqueous subphase; stronger binding of cytochrome c at an initial surface pressure of π_i = 30 mN/m (circles) than at π_i = 5 mN/m (squares, figure taken from ref. 12).

of labelled cytochrome c is strongly diminished in liquid-crystalline domains of the monolayers.

The reflection method provides the possibility for a more direct study of the binding of cytochrome c to monolayers of dipalmitoyl phosphatidic acid (DPPA) [8,12]. The reflection spectra obtained from the surface of the aqueous solution of cytochrome c on which the monolayers were formed by spreading show the Soret band of the porphyrin. The amount of protein bound at constant monolayer area depends on the protein concentration and on the initial surface pressure of the monolayer. Fig. 4 shows the adsorption isotherms of cytochrome c bound to monolayers of DPPA as determined from measurement of the reflection spectra for initial surface pressures of 5 mN/m (squares) and 30 mN/m (circles). According to these data cytochrome c is bound stronger to the DPPA monolayer at π_i = 30 mN/m than at π_i = 5 mN/m. With monolayers of dimyristoyl phosphatidic acid a similar result was obtained, i.e. stronger binding of the cytochrome c to monolayers in the liquid-crystalline state than in the liquid condensed state.

The reflection method yields a result completely different from that of fluorescence microscopy. It should be stressed, however, that the monolayers were prepared in different ways in these investigations. Nevertheless, the adsorption isotherm determined from the reflection spectra should be more reliable since the reflection spectroscopy like absorption provides information about the ground state of the adsorbed molecules. The evaluation requires the knowledge of the average orientation of the molecules. Measurement of the reflection spectra with plane polarized light under 45^o incidence indicate that the plane of the porphyrin chromophore is oriented parallel to the monolayer plane in all cases [12]. Therefore, the fluorescence of the labelled cytochrome c might be diminished by some quenching process which is active when the protein is bound to monolayers in the liquid-crystalline state only.

These examples show that the interaction of dyes and proteins with monolayers at the surface of the solutions may be investigated very efficiently using the reflection method. The sensitivity of fluorescence techniques is not reached with this method. The results provide, however, completely new insights in the processes occurring at interfaces and complement the information accessible by other techniques. The adsorption of polyvalent dye ions is partly determined by the distribution of the binding sites in the monolayer or solid surface, respectively. Via interaction of the mixed monolayer with the adsorbate a charge pattern complementary to that of the adsorbate may be imprinted in the monolayer.

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