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Interaction of proteins with lipid monolayers at the air-solution interface studied by reflection spectroscopy

Z. Kozarac*, A. Dhathathreyan, and D. Möbius**

Max-Planck-Institut für Biophysikalische Chemie, Postfach 2841, D-3400 Göttingen, Federal Republic of Germany

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Abstract. The interaction of a fluorescein-labelled insulin and of cytochrome C with the air-solution interface and with lipid monolayers at the air-solution interface has been studied by measuring the change in surface pressure at constant area and by reflection spectroscopy. Chromophores at the interface only give rise to enhanced light reflection without contribution to the signal from chromophores in the bulk. The accumulation of labelled insulin at the solution surface is very weak as concluded from the shape of the spectrum and reflection intensity. No interaction with a monolayer of dipalmitoyl-phosphatidylcholine at initial surface pressure of 5 mN/m was detected. In contrast, the interaction with monolayers of dioctadecyldimethyl-ammonium bromide at initial surface pressures between 5 and 40 mN/m is much stronger, leading to a remarkable increase of surface pressure at constant area and strong reflection signal. The technique was also used to detect cytochrome C at the air-solution interface.

Key words: Reflection, lipid-protein interaction, surface pressure, monolayer

Introduction

Many proteins accumulate at the surface of aqueous solutions, thereby changing the surface tension (Doty and Schulman 1949). When a lipid monolayer is present on top of the protein solution the interaction of the protein molecules with the lipid molecules may cause a change of surface pressure at constant area or of monolayer area at constant surface pressure (Quinn and Dawson 1970). Changes of the electrical interfacial potential have also been observed (Ter-Minassian-Saraga 1986), and fluorescence techniques have been used to investigate protein binding (Teissie 1981).

An increase of the surface pressure π at constant monolayer area A is widely interpreted as protein penetration (Verger and Patus 1982), that is partial incorporation of the protein into the hydrophobic region of the lipid monolayer or at least partial occupation of the interface by protein molecules. In contrast, adsorption is interpreted as attachment of the protein to the polar head groups of the lipid layer which should not cause a change of π at constant A. Recently it has been demonstrated with a water soluble porphyrin tetrasulfonate interacting with monolayers containing positively charged head groups that an increase in A at constant π can be due to a change in lipid organization by the adsorbate without penetration (Möbius and Grüniger 1984; Orrit et al. 1985).

It is therefore important to monitor the density of protein molecules at the interface simultaneously with the change of π at constant A (or change of A at constant π) after formation of the lipid monolayer. The reflection method is particularly suited for this purpose since only chromophores at the interface and not those in the bulk contribute to the enhanced reflection (Grüniger et al. 1983). We report on studies of the interaction of fluorescein-labelled insulin (FLI) with monolayers of dioctadecyl-dimethyl-ammonium bromide (DOMA) and dipalmitoyl-phosphatidyl-choline (DPPC) by both π/A and light reflection methods. The accumulation of cytochrome C (CYT) at the air-solution interface was also detected by the reflection spectrum from the interface.

This is a part of the general studies on lipid protein interactions using reflection spectroscopy at the air-water interface. The intention of this paper is to demonstrate the potential of the reflection method for obtaining additional information on lipid protein interactions rather than a detailed analysis of such interactions.

* Permanent address: Center for Marine Research, "Rudjer Boskovic" Institute, Zagreb, Croatia, Yugoslavia
** To whom offprint requests should be sent

Materials and methods

The reflection spectra were measured with the instrument described elsewhere (Grüniger et al. 1983; Möbius et al. 1985) from the surface of solutions of the labelled insulin FLI (purchased from Sigma, concentration 4 mg/l in 2 mM phosphate buffer, pH = 7.15 ± 0.05) or CYT (from Sigma, concentration 28 mg/l in 2 mM phosphate buffer, pH = 7.15 ± 0.05) as difference ΔR in reflectivity from the solution surface and reflectivity from a clean water surface against wavelength. Monolayers of the lipids DPPC (purchased from Larodan, solution 3.42×10^{-3} M in chloroform) and DOMA (from Sigma, solution 3.47×10^{-3} M in chloroform) were formed by spreading 5 µl of the solution on a clean water area of 140 cm^2, followed by compression of the film to the desired initial surface pressure and subsequent transfer to the surface of the FLI or CYT solution using the technique of Fromherz (1975). The surface pressure was measured at constant area with a Wilhelmy balance provided with a plate of filter paper (Möbius et al. 1969). Insulin without fluorescein label (purchased from Sigma) was used under the same conditions as FLI for measuring surface pressure changes at constant area. All the measurements were done at room temperature (18 °C).

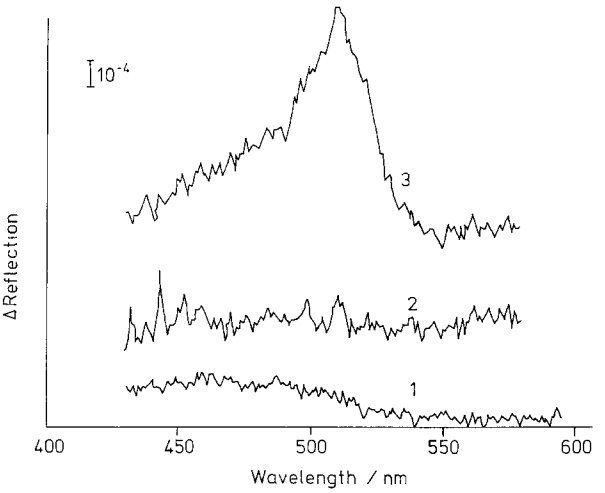

Fig. 1. Reflection spectra plotted as difference ΔR in reflectivity of the solution surface and of a clean water surface against wavelength obtained from a solution of the fluorescein labelled insulin FLI (4 mg/l in 2 mM phosphate buffer, pH = 7.15 ± 0.05) after reaching equilibrium (no further change in surface pressure and ΔR); solution surface (curve 1); solution with monolayer of dipalmitoyl-phosphatidyl-choline (DPPC), initial surface pressure 5 mN/m (curve 2); solution with monolayer of dioctadecyl-dimethyl-ammonium bromide (DOMA), initial surface pressure 5 mN/m (curve 3). Curves 2 and 3 are shifted up for clarity. According to the spectra, there is very small accumulation of FLI at the solution surface, no binding to the DPPC monolayer, and strong interaction with the DOMA monolayer

Results and discussion

Reflection spectra obtained from FLI solutions after reaching equilibrium (i.e. no change in surface pressure and enhanced reflectivity) are shown in Fig. 1: solution surface (curve 1), in the presence of a monolayer of DPPC (curve 2), and of DOMA (curve 3), initial surface pressure $\pi_i = 5$ mN/m. At this pressure of 5 mN/m both DPPC and DOMA are in an expanded state. It was found from the $\pi - A$ isotherms that DOMA shows a strong binding with insulin in contrast to DPPC, thus indicating that insulin binds to positively charged layer. Such preferential binding of proteins to charged lipid layers has been reported earlier (Rietvald et al. 1986).

The traces have been shifted up for clarity. In the absence of a monolayer, two small bands at 455 nm and 490 nm are barely detectable, and in the presence of a DPPC monolayer there is no signal above noise. We conclude from this result that only a very small amount of FLI is accumulated at the solution surface and that no FLI is bound to the DPPC monolayer. In the presence of the DOMA monolayer, however, a strong band at 505 nm is cleary seen in the spectrum which is attributed to the fluorescein label. The difference to the spectrum of curve 1 may be due to a different environment of the fluorescein chromophore after interaction with the DOMA monolayer as compared to the solution surface. The equilibrium intensity ΔR(505) at the maximum increases with increasing π_i of the DOMA monolayer with constant shape of the spectrum.

When the monolayer of the ammonium salt DOMA is transferred to the FLI solution, the surface pressure increases with time due to the interaction of FLI with the monolayer depending on the initial surface pressure. Simultaneously, the reflection ΔR(505) increases. In Fig. 2, these time dependences are shown for π_i of 40 mN/m (top) and 5 mN/m (bottom). The kinetics of changes in surface pressure and reflectivity are quite different, but they are parallel for 5 mN/m. This behaviour indicates different binding to and penetration into the DOMA monolayer of the labelled insulin at the different initial surface pressures. The corresponding surface pressure changes obtained with non-labelled insulin under the same conditions with $\pi_i = 5$ mN/m, shown in Fig. 2 (bottom, triangles) are identical with those for the labelled insulin. At the pressure of 40 mN/m DOMA is in the condensed phase. If it is assumed that the bound FLI has the same average orientation at initial pressures of 5 mN/m and 40 mN/m then from Fig. 2 it is seen from

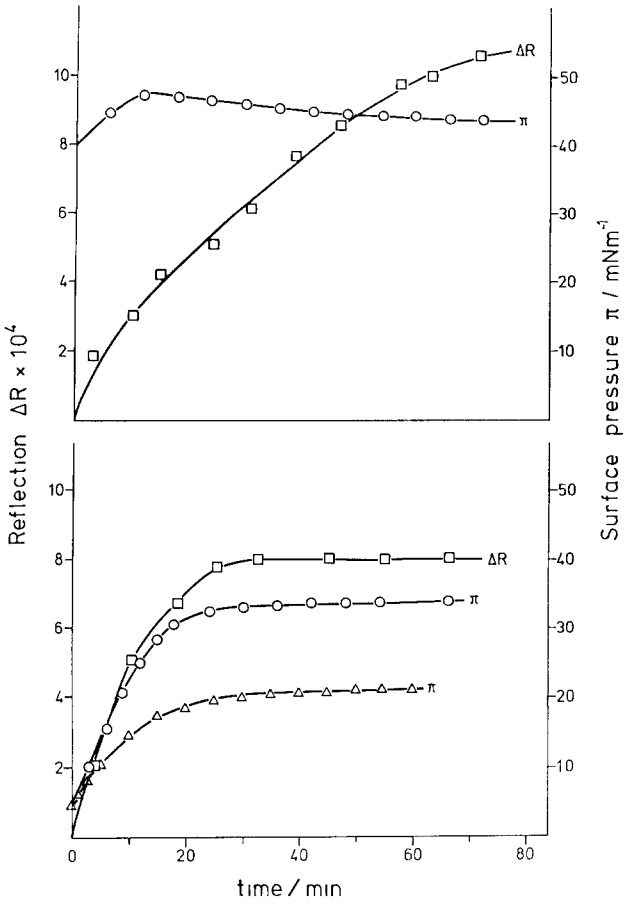

Fig. 2. Interaction of the fluorescein labelled insulin FLI (4 mg/l in 2 m*M* phosphate buffer, pH = 7.15 ± 0.05) with monolayers of dioctadecyl-dimethyl-ammonium bromide at the air-solution interface at different initial surface pressures, surface pressure π (*circles*) and reflection ΔR (505) (*squares*) at the maximum of the reflection spectrum. At π_i = 40 mN/m, the surface pressure increases slightly with time and decreases somewhat after 10 min, the reflection ΔR (505), however, increases slowly until it reaches saturation after more than 70 min only. In contrast to this behaviour, the time dependence of surface pressure and reflection ΔR (505) are parallel for π_i = 5 mN/m. The reflection spectra at equilibrium are the same in both cases. The time dependence of surface pressure obtained with unlabelled insulin (*triangles*) under the same conditions is identical to that of FLI

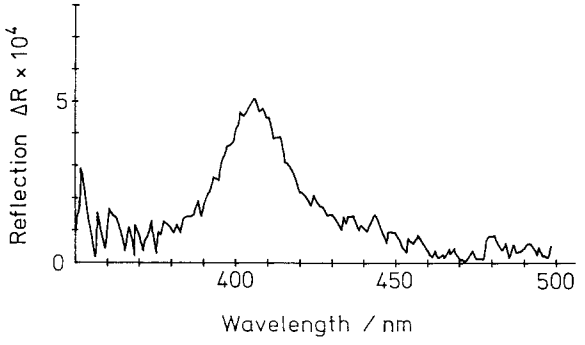

Fig. 3. Reflection spectrum obtained from the air-solution interface of cytochrome C (28 mg/l in 2 m*M* phosphate buffer, pH = 7.15 ± 0.05). The Soret band of the porphyrin chromophore in the protein at 410 nm can easily be identified showing accumulation of the cytochrome C at the solution surface

the reflection signal that there is an increase of the FLI bound to DOMA by a factor of 1.4 at π_i = 40 mN/m as compared to 5 mN/m.

The optical method is of particular value in the case of π_i = 40 mN/m where the kinetics of surface pressure and reflection changes are considerably different. It must be kept in mind, however, that the measurement of enhanced light reflection under normal incidence is not sufficient for a quantitative evaluation of the density of chromophores, since the reflection ΔR depends on their orientation relative to the surface. The different kinetics of π and ΔR (505) could therefore be due to slow orientational changes of the label not involving a large reorganization of the lipid layer. The orientation of chromophores at the air-water interface has been determined in the case of dye monolayers (Orrit et al. 1985; Möbius et al. 1985) using plane polarized light under oblique incidence, and this type of measurement will be applied to the protein/lipid monolayers in the near future.

The method has also been applied to study the accumulation of cytochrome C at the surface of the aqueous solution, and Fig. 3 shows the reflection spectrum obtained in this case at equilibrium. The maximum of the porphyrin Soret band is observed at 410 nm as in the aqueous solution. The time dependencies of surface pressure π and ΔR (410) after sweeping the surface of the CYT solution are shown in Fig. 4. A striking difference is seen between the change of the optical signal and that of the surface pressure, in particular on a short time scale (inset). For short times (10 min) the reflection ΔR (410) was not determined by measuring the spectrum between 350 nm and 500 nm but by monitoring the signal at 410 nm directly. This may introduce some error due to possible base line drift. From these results it must be concluded that the cytochrome C is rapidly accumulated at the air-water interface. The intermediate decrease of the ΔR (410) signal could be due to some reversible reorientation, and measurements with plane polarized light are required to clarify this point. The change in surface pressure, however, is mainly due to slow structural changes of the protein.

The results demonstrate the sensitivity of the technique and illustrate the possibility of using the reflection method as an important additional tool for investigation of the interactions of proteins with lipid monolayers. As mentioned earlier the present results provide no quantitative information regarding the orientation of the chromophores relative to the surface. Hence it is presently not possible to quantify the fractions of molecules that are adsorbed or inserted into the lipid layer. Reflection spectroscopy, however offers a method which is sensitive regarding surface densities and can therefore be used in addition to other monolayer measurements like π/A or surface potential-area

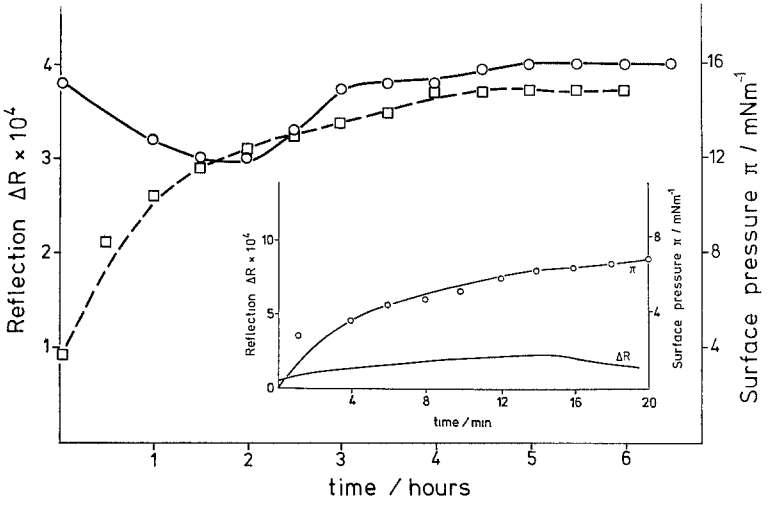

Fig. 4. Time dependence of reflection $\Delta R(410)$ and surface pressure π during 7 h after sweeping the surface of a solution of cytochrome C (28 mg/l in 2 mM phosphate buffer, pH = 7.15 ± 0.05). The initial period of 20 min is shown in the inset. According to the optical signal, the cytochrome C is rather rapidly accumulated at the solution surface, whereas the surface pressure rises slowly, presumably due to slow structural changes of the protein

measurements to study adsorption versus penetration processes in monolayers at the air-water interface. It is seen from the case of CYT (Fig. 4) that there is accumulation of the protein at the interface within a short interval of time as observed from the optical signal whereas the $\pi - A$ diagram is not sensitive enough to this change within this time. Thus the bound molecules at the interface could be detected using the reflection spectra in contrast to the conventional $\pi - A$ or other monolayer measurements.

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