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An infrared spectroscopic study on the lipid–protein interaction in an artificial lamellar system

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SUMMARY

Infrared spectra of lipid–protein films consisting of hemoglobin and arachidic acid and methylstearate, respectively, were measured in the range from 1800–1300 cm^{-1}. The interactions of hemoglobin with this hydrophilic and hydrophobic surface, respectively, are discussed; the degree of uncoiling of the protein may be deduced by comparison with its adsorption and penetration properties.

Recently a technique was developed which allows adsorption of protein molecules to a monomolecular lipid film and the removal of the lipid–protein film from the protein solution for further investigation[1-3]. These lipid–protein films are now studied by infrared spectroscopy in order to obtain more detailed information about the nature of the interaction between the protein molecules and the lipid films. The study is directed primarily to an investigation of the elementary interactions between lipids and proteins, and does not intend to postulate any kind of structural model of biological membranes. For this reason, our present investigations have been restricted to well-known proteins and simple lipids. There is, of course, no reason why this kind of studies should not be extended to membrane constituents.

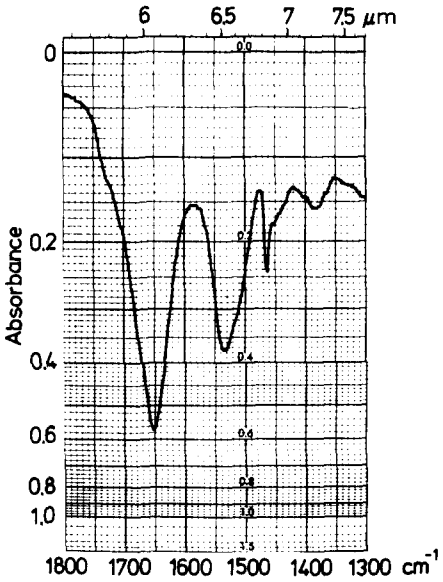
Monomolecular films were made on the water surface of a multicompartment trough[2]. 5 mM solutions of arachidic acid and methylstearate in chloroform (Fluka AG, Buchs and Merck AG, Darmstadt, p. a.) were used. The films were compressed to a surface pressure of 30 dynes/cm and transferred over a protein solution (5 μM human hemoglobin purchased from Pentex Biochemicals, Kankakee, Ill.). After 15 min the lipid–protein film thus produced was separated from the protein subphase by shifting it over several compartments filled with water. The surface pressure was controlled by a Wilhelmy

balance. The quantity of protein adsorbed was determined by the method of Lowry *et al.*[4] after the films had been transferred to a glass slide and the protein had been resolubilized with 0.1 M NaOH.

Because of the minute absorbance of monomolecular films, the Frustrated Multiple Internal Reflection (FMIR) technique was applied in order to produce infrared spectra of reasonable intensity[5]. We used Germanium plates (20 mm x 50 mm x 1 mm, two 45° bevels; Harrick Scientific Corporation, Ossining, N.Y.), which were cleaned ultrasonically, sprayed with distilled water, dried and finally treated in a plasma cleaner (Harrick Scientific Corporation). The plates, now hydrophilic, were then made hydrophobic by covering them with a monomolecular cadmium–arachidate film (5 mM $CdCl_2$, pH 7). The germanium plates prepared in this way were covered with eight lipid–protein films by repetitive dipping in and out through the film[5,6]. During this procedure the surface pressure was kept constant and the transfer of the film onto the plate was followed by automatic registration of the area decrease of the lipid–protein films on the water surface The measurements were performed in an infrared spectrometer (Perkin–Elmer, Model 621) equipped with an FMIR accessory. A germanium plate covered with a single cadmium–arachidate film served as a blank.

Figs 1 and 2 show the infrared absorption spectra of eight transferred hemoglobin–arachidate and hemoglobin–methylstearate films, respectively, in the region of 1800–1300 cm^{-1}. The hemoglobin was adsorbed at pH 7, and the resulting lipid–protein film was transferred onto the germanium plate at pH 4. It is known that polypeptides and proteins of mainly α-helical structure, like hemoglobin, exhibit absorption bands near 1650 cm^{-1} (Amide I) and 1540 cm^{-1} (Amide II), which are assigned to vibrations of the amide groups[8–10]. In native hemoglobin (in KJ) these bands are found at 1650 cm^{-1} and 1525 cm^{-1}, respectively. In the lipid–protein films these bands are also present: In both kinds of film (Figs 1 and 2) the Amide I band is found at 1650 cm^{-1}, whereas the Amide II band of the hemoglobin–arachidate film is skewed and slightly shifted as compared to the hemoglobin–methylstearate film (1535 cm^{-1} for hemoglobin–arachidate and 1525 cm^{-1} for hemoglobin–methylstearate, see Figs 1, 2). The hemoglobin–methylstearate film exhibits an additional band in the amide region at 1620 cm^{-1}.

Multilayers of pure arachidic acid deposited at pH 4 show a strong band at 1690 cm^{-1} (refs 11, 12), which is assigned to C=O stretching of the carboxyl groups[13]. In the hemoglobin–arachidate film this CO band can be noticed as a weak shoulder at 1720 cm^{-1} (Fig. 1). (The difference in wavelength may be explained by the formation of dimers in pure arachidate films[12,13].) However, the intensity of this CO band in the lipid–protein film is considerably lower than could be expected. In multilayers of dissociated arachidate (sodium and cadmium arachidate, respectively, deposited at pH 7) a strong carboxylate band is found at 1540 cm^{-1} (refs 11, 13). A contribution of this lipid band to the spectrum of the lipid–protein film could explain the skewing of the Amide II band mentioned above.



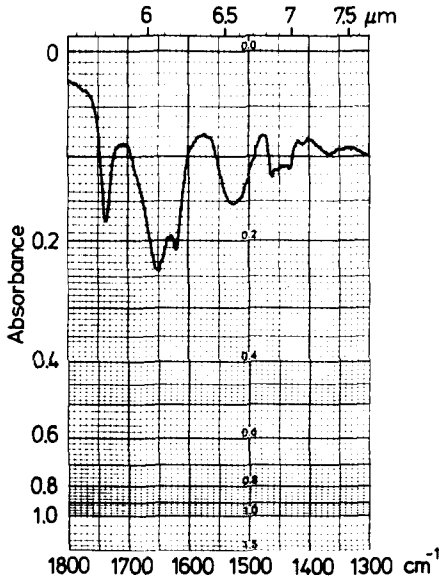


Fig. 1. Infrared spectrum (absorbance *versus* wavelength) of a multilayer assembly consisting of four double layers of hemoglobin–arachidic acid transferred onto a germanium plate. The spectrum is measured by means of the FMIR technique. $2.3 \cdot 10^{12}$ hemoglobin molecules per cm^2 are adsorbed to one arachidic acid film.

Fig. 2. Infrared spectrum (absorbance *versus* wavelength) of a multilayer assembly consisting of four double layers of hemoglobin–methylstearate transferred onto a germanium plate. The spectrum is measured by means of the FMIR technique. $1.1 \cdot 10^{12}$ hemoglobin molecules per cm^2 are adsorbed to one methylstearate film.

The sharp band at 1735 cm^{-1} found in the hemoglobin–methylstearate film (Fig. 2) can be assigned to CO stretching of the ester groups[12,13,15]. The additional band in the Amide I region at 1620 cm^{-1}, however, is found neither in the native protein nor in the pure ester film.

Native hemoglobin exhibits two bands of low intensity at 1445 cm^{-1} and 1385 cm^{-1} (ref. 12), which are also found in both kinds of lipid–protein film, but they are modified by superimposed C–H bending of the lipids (1462 cm^{-1}, C–H scissor of the paraffin chain in both types of film; 1432 cm^{-1}, C–H bending of the methyl groups of the ester in the hemoglobin–methylstearate film)[12,13].

A meaningful interpretation of the infrared spectra of lipid–protein films is only possible if these results are combined with earlier, macroscopic measurements on adsorption and penetration properties of the hemoglobin molecules on lipid films[1,2]. The following assumptions concerning the structure of hemoglobin molecules and the interaction between protein molecules and a lipid film may explain these results:
The hydrophilic side of an arachidate film consists of a closely packed layer of carboxyl groups. The hemoglobin adsorbs onto this layer with the hydrophilic amino acid side chains,

which are oriented to the outside of the molecule. The binding forces are partly ionic. The protein molecules do not uncoil during this process. In the methylstearate film, the part of the lipid molecules facing the water is dominated by the methyl groups. This interface is therefore rather hydrophobic. The protein is bound by hydrophobic interaction of its hydrophobic amino acid side chains with the methyl groups of the film. During this process the protein partly uncoils and a new, non-random arrangement of the amino acids takes place.

This concept is supported by the following results: Upon addition of 30% of the paraffin Eicosan ($C_{20}H_{42}$) to a methylstearate film, the protein exhibits the same adsorption and penetration properties as upon use of a pure ester film[1]. Thus in this case hydrophobic parts of the lipid are responsible for the interaction. In the pure ester film these hydrophobic parts are represented by the methyl groups. This is confirmed by the study of CPK molecule models.

The infrared spectrum of the hemoglobin–methylstearate film exhibits a new band at 1620 cm^{-1}. Since this band is present neither in the native hemoglobin nor in the pure ester film it must be the result of a change in structure of the polypeptide chain. It is known that in the case of the β-pleated sheet conformation of a polypeptide chain there is a band at 1635–1625 cm^{-1} (refs 8–10, 16, 17). Whether the band we found at 1620 cm^{-1} is correlated to a β-pleated sheet conformation, with a maximum slightly shifted by its surroundings, cannot be decided as yet. However, one may conclude that some uncoiling of the native hemoglobin structure takes place. Macroscopically, this destruction of the native protein when adsorbed to a methylstearate film at a high surface pressure is expressed by the very high speed with which the protein penetrates into the lipid film upon reduction of the surface pressure to 10 dynes/cm (ref. 2).

The penetration velocity of a hemoglobin–methylstearate film is considerably greater than that of a hemoglobin–arachidate film. This enhancement suggests that the protein, when adsorbed to an arachidate film, is altered far less than upon adsorption to a methylstearate film. Accordingly, one finds that the hemoglobin–arachidate film shows an Amide I band, which does not differ from that of the native hemoglobin. Unfortunately, the infrared spectra of polypeptides with an α-helix or random coil conformation can hardly be differentiated[8–10]. But, since the velocity of penetration is so much smaller as compared to the hemoglobin–methylstearate film, and can be enhanced considerably by adding 5 M urea[2] it may be concluded, that the Amide I band of the hemoglobin–arachidate film is derived from the native α-helix.

From the disappearance of CO stretching of arachidate in the hemoglobin–arachidate film and the skewing of the Amide II band mentioned above, one may conclude that, while the protein is bound the acid partly dissociates. This may be related to an ionic interaction of the protein and the lipid film[17,18].

The results discussed so far are mainly concerned with the action of the lipid layer on the structure of the protein molecules. In this context it should be mentioned briefly that the infrared spectra also contain information about the loosening up of the

structure of lipid layers by the embedded protein molecules. This is demonstrated by the narrow band of the hemoglobin–arachidate film at 1462 cm^{-1}. In pure arachidate multilayers this band (C–H scissors of the paraffin chain) is split up by lattice forces into two sharp bands at 1458 and 1467 cm^{-1} (ref. 12).

The results of infrared spectroscopy, together with former results of studies on the adsorption and penetration process, show that a soluble globular protein behaves quite differently towards a polar and a non-polar lipid film, respectively. The protein is bound to both interfaces but, as the kind of bonding must be different, a different conformation in the state of adsorption is also found. Polar or ionic bonding does not cause destruction of the protein structure, whereas non-polar bonding changes the conformation in a specific way without destroying it completely. It might be interesting to compare these results with similar infrared studies of biological systems[19–22].

A disadvantage of the studies described here is that the lipid–protein films are deposited as multiple layers, and therefore only indirect inferences on the state of the films on the water surface are possible. However, the penetration experiments mentioned, which do give information about the properties of lipid–protein films on the water surface, may easily be correlated to the results of the infrared spectra of deposited films. It therefore seems reasonable that the more detailed results obtained from the spectra, hold for lipid–protein films on the water surface also.

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