Absorption Spectra Indicate Conformational Alteration of Myoglobin Adsorbed on Polydimethylsiloxane

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ABSTRACT To assess the effects of adsorption on protein structure, ultraviolet optical absorption spectra of myoglobin (Mb) bound to polydimethylsiloxane (PDMS) were measured. A flow cell, which enabled adsorption under controlled hydrodynamic conditions, was used in conjunction with a conventional spectrophotometer to obtain the spectra. Adsorption to PDMS reduced significantly the absorbance in the Soret region of the Mb spectrum, whereas the spectrum in the region near 280 nm was essentially unaffected. This result showed that disruption of the native structure of Mb occurs following interaction with PDMS. Furthermore, the change in the absorption spectrum may indicate loss of heme from the heme pocket of the adsorbed protein. Mb structure was altered from its solution configuration within fifteen min of contact with the surface. Exchange of adsorbed Mb with Mb in solution had little or no effect on the absorption spectrum of the surface-confined protein, indicating that exchange occurs only between conformationally altered species or between native species.

INTRODUCTION

Protein adsorption

Protein adsorption at the solid-liquid interface plays an important role in many applications (Cooper and Peppas, 1982; Andrade, 1985; Horbett and Brash, 1987). One key element to understanding the physical and chemical properties of adsorbed proteins is elucidation of the effects of adsorption on their three-dimensional structure. Many previous studies of adsorbed protein conformation have involved proteins (for example, fibrinogen, fibronectin, and serum albumin) whose structures are incompletely characterized in solution, making interpretation of adsorbed protein structure difficult. Furthermore, the limitations of current structure-sensitive techniques have prevented precise examination of adsorbed protein conformational issues. Consequently, the relationship between structure and function of adsorbed proteins remains incompletely understood.

To address issues of adsorbed protein structure, we have chosen to study, as a model system, adsorption of sperm whale myoglobin (Mb) onto polydimethylsiloxane (PDMS). In three previous studies of this system (Darst et al., 1986; Darst et al., 1988; Anderson, 1991) results were obtained that could be interpreted as evidence for conformational changes of the protein after adsorption. In the first study, nonlinearities of the time-dependent fluorescence of fluorescein-labeled Mb and increasing tenacity of the PDMS-bound Mb with adsorption time were postulated to involve structural changes of the adsorbed protein. In the second study, the binding of monoclonal antibodies to PDMS-bound Mb was inhibited compared to binding to Mb in solution. It was not possible, however, to distinguish conformational changes from non-random orientation of the adsorbed Mb. In the third study, the fluorescence of a Mb analog containing a chlorophyll derivative instead of heme was reduced after adsorption of the protein to PDMS. One interpretation of these fluorescence results was that the conformation of the protein was altered following adsorption to PDMS such that the quantum yield of the chlorophyll was decreased. Because of the complexity of interpreting antibody binding and fluorescence results in terms of adsorbed protein conformation, it was necessary. to examine this system with other techniques to obtain more definitive information concerning the structure of PDMS-bound Mb.

In the present study, an optical property peculiar to heme-proteins was exploited to investigate the structure of Mb bound to PDMS. The absorbance of Mb in the ultraviolet and visible wavelengths depends directly on the physical environment of the heme and, consequently, the structure of the heme pocket. For this reason, the absorption spectrum of PDMS-bound Mb can serve as a useful indicator of conformation and is more directly interpreted in terms of protein structure than the antibody binding (Darst et al., 1988) and fluorescence (Darst et al., 1986; Anderson, 1991) results reported previously. Experiments designed to measure the absorbance of PDMS-bound Mb described here were performed to challenge these previous results and to provide more evidence concerning the time-dependence of alteration of adsorbed Mb structure.

Absorbance of Mb in solution

Absorption spectra of Mb in solution have been extensively characterized (Antonini and Brunori, 1971; Hanania et al., 1966; Eaton and Hochstrasser, 1968) and are known to be rich in information concerning the physical state of the pro-

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tein. Mb absorption bands in the ultraviolet and visible wavelengths can be used to reveal physical properties such as the oxidation state of the heme iron (Antonini and Brunori, 1971), the identities of ligands bound to the heme (Eaton and Hochstrasser, 1968), and the structural integrity of the protein (Schechter and Epstein, 1968). This final property is critical for the present study, but the other features of the absorption spectra would be useful for examining the function of Mb in the adsorbed state.

The iron atom contained within the heme can exist in either an oxidized (ferric) or reduced (ferrous) form. In all experiments discussed in this work, the heme iron is in the ferric state. This form of Mb, often referred to as metMb, can bind various ligands from solution, including cyanide, azide, fluoride, ammonia, and formate (Hanania et al., 1966; Eaton and Hochstrasser, 1968). In the absence of specific ligands, metMb contains a water molecule or a hydroxide ion bound to the heme, depending on the pH of the solution. For all studies described herein, solutions are maintained at pH 7.4 and the metaquo-Mb (i.e., water-liganded) form dominates. For convenience, and because heme iron is in the ferric form, pH is kept constant at 7.4, and no specific ligands are involved in these experiments, the term Mb will be used throughout to refer to metaquo-Mb.

The absorption spectrum of Mb in phosphate-buffered saline (PBS) at pH 7.4 contains two strong bands in the ultraviolet region and several weaker bands in the visible region (Antonini and Brunori, 1971; Gouterman, 1978). The extinction coefficients of the visible bands are too low to allow their measurement for adsorbed Mb with the experimental system used here. For this reason, discussion is limited to the two ultraviolet bands.

The peak centered at 280 nm in the Mb absorbance spectrum is due to aromatic amino acids (primarily tryptophan and tyrosine) in the polypeptide and to the heme. Its location in the Mb spectrum is characteristic of most proteins. The other absorption peak centered at approximately 410 nm is due entirely to the heme. This narrow, intense band is characteristic of all heme proteins and is commonly referred to as the Soret band. The position, intensity, and shape of this peak depend strongly on the oxidation state of the heme iron and the identity of any ligand bound to the heme.

The Mb absorption spectrum also is sensitive to the structure of the protein. Shown in Fig. 1 is a comparison of the spectra of Mb in PBS and Mb in PBS and 8 M urea (protein concentration is identical for the two spectra). This concentration of urea disrupts significantly the secondary and tertiary structure of Mb (Schechter and Epstein, 1968; Herskovits et al., 1970b). The loss of native protein structure causes a profound decrease in the extinction coefficient and a blue shift of the absorption maximum of the Soret band, whereas the absorbance of the 280 nm band is relatively unaffected.

The spectral shift shown in Fig. 1 is specific to urea denaturation; it is recognized, however, that the spectrum undergoes qualitatively similar changes when Mb encounters other denaturing conditions, including high temperature

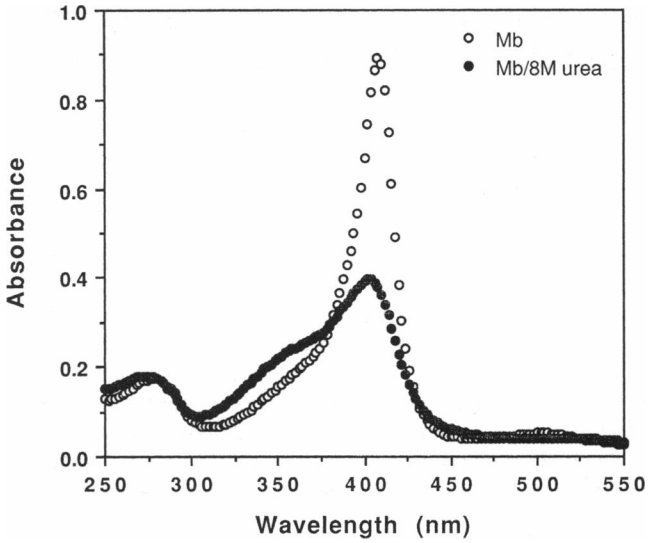

FIGURE 1 Absorption spectra for Mb in PBS and in PBS containing 8 M urea. The protein concentration in both solutions was 85 μg/ml (4.8 μM).

(Acampora and Hermans, 1967), low pH (Acampora and Hermans, 1967; Breslow and Gurd, 1962), guanidine-HCl (Schechter and Epstein, 1968), and alcohols (Herskovits et al., 1970a). The Soret peak alteration results from changes to the physical environment of the heme after unfolding of Mb. Transfer from a hydrophobic to an aqueous environment, scission of the iron-nitrogen bond, interaction with denaturant (if present), and contact with other heme molecules can alter heme absorbance. The Mb absorption spectrum, therefore, is a direct indicator of heme environment and, consequently, integrity of the protein's structure.

Specific structural changes that occur to Mb after denaturation have not been characterized completely at the atomic level. It is known, however, that: α-helical structures are disrupted, based on circular dichroism studies (Schechter and Epstein, 1968); buried histidine residues become exposed, based on acid titration measurements (Breslow and Gurd, 1962); fluorescence of tryptophan residues is altered (Schechter and Epstein, 1968; Hochstrasser and Negus, 1984); and intrinsic physical properties, including heat capacity (Privalov et al., 1989) and intrinsic viscosity (Privalov et al., 1989) are modified. Such results indicate that denaturation of Mb in solution encompasses significant alteration of its three-dimensional structure. Although the Mb absorption spectrum alone does not contain this level of detail, it is possible nevertheless to determine whether alteration of heme environment occurs. Indeed, the similarity between spectra of isolated heme in solution (Gouterman, 1978) and denatured Mb suggests that the heme may be lost entirely from the polypeptide during denaturation. Additional evidence exists to support this hypothesis (Schechter and Epstein, 1968).

The goal of the work described here is to measure the absorption spectrum of Mb bound to PDMS. Mb absorbance can be interpreted directly in terms of the environment of the heme and, therefore, the integrity of the heme pocket. For this

reason, absorbance measurements are considered to be a more straightforward indicator of Mb structure than previous fluorescence or antibody binding measurements.

MATERIALS AND METHODS

The flow cell

Several specific criteria governed design of the flow cell. First, it was desired to conduct adsorption experiments under laminar flow conditions similar to those used during previous total internal reflection fluorescence (TIRF) studies of adsorbed Mb. Second, it was necessary that the flow cell fit within the optical train of a conventional ultraviolet/visible spectrophotometer. Third, it was essential that the spectrophotometer beam sample enough adsorbed protein to allow sufficient signal to be obtained. Finally, it was necessary that the adsorbing surfaces be optically transparent to wavelengths at least as low as 260 nm to allow adequate measurement of the 280-nm band.

These specifications were used to design and construct a cell consisting of a stack of seven PDMS-coated quartz slides alternating with six Teflon spacers, each of which was machined to form a chamber when bounded by a slide on each side. The cell is shown schematically in Fig. 2. Not shown in this figure (for purposes of clarity) are the end caps which couple the entrance and exit ports at the ends of the Teflon chambers to a flow system and the hardware used to compress the stack of slides and spacers. When assembled, the flow cell contains six parallel thin slits through which protein solution can flow and twelve surfaces onto which protein can adsorb. This cell is similar in design to a multiple-surface cell used previously (McMillin and Walton, 1974) to measure circular dichroism spectra of adsorbed proteins.

To alleviate problems with leaking, a thin layer of vacuum grease was used to couple each slide to the Teflon spacers and to couple the end caps to each end of the slide/spacer stack. Under these conditions, leaking could be avoided, but only at low peristaltic pump flow rates. This limited the range of shear rates that could be examined with this cell.

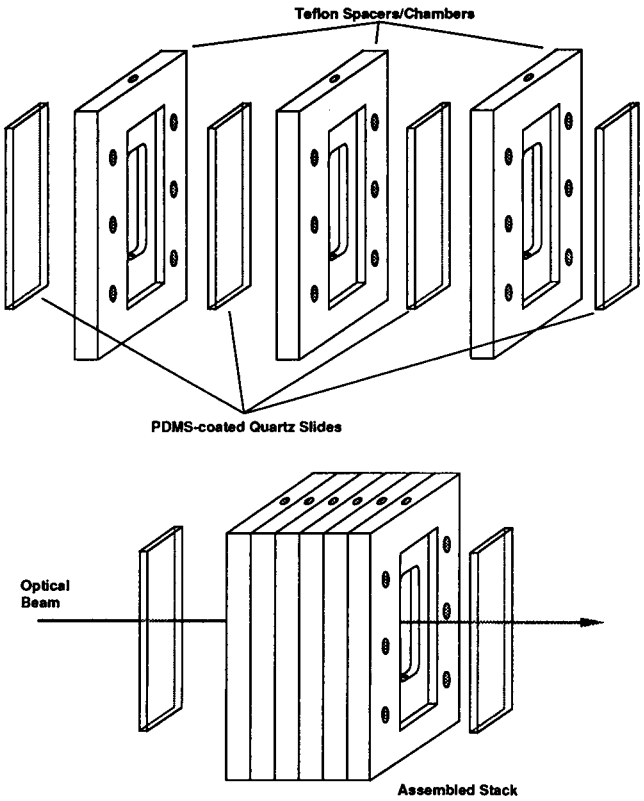

FIGURE 2 Schematic diagram of the flow cell used for the absorption spectra measurements. (*Top*) Exploded view of the spacer/slide stack. (*Bottom*) Assembled stack. The stack was compressed with end plates (not shown) aligned with bolts threaded through the holes in the spacers. Caps were added to the top and bottom of the stack to couple the ends of the Teflon spacers to the flow system. The flow cell was placed in the beam of the spectrophotometer.

Flow system and spectrophotometer

The flow system used for these experiments was similar to that used for previous TIRF experiments (Lok et al., 1983). The system consists of two reservoirs containing protein solution and buffer solution, respectively, held in a constant temperature bath. Before addition of Mb to the protein reservoir, buffer solutions were sparged with helium to reduce oxygen content and to inhibit bubble nucleation in the flow system. After 30 min of helium flow, the spargers were lifted above the fluid surface and the gas flow was switched to nitrogen. This protocol provided a gaseous blanket over the protein and buffer solutions, reducing contact with ambient air. Fluid was pumped by a peristaltic roller pump (Rainin, Middleton, WI) and directed to the flow cell or a waste receptacle by a four-way valve near the entrance to the flow cell.

The flow system and cell were coupled with a HP8452A diode-array, UV/Vis spectrophotometer (Hewlett Packard, Palo Alto, CA) in conjunction with a Vectra personal computer (Hewlett Packard). The flow cell was oriented vertically in the spectrophotometer with the entrance port at the bottom. Data were collected and stored digitally on the Vectra using the spectrophotometer controller software. The HP8452A measures a complete spectrum (from 190 to 820 nm) approximately every 0.1 s. For the experiments conducted here, a spectral averaging time of 25 s was used. Thus, each resulting spectrum was the average of 250 consecutive measurements taken over a 25-s interval. The signal-to-noise ratio in the spectra was observed to decrease with decreasing averaging times.

Myoglobin

Sperm whale Mb was obtained from Sigma Chemical Co. (St. Louis, MO) and used as received. Stock solutions of Mb were prepared by dissolving the protein in water purified with a standard MilliQ system (Millipore, Bedford, MA). Mb solutions were centrifuged to remove insolubles and stored at 4°C. Protein concentration was determined spectrophotometrically. A few crystals of KCN were added to a diluted aliquot and the absorbance at 540 nm was measured. An extinction coefficient of 10.4 mM^{-1} cm^{-1} (Hanania et al., 1966) and a Mb molecular weight of 17,800 were used for all concentration calculations. Protein solutions used in the adsorption experiments were prepared by diluting an aliquot of stock solution into PBS sparged previously with helium (as described above).

PDMS-coated quartz slides

CO-quartz slides (1 inch × 3 inches × 1 mm) were obtained from ESCO Products (Oak Ridge, NJ). Slides were cleaned using procedures identical to those described previously (Lok et al., 1983; Cheng et al., 1987). Because it was necessary to coat the slides on two sides with PDMS, spin-coating protocols were modified as follows. PDMS was deposited on the first side of the slide as described previously (Cheng et al., 1987). Immediately after the 1-min spin on the photoresist spinner, the coated slide was inverted, placed again on the spinner, and coated with PDMS on the opposite side. After PDMS had been applied to both sides, the slide was then placed under a heat lamp for curing. This procedure was found to yield slides of superior optical quality compared to slides coated on one side and cured and subsequently coated and cured on the second side. PDMS-treated slides were used within 2 weeks. Fresh slides were used for each experiment.

Solutions

Protein solutions were prepared in PBS having the same composition described previously (Darst et al., 1986). Buffer and protein solutions were treated as described above before adsorption experiments.

Experimental conditions

All adsorption experiments were conducted under the following conditions: temperature, 25°C; wall shear rate, 10 s^{-1}; protein concentration, 8.5 mg/ml (480 nM). It was not possible to examine adsorption at shear rates higher than 10 s^{-1} because increased flow rates through the cell resulted in leaking.

Experiments were conducted initially as follows. After degassing of PBS solutions and addition of Mb to the protein reservoir, PBS was passed through the flow cell. After several minutes of flow, a blank spectrum was recorded. This spectrum served as the baseline for all subsequent spectra. Shortly thereafter, the PBS solution was replaced with a Mb solution (t = 0). Because the protein solution and the adsorbing surfaces were both in the path of the spectrophotometer beam, it was not possible to measure spectra of adsorbed Mb continuously as a function of time. Therefore, it was necessary to replace the Mb solution with PBS again to record the spectrum of adsorbed Mb alone. The time at which the Mb solution was replaced with PBS is considered to be the adsorption time. After ~10 min of PBS flow, the adsorbed protein spectrum was recorded.

During experiments using this protocol, it was determined that spectrophotometer lamp intensity decreased slowly with time. Because absorbance of adsorbed Mb was sufficiently low, the drift in lamp intensity influenced the resulting spectra. To account for intensity drift, the baseline spectrum was measured at 5- or 10-min intervals for 1 h before starting an adsorption experiment. The rate of drift in baseline absorbance was steady over several hours but varied from day to day. Therefore, it was necessary to measure the absorbance drift before each experiment and subtract the time-dependent baseline from the adsorbed protein spectra (extrapolating drift for experiments longer than 1 h). Because the magnitude of absorbance drift was substantial and the drift rate was not constant over tens of hours, long adsorption experiments were more susceptible to error and, therefore, were discontinued. It was determined, however, that absorption spectra of PDMS-bound Mb changed little after about 15 min of adsorption (see below) and, thus, experiments lasting more than several hours were not essential to this study.

RESULTS AND DISCUSSION

The spectrum of Mb is affected significantly by adsorption

Adsorption to PDMS was determined to alter the absorption spectrum of Mb. Shown in Fig. 3 is a comparison of two spectra, of Mb adsorbed to PDMS and of Mb in solution. Each spectrum shown is for an equivalent number of Mb molecules in the path of the spectrophotometric beam. The adsorbed Mb spectrum shown was recorded after adsorption of Mb to PDMS for 2 h. The optical density of the Mb solution spectrum was adjusted to represent 12 surfaces covered with native, intact Mb with a surface concentration, determined previously (Darst et al., 1986), of 94 ng/cm^2. If no change to the optical properties of Mb occurred after adsorption, then the two spectra in Fig. 3 would be identical. It is clear, however, that the spectra are significantly different.

Two qualitative features are apparent in Fig. 3. First, the optical density and shape of the Soret peak are altered after adsorption. The shape of this portion of the spectrum is comparable to the shape of the spectrum of Mb exposed to urea, although the reduction in intensity is more substantial. Second, the 280-nm peaks, in contrast to the Soret peaks, appear similar in terms of location, shape, and optical density. This observation is also consistent with the behavior of the 280-nm peak during denaturation of Mb in solution (see Fig. 1).

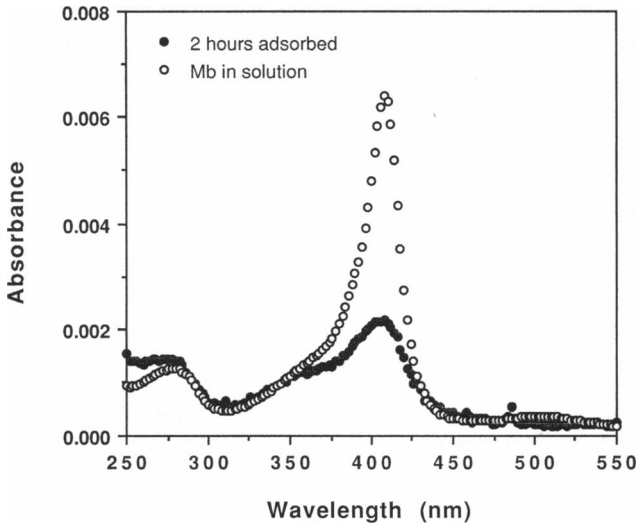

FIGURE 3 Comparison of the absorption spectra of Mb in solution and Mb adsorbed to PDMS. The solution spectrum was recorded in PBS, pH 7.4 at the protein concentration given in Fig. 1. The adsorbed Mb spectrum was measured after 2 h of adsorption to PDMS under conditions described in the text.

It is difficult, however, to extrapolate a change of heme environment to predict precise conformational changes experienced by the polypeptide portion of Mb following adsorption. Indeed, one could postulate various mechanisms to account for altered heme environment, ranging from a minor change involving relaxation of the folded structure of the heme pocket which allows exposure of heme to either the aqueous or PDMS phase to a drastic change involving loss of heme from the polypeptide and complete disruption of all tertiary and secondary structure. Previous study of the Mb/PDMS system (Darst et al., 1988) suggests that alteration of Mb structure is not as extreme as implied by the latter mechanism, but does not relieve entirely the ambiguity surrounding adsorbed Mb structure.

It is also not clear from this result whether all heme remains at the interface with the Mb polypeptide or whether some of it is lost to solution. It was shown previously (Darst et al., 1986) that the surface concentration of the polypeptide portion of Mb adsorbed on PDMS is constant between 30 min and 10 h of adsorption. If the heme were lost from the surface and carried away by the flowing solution, reduction of the absorbance of the Soret band, as well as the 280 nm band would be expected. Because absorbance of the latter is not decreased significantly by adsorption, it is likely that reduced absorbance in the Soret band is due primarily to alteration of the environment of the heme.

Mb structure is altered before 15 min

The absorption spectrum of Mb was monitored initially for times ranging from 2 to 8 h to ascertain whether changes occurred during the same time period as the fluorescence of FITC-Mb (Darst et al., 1986) and chlorophyll-substituted Mb (Anderson, 1991). Because of the problems associated with lamp intensity drift, it was difficult to measure spectra accurately at these longer times. The spectra (not shown) did not appear to change significantly, however, over this time period. This result highlighted the need for examination of Mb absorbance at shorter surface-contact times.

Shown in Fig. 4 are absorption spectra of Mb adsorbed to PDMS for times ranging from 15 min to 2 h. These results indicate that alteration of heme environment and Mb structure, discussed in the previous section, occurs before 15 min of adsorption to PDMS. Also, based on the similarity between the 15 min and 2 h spectra, it is concluded that no significant changes in heme environment occur between these two times. These findings are in contrast with fluorescence results (Darst et al., 1986; Anderson, 1991), which indicated that structural changes to PDMS-bound Mb may have occurred after 2 h of adsorption, although the fluorescence data measured in these studies were not unambiguously influenced by conformational changes alone.

It would have been revealing to examine the spectrum of Mb adsorbed for times shorter than 15 min to determine more precisely when heme pocket structure is first affected, but experimental protocols and the nature of PDMS-bound Mb imposed this time as a lower limit. This time was necessary to allow complete replacement of PBS with Mb solution in the flow cell and to ensure that Mb was bound with sufficient tenacity to the surface so that no significant desorption occurred during the PBS flush of the flow cell after adsorption. Mb has been shown to unfold in solution in times ranging from milliseconds to minutes (Shen and Hermans, 1972). It is conceivable, therefore, that unfolding of surface-bound Mb could occur on a similar time scale, although confinement to the surface may inhibit molecular motions within the protein. To examine adsorbed Mb conformation before 15 min, it would be necessary to devise a method or apparatus which allows spectra to be measured during adsorption. An internal reflection, UV/visible spectrophotometer would be useful for such measurements.

Exchange has no effect on adsorbed Mb absorbance

Adsorption of a protein to a surface is a dynamic process. For example, although the surface concentration of Mb reaches a maximum early in the adsorption process (after a few minutes for the conditions used in these experiments), it is still possible for Mb in solution to exchange with surface-bound Mb. Indeed, adsorbed Mb does exchange with Mb in solution (Darst, 1987); the rate of exchange, however, decreases as a function of time, eventually approaching 0 after several hours of adsorption. Therefore, during the range of times over which Mb absorbance was measured in this study, it is likely that Mb in solution was exchanging with adsorbed Mb and, thus, the time that an individual protein was actually in contact with the surface varied among the adsorbed protein population.

To examine whether exchange had an effect on absorbance, Mb was adsorbed to PDMS from solution for 15 min and then PBS was re-introduced into the flow cell and the absorption spectrum of surface-confined protein was monitored as a function of time. With this protocol, the absorption spectrum at each time was due only to protein that was adsorbed at 15 min, because no exchange could occur after replacement of the Mb solution with PBS.

The results of this experiment are shown in Fig. 5. There

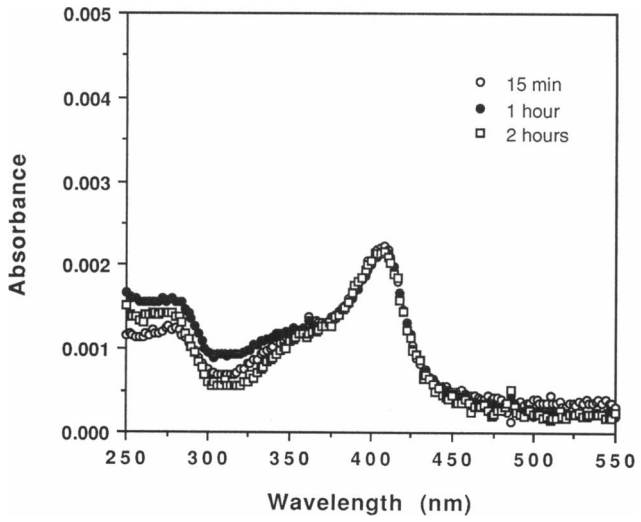

FIGURE 4 The absorption spectra of Mb adsorbed to PDMS for times ranging from 15 min to 2 h. The time shown in the legend corresponds to the start of the PBS flush of the flow cell.

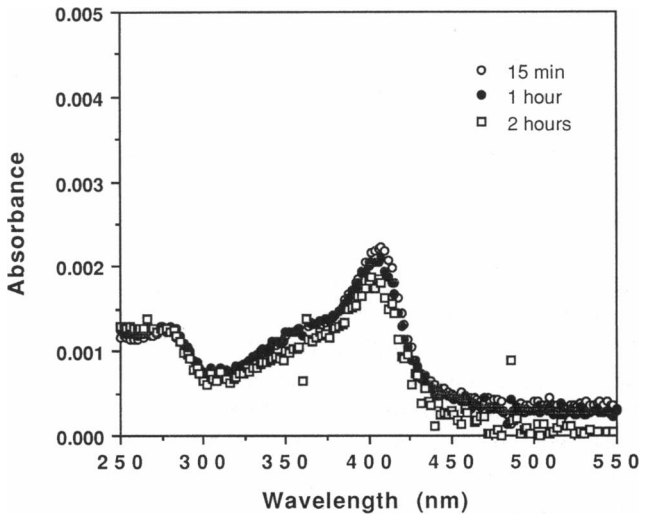

FIGURE 5 The time-dependent absorption spectra of Mb adsorbed to PDMS for 15 min followed by PBS flow through the cell. The time shown in the legend indicates the total time of contact between the protein and the PDMS.

is little change to the spectrum of Mb confined to PDMS between 15 min and 2 h. Indeed, this figure appears very similar to Fig. 4 except for the slight decrease in absorbance in the Soret peak between 15 min and 2 h. This decrease could indicate either that the average environment of heme changed (for example, more dimers formed) or that some heme was lost from the interface over this time period. The results of this experiment indicate that the absorbance data presented above were not affected substantially by exchange with the surrounding protein solution. This is not to say that exchange did not occur; it indicates only that, if exchange occurred, it did not alter the absorption spectrum of the adsorbed proteins.

CONCLUSIONS

The primary conclusion from these results is that the native structure of Mb is affected by adsorption to PDMS. The absorbance measurements indicate that the environment of heme in the adsorbed protein is altered, which can only occur if the structure of the protein changes to expose the heme to the aqueous or PDMS phase or the heme leaves the pocket altogether. This result is consistent with a recent study (Arai and Norde, 1990) which concluded, based on thermodynamic arguments, that Mb structure should be altered after adsorption from aqueous solution to a low energy surface, like PDMS. The nature and extent of conformational changes of adsorbed Mb were not specified in that work. In addition, these results eliminate the ambiguity associated with previous fluorescence studies of adsorbed Mb (Darst et al., 1986; Anderson, 1991). For example, one issue that is now clear is that the structure of Mb is altered significantly within 15 min of contact with PDMS.

The absorbance measurements suggest that the heme group may be lost from Mb adsorbed to PDMS. As discussed in the Introduction, loss of heme from Mb in solution is accompanied by significant changes to the three-dimensional structure of the protein. Results of a previous study (Darst et al., 1988) of PDMS-bound Mb using site-specific monoclonal antibodies to probe the conformation of the adsorbed protein indicated that, if structural changes to the protein occurred following contact with the surface, they were small and confined to local regions. A cursory examination indicates these two results are contradictory.

The two interpretations can be rendered consistent, however, by consideration of the following. First, it is known that apoMb (Mb without the heme) shares considerable three-dimensional structure with native Mb. Based on circular dichroism measurements (Schechter and Epstein, 1968), it has been shown that apoMb retains ~80% of the α-helical content of Mb. Therefore, loss of heme in solution does not result in complete disruption of the native secondary structure of the protein. Second, confinement of Mb at the polymer surface may limit the extent of structural change by restricting the protein to a subset of the dynamic conformations normally sampled in solution after removal of the heme. Third, it has been demonstrated that the affinities of the antibodies used previously (Darst et al., 1988) to probe adsorbed Mb structure are not substantially altered by removal of the heme from the protein. The affinities for apoMb of two of the antibodies used in that study are reduced by a factor of ~2–3 compared to their affinities toward Mb (Jay A. Berzofsky, National Cancer Institute, National Institutes of Health, personal communication). It was observed that affinities for three of the antibodies were reduced slightly toward PDMS-bound Mb, whereas the affinities for the other two were reduced by greater than two orders of magnitude. The results with the first three antibodies are consistent with the interpretation of the present study (i.e., loss of heme from adsorbed Mb), whereas the results with the other two antibodies suggest more substantial disruption of the antibody-binding sites in adsorbed Mb or orientation of the adsorbed protein such that the antibody-binding sites are inaccessible. These results taken together suggest that Mb may retain a structure similar to apoMb on the PDMS surface.

It is often assumed in the literature that proteins adsorbed to hydrophobic surfaces experience conformational changes. To understand the function or to control the behavior of adsorbed proteins, however, it is essential that the nature and extent of conformational change be elucidated. The present work has revealed several conclusions concerning the nature and extent of the alteration of the three-dimensional structure of adsorbed Mb. Nevertheless, many unanswered questions remain about this system and other protein/surface combinations. It is likely that the answers to these questions will be unattainable without the advancement of current techniques and the development of novel methodologies for examining the three-dimensional conformation of adsorbed proteins. This should be a major focus of future studies of proteins at interfaces.

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