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LETTER TO THE EDITOR

Spectroscopic Investigation of the Structure of a Protein Adsorbed on a Hydrophobic Latex

Spectroscopic methods provide a powerful tool for investigating the structural properties of immobilized proteins. For that purpose, there was a strong need for a hydrophobic particle that allows determination of the adsorbed protein conformation by fluorescence and circular dichroism. Among the various hydrophobic suspensions that are available, perfluoro-alkoxy fluoro carbon Teflon latex satisfies the requirements of low light absorption and scattering. As an example, preliminary results of structural changes of a proteolytic enzyme dissolved in aqueous solution and in the adsorbed state are given.

Key Words: **protein; adsorption; hydrophobic particle; fluorescence; circular dichroism.**

INTRODUCTION

An experimental problem in spectroscopic studies of the structure of proteins adsorbed on finely dispersed particles is caused by scattering and/or absorption of light by the sorbent particles. In fluorescence spectroscopy it leads to aberrant values for the measured fluorescence anisotropy (1, 2). In a few circular dichroism (CD) studies the problem has been circumvented by having the protein desorbed prior to the measurement (3–5). However, the structure of the desorbed protein may well differ from that of the protein in the adsorbed state (6). So far, succesful CD measurements on adsorbed proteins have been reported using dilute dispersions of ultrafine silica particles for which absorption and light scattering are minimized (6, 7). These silica particles are *hydrophilic* and because the hydrophobicity of the sorbent is an important parameter in the structural behavior of proteins at interfaces (8) there was a strong need for *hydrophobic* surfaces that allow spectroscopic determination of the adsorbed protein structure. In literature a few CD and fluorescence studies using hydrophobic surfaces have been reported (9–11). However, these studies were disturbed by considerable light absorption by the polymeric sorbent matrices. Furthermore, in a few cases the particles used were also good scatterers.

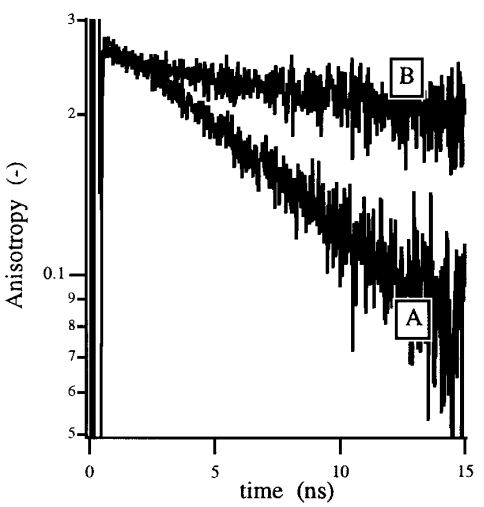

FIG. 1. Fluorescence anisotropy decay of dissolved protein (curve A) and protein adsorbed on Teflon particles (curve B: $\Gamma = 2.0$ mg m^{-2}).

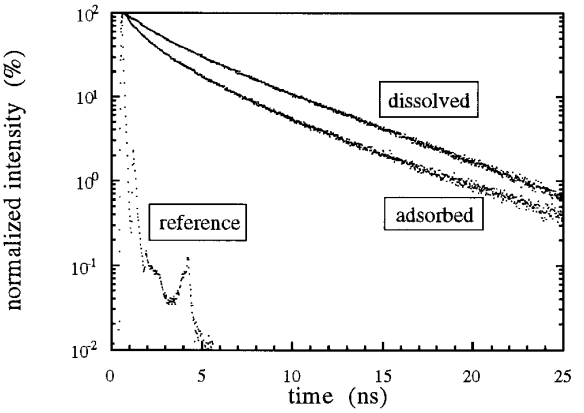

FIG. 2. Normalized fluorescence decay of dissolved and adsorbed protein ($\Gamma = 0.5$ mg m^{-2}). The curve for $\Gamma = 2.0$ mg m^{-2} coincides with that of the free protein (not shown).

In this letter we report on hydrophobic sorbent particles that allow obtaining unambiguous spectroscopic results for the adsorbed protein. The sorbent material is a copolymer of tetrafluoroethylene and perfluorovinylether. Thus the polymeric matrix of the particles is a perfluoroalkoxy (PFA) fluorocarbon resin (further denoted Teflon) that does not contain any UV absorbing double bonds and aromatic groups. Because of the amorphous character of this material its refractive index (1.35) is close to that of water (1.33). Hence, it has the desired properties of negligible absorption and very low scattering.

EXPERIMENTAL

Protein and sorbent. The protein studied is wild-type Subtilisin 309 (molecular mass: 28 kDa, pI 10) which has been inhibited with phenylmethanesulfonylfluoride to prevent autolysis during the course of the experiment (5, 12, 13). The adsorbent used is negatively charged Teflon latex (kindly donated by Du Pont de Nemours) which is free of spurious contaminants. The latex particles (density $\rho = 2.0$ g cm^{-3}) have a diameter of about 215 nm and are highly monodisperse as inferred from electron microscopy. From the contact angle θ of a sessile drop of water on a tablet of the dried material ($\theta = 96°$), it is concluded that Teflon is hydrophobic (12).

The coverage Γ of the Teflon surface by the protein is adjusted while the total protein concentration is maintained at 0.10 g l^{-1} (in $Na_2B_4O_7$–HCl buffer at pH 8.0, $I = 0.01$ M and $T = 20°C$). To this end, the amount of Teflon particles is 0.25% w/w for $\Gamma = 2.0$ mg m^{-2} ($=\Gamma_{plateau}$ in the isotherm) or 1.40% for $\Gamma = 0.5$ mg m^{-2} according to the characteristics of the adsorption isotherm (12). The equilibrium concentration of protein in solution (after 10 min contact time) at $\Gamma = 2.0$ mg m^{-2} was 0.025 g l^{-1} (75% of the protein adsorbed) while for $\Gamma = 0.5$ mg m^{-2} practically all

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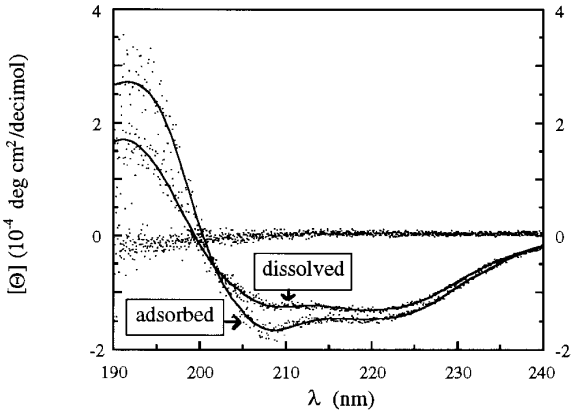

FIG. 3. Molar residual ellipticity Θ of dissolved and adsorbed (Γ = 0.5 mg m^{-2}) protein. The solid lines are the fits to the secondary structure yielding 35% α-helix for the dissolved and 50% α-helix for the adsorbed protein. Blank curves of buffer and buffer + Teflon are the lines near zero. The curve for Γ = 2.0 mg m^{-2} coincides with that of the free protein (not shown).

protein was adsorbed. The properties of the adsorbed protein are compared with those of the protein in aqueous solution.

Time-resolved fluorescence. Measurements were carried out using a time-correlated single photon counting setup as described previously (14), although a few alterations are made. The excitation wavelength was set at 300 nm in order to selectively excite the naturally occurring tryptophans in the protein. The detection wavelength was set at 348.8 nm. The reference compound to yield the dynamic instrumental response function of the setup was para-terphenyl (PTF), dissolved in a mixture of 30% carbon tetrachloride and 70% cyclohexane. The fluorescence of this reference is characterized by a 16 ps single exponential decay (15). Per experimental decay 1024 channels of the multichannel analyzer were used with a time spacing of 25 ps per channel.

Circular dichroism. CD spectra were recorded between 195 and 240 nm on a Jobin-Yvon Mark V dichrograph in cuvettes of 1 mm path length.

RESULTS AND DISCUSSION

Figure 1 shows the fluorescence anisotropy decay of the dissolved protein (curve A) and of the adsorbed protein (Γ = 2.0 mg m^{-2}: curve B). The finite value of the fluorescence anisotropy in the presence of Teflon latex within the time scale of the measurement evidences the adsorbed state of the protein. The relatively high initial anisotropy in the presence of Teflon indicates marginal interference of light scattering with the fluorescence anisotropy.

Figure 2 shows the fluorescence decay of the dissolved and adsorbed proteins. The curves have been normalized with respect to initial fluorescence. This has been done because a small amount of background fluorescence from Teflon was observed and because the protein fluorescence is scattered to a small extent by the Teflon-particles. This has been confirmed by steady-state fluorescence experiments (data not shown). It is demonstrated in Fig. 2 that the fluorescence of the adsorbed protein is not quenched by the adsorbing surface. Specifically, the average fluorescence lifetime of the adsorbed protein at Γ = 2.0 mg m^{-2} is virtually the same as that for the dissolved protein. Hence, the fluorescence quenching which is observed for Γ = 0.5 mg m^{-2} must be attributed to another origin, probably related to structural rearrangements in the adsorbed protein molecules at this relatively low surface coverage.

The results with CD spectroscopy are shown in Fig. 3. The spectra for the buffer and the Teflon latex in buffer are practically zero. It can be inferred from Fig. 3 that the α-helix content in the protein increases upon adsorption at low surface coverage (Γ = 0.5 mg m^{-2}) while it remains essentially unaltered at full surface coverage (Γ = 2.0 mg m^{-2}). This is a remarkable result since a decrease in the α-helix content is generally found for proteins adsorbing on a hydrophilic silica surface. It also contradicts the general assumption that denaturation upon protein adsorption is an *unfolding* process. In conclusion, a new type of hydrophobic particles is presented which allows the spectroscopic determination of structural properties of adsorbed proteins.

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