Protein Adsorption onto Biomaterials
Investigated by In Situ ATR-IR Spectroscopy

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Abstract: A process of protein adsorption onto a polymer surface from an aqueous solution was investigated by time-resolved in situ attenuated total reflection infrared (ATR-IR) spectroscopy. Following two adsorption modes were found in the present study: In the initial stage within 6 min, abrupt adsorption with protein denaturation was observed, while in the rate stage, gradual adsorption process was detected.

Keywords: Protein Adsorption, Polymer Surface, Infrared Spectroscopy

1. INTRODUCTION
A mechanism of protein adsorption onto a surface of polymeric materials has considered to be an important knowledge for design of biocompatible polymers. In the present study, a process of protein adsorption onto a polymer surface from an aqueous solution was investigated by time-resolved infrared spectroscopy. In order to detect adsorbed protein molecules selectively excluding natural protein in solution, attenuated total reflection infrared (ATR-IR) spectroscopy was applied.

2. EXPERIMENTAL
2.1. Samples
Polystyrene (PS) with a weight-averaged molecular weight of $2.4 \times 10^4$ was purchased from Wako Pure Chemical Industries and used as received. A PS film was prepared on a flat surface of a hemispherical ZnSe prism by dip-coating from a toluene solution. The film sample was annealed at ca. 120°C. A thickness of the film was determined from interference fringe of a transmission IR spectrum and adjusted to ca. 0.12 µm.

Lysozyme (LZM) form chicken egg white was purchased from Wako Pure Chemical Industries. Phosphate buffered saline powder (NaH₂PO₄ of 3.6 wt%, Na₂HPO₄ of 13.3 wt% and NaCl of 83.0 wt%) was obtained from Wako Pure Chemical Industries and dissolved in a deuterated water (99.9 %). A protein solution of 20 g·L⁻¹ LZM in the buffered D₂O with a pH of 7.4 was prepared.

2.2. In situ ATR-IR spectroscopy
All the ATR-IR spectra were measured by using a homemade flow cell designed for one-bounce ATR-IR detection. Detailed information of the cell was described elsewhere. [1] The ZnSe prism with the adhered polymer film was mounted on the cell. The buffered D₂O without protein was filled in the cell until stable. An IR beam was introduced into the prism at an incident angle of 45°. An IR absorption by near-field light of evanescent wave generated at the prism/polymer interface was monitored by a Fourier transformation IR spectrometer (Thermo Electron, Nexus 470) equipped with a liquid-nitrogen-cooled HgCdTe detector. It should be noted that a penetration depth of the evanescent wave (ca. 0.9 µm at 1700 cm⁻¹) [1] is enough larger than the thickness of the film (ca. 0.12 µm). Thus the polymer/solution interface can efficiently be exposed by the near-field light. All the ATR-IR spectra were defined in an absorbance unit as

$$A = -\log_{10} \frac{R}{R_0}$$

where $R$ and $R_0$ are intensities of the ATR-IR light with and without protein in the solution, respectively. Note that the $R_0$ spectrum reflects wet polymer film by buffered D₂O. With starting a time-resolved measurement, the buffered D₂O was exchanged to the protein solution. A series of time-resolved ATR-IR spectra during an adsorption process of protein molecules onto the polymer surface was measured. A total of 96 scans was co-added to obtain each spectrum at a wavenumber resolution of 4 cm⁻¹, and a total of 120 spectra, one every 60.0 s, were measured.

3. RESULTS AND DISCUSSION
Figure 1 shows time-resolved in situ ATR-IR spectra in the amide I (1700-1600 cm⁻¹) and the amide II (1600-1500 cm⁻¹) regions of an adsorption process of
Table 1. Fitting result shown in Figure 2 by a double exponential function

<table>
<thead>
<tr>
<th></th>
<th>$A_f$</th>
<th>$A_s$</th>
<th>$\tau_f$</th>
<th>$\tau_s$</th>
</tr>
</thead>
<tbody>
<tr>
<td>amide I</td>
<td>0.0718</td>
<td>0.0385</td>
<td>6.11</td>
<td>36.69</td>
</tr>
</tbody>
</table>

f: fast mode, s: slow mode.

Both amide I and amide II bands gradually increase with time, implying process of protein adsorption onto PS surface. Area intensities of the amide I band shown in Figure 1 are plotted in Figure 2 as a function of time, and fitted by a following double exponential function \[A(t) = A_f \left(1 - \exp \left(\frac{-t}{\tau_f}\right)\right) + A_s \left(1 - \exp \left(\frac{-t}{\tau_s}\right)\right)\]

where $A(t)$ and $t$ correspond to the band intensity and time, respectively. Fitting parameters of $A$ and $\tau$ are amplitude and relaxation time, respectively, and subscripts or $f$ and $s$, respectively, denote fast and slow modes, i.e., $\tau_f < \tau_s$. Fitting results shown in Figure 2 are summarized in Table 1. Here, it is clearly demonstrated two relaxation modes of $\tau_f \sim 6.11$ min and $\tau_s \sim 36.69$ min. These two modes will be discussed below.

In order to clarify time-dependent structural change of the adsorbed protein molecules onto the PS surface, each spectrum in the amide I region was fitted by following Gaussian components

\[A(\nu) = \sum A_i \exp \left(-\frac{(\nu - \nu_i)^2}{2\Gamma_i^2}\right)\]

where fitting parameters of $A_i$, $\nu_i$, and $\Gamma_i$ are peak height, peak position and peak width, respectively. A typical fitting result of the time-resolved spectra shown in Figure 1 at 120 min is depicted in Figure 3, and the band assignments are summarized in Table 2.

In the case of LZM solution measured by transmission IR spectroscopy, i.e., natural protein, ratios of the secondary structure are calculated as follows; $\alpha$-helix of 41\%, $\beta$-sheet of 25\%, $\beta$-turn of 19\% and unordered structure of 13\%. These results indicate good agreement with previous studies by X-ray diffraction, UV circular dichroism and other IR spectroscopy.

By using the procedure described above, denaturation
process of the adsorbed protein molecules was estimated. Figure 4 shows secondary structure change of the adsorbed protein as a function of time. It is of particular note that the secondary structure suddenly denatured within ca. 6 min. This time domain is close to $\tau_f$, suggesting that the initially adsorbed protein molecules within ca. 6 min denatured at the polymer surface.

REFERENCES