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PROTEIN-CATIONIC DETERGENT INTERACTION

FOURIER TRANSFORM INFRARED AND LASER RAMAN SPECTROSCOPIC STUDIES
ON THE INTERACTION BETWEEN PROTEINS AND DODECYPDYPYRIDINIUM
BROMIDE

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Fourier transform infrared and laser Raman spectroscopies were used to study
the effects of dodecylpyridinium bromide on the conformation of haemoglobin,
myoglobin, bovine serum albumin, ribonuclease, ovalbumin, lysozyme, trypsin
and β-lactoglobulin in aqueous solution. Addition of the cationic detergent caused
a decrease in α-helix conformation in highly helical proteins. At low detergent con-
centrations stabilization of β-sheet conformation was observed.

The binding of dodecylpyridinium bromide to several globular proteins has
been described in the accompanying paper (Wasylewski, 1979). This paper presents
data on the conformational changes caused by DPB1 in native globular proteins,
investigated by infrared and laser Raman spectroscopy.

Vibrational spectra commonly obtained by infrared absorption and Raman
scattering techniques visualize changes in protein conformation (Walton & Blackwell,
1973). Proteins in 2H2O solution have characteristic infrared absorption bands
around 1650 cm⁻¹ and 1450 cm⁻¹ which are usually designated amide I and amide II,
respectively (Ruegg et al., 1975). Their fine structure and the exact position of the
amide I band can be used to derive information about changes within the structure
of proteins in detergent solution.

Laser Raman spectroscopy has been used to observe conformational changes
resulting from the binding of DPB with trypsin and β-lactoglobulin investigated
in aqueous solution. As a rule, the characteristic band around 1250 cm⁻¹, which
is usually called amide III, was analysed to infer the nature of the changes in secondary
structure of the proteins.

1 Abbreviation used: DPB, dodecylpyridinium bromide.

[205]
MATERIALS AND METHODS

Reagents. Heavy water, $^2$H$_2$O, 99.75% purity was obtained from the Institute for Nuclear Research (Swierk, Poland). Other reagents were the same as in the accompanying paper (Wasylewski, 1979).

Fourier transform infrared spectroscopy. The proteins were dissolved in $^2$H$_2$O containing 0.1 M-NaCl or in this solution containing DPB. To obtain a uniform set of data and avoid complication caused by possible partial hydrogen-deuterium exchange in the peptide groups, prior to the spectroscopic experiments all samples were stored for 70 h (by this time the exchange in peptide groups is known to be complete; Timasheff et al., 1967).

The infrared spectra were obtained interferometrically on a Digilab FTS-14 Fourier transform infrared spectrophotometer at a resolution of 2 cm$^{-1}$. All recorded spectra represent the mean value obtained from 300 scans. The samples were placed in 0.03 - 0.1 mm thick CaF$_2$ liquid cell. The absorption spectra of $^2$H$_2$O or DPB in $^2$H$_2$O were then digitally subtracted from the solution spectrum of the proteins. The concentration of all protein solutions was 2% by weight. The spectra were recorded from 1300 to 1800 cm$^{-1}$ and expanded within the 1550 to 1750 cm$^{-1}$ range for analysis of the amide I band fine structure.

Laser Raman spectroscopy. Aqueous protein solutions (3 - 5% by weight) were prepared in 0.1 M-NaCl containing DPB. The samples of proteins in detergent solution were kept for 24 h at 5°C to obtain protein-detergent associates. Prior to recording the spectra the samples were ultracentrifuged at 100 000 g for 0.5 h to remove larger scattering particles, and immediately transferred to the Raman cell (1.5 x 50 mm).

The Raman spectra were obtained with a Spectra Physics Model argon ion laser on a Cary 82 spectrometer. The spectral width was 10 cm$^{-1}$. All the spectra reported were obtained with a 514.5 nm line argon laser with a power of 50 mW on the samples. The spectra were recorded from 400 to 1600 cm$^{-1}$ or from 1200 to 1600 cm$^{-1}$.

RESULTS AND DISCUSSION

Fourier transform infrared spectroscopy. The infrared spectra of proteins differing in secondary structure, in $^2$H$_2$O solution or in DPB in $^2$H$_2$O are shown in Figs. 1 and 2. The infrared frequencies of the amide I and amide II bands together with the amide I bandwidth, symmetry of the contours and their fine structure are listed in Table 1.

The infrared spectra of highly helical native bovine serum albumin, haemoglobin, myoglobin and lysozyme are presented in Fig. 1. The amide I bands of these proteins occur at 1650 to 1652.5 cm$^{-1}$ with asymmetric contours, denoted as the contribution of the half bandwidth from the low and high frequency sides of the band (Table 1). Timasheff et al. (1967) observed the same frequencies for these proteins.

Addition of DPB at a concentration of 10$^{-2}$ M (DPB-albumin molar mixing ratio of 34) shifted the amide I frequency from 1652.5 to 1650 cm$^{-1}$. An increase
Fig. 1. Infrared absorption spectra of the indicated proteins in the following solutions: A, $^2$H$_2$O containing 0.1 m-NaCl; B, the same solution containing $10^{-3}$ m-dodecylpyridinium bromide; and C, containing $5 \times 10^{-3}$ m-dodecylpyridinium bromide.
Fig. 2. Infrared absorption spectra of the indicated proteins in the following solutions: A, $^2$H$_2$O containing 0.1 M-NaCl; B, the same solution containing $10^{-3}$ M-dodecylpyridinium bromide; and C, containing $5 \times 10^{-2}$ M-dodecylpyridinium bromide.
**Table 1**

Infrared absorption bands of native proteins and proteins in dodecylpyridinium bromide solutions

For each protein the values in parentheses are the contribution of the half bandwidth from the low and high frequency sides of the bands, respectively. Conditions: all proteins were dissolved in $^2$H$_2$O containing 0.1 m-NaCl or in detergent solution in this solvent.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Conditions</th>
<th>Amide I</th>
<th>Amide II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine serum albumin</td>
<td>$^2$H$_2$O</td>
<td>1652.5</td>
<td>42.5 (26.3/16.2)</td>
</tr>
<tr>
<td>Bovine serum albumin</td>
<td>$10^{-2}$ M-DPB</td>
<td>1650</td>
<td>45 (27/18)</td>
</tr>
<tr>
<td>Bovine serum albumin</td>
<td>$5 \times 10^{-2}$ M-DPB</td>
<td>1647 1651 1654</td>
<td>46 (23/23)</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>$^2$H$_2$O</td>
<td>1651</td>
<td>43 (25/18)</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>$10^{-2}$ M-DPB</td>
<td>1649 1644</td>
<td>51 (25.5/25.5)</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>$^2$H$_2$O</td>
<td>1650</td>
<td>43 (25/18)</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>$10^{-2}$ M-DPB</td>
<td>1647 1643</td>
<td>47 (25/22)</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>$5 \times 10^{-3}$ M-DPB</td>
<td>1647 1643 1653</td>
<td>44 (21.5/22.5)</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>$^2$H$_2$O</td>
<td>1652</td>
<td>1654</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>$10^{-2}$ M-DPB</td>
<td>1651 1643, 1646, 1655</td>
<td>43 (18/25)</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>$5 \times 10^{-3}$ M-DPB</td>
<td>1650 1653</td>
<td>43 (22.5/20.5)</td>
</tr>
<tr>
<td>Ovalbumin</td>
<td>$^2$H$_2$O</td>
<td>1641</td>
<td>48 (20/28)</td>
</tr>
<tr>
<td>Ovalbumin</td>
<td>$10^{-2}$ M-DPB</td>
<td>1642</td>
<td>56 (27.5/28.5)</td>
</tr>
<tr>
<td>Ribonuclease</td>
<td>$^2$H$_2$O</td>
<td>1641 1639, 1645, 1654</td>
<td>46 (16/30)</td>
</tr>
<tr>
<td>Ribonuclease</td>
<td>$10^{-2}$ M-DPB</td>
<td>1639 1643-45, 1655, 1675</td>
<td>46 (17/29)</td>
</tr>
<tr>
<td>Trypsin</td>
<td>$^2$H$_2$O</td>
<td>1642.5</td>
<td>1637 1637, 1646</td>
</tr>
<tr>
<td>Trypsin</td>
<td>$10^{-2}$ M-DPB</td>
<td>1637 1632, 1643, 1652</td>
<td>57 (22/35)</td>
</tr>
<tr>
<td>Trypsin</td>
<td>$5 \times 10^{-3}$ M-DPB</td>
<td>1641.5 1633, 1649, 1655</td>
<td>54 (20/34)</td>
</tr>
<tr>
<td>$\beta$-Lactoglobulin</td>
<td>$^2$H$_2$O</td>
<td>1636 1645, 1675</td>
<td>51 (20/31)</td>
</tr>
<tr>
<td>$\beta$-Lactoglobulin</td>
<td>$10^{-2}$ M-DPB</td>
<td>1632.5 1645, 1675</td>
<td>50 (17/33)</td>
</tr>
<tr>
<td>$\beta$-Lactoglobulin</td>
<td>$5 \times 10^{-3}$ M-DPB</td>
<td>1632.5 1646, 1653, 1675</td>
<td>52 (17/35)</td>
</tr>
</tbody>
</table>

in the detergent concentration to $5 \times 10^{-4}$ M (molar mixing ratio of 170) shifted the amide I band frequency to a value of 1647 cm$^{-1}$ and resulted in an increase of the amide I bandwidth. The band was symmetrical.

Similar results were observed for myoglobin, haemoglobin and lysozyme, the magnitude of these changes was, however, smaller than for albumin (Table 1).
This can be explained by lower binding of DPB to these proteins than to albumin (Wasylewski, 1979).

Proteins in unordered form in \( ^2 \text{H}_2 \text{O} \) solution are known to have an amide I band at 1643 cm\(^{-1} \), and a band corresponding to the \( \alpha \)-helix conformation in the region between 1650 - 1655 cm\(^{-1} \) as opposed to about 1630 cm\(^{-1} \) for \( \beta \)-sheet conformation (Timasheff et al., 1967; Susi et al., 1967). The lower the stability of the \( \alpha \)-helix form in a protein solution, the larger is the half bandwidth of the amide I band (Chirgadze et al., 1976).

Addition of DPB to highly helical proteins caused a shift of the amide I band frequency to the low frequency side together with an increase in half bandwidth and changes in its symmetry (Table 1).

It can be concluded that addition of cationic detergent to highly helical protein solutions caused a decrease in \( \alpha \)-helix structure. This observation is in agreement with optical dispersion measurements (Nozaki et al., 1974) for the binding of bovine serum albumin with another cationic detergent, tetradecyltrimethylammonium chloride.

The infrared spectra of much less ordered ovalbumin and proteins showing a high content of \( \beta \)-sheet conformation, ribonuclease, trypsin and \( \beta \)-lactoglobulin, are presented in Fig. 2. Addition of cationic detergent to ovalbumin solution caused a shift in the amide I band frequency from 1641 to 1642 cm\(^{-1} \) together with an increase in the half bandwidth (this band was symmetrical) which points to disordered form of this protein. Addition of DPB at a concentration of \( 10^{-2} \text{ M} \) to ribonuclease, trypsin or \( \beta \)-lactoglobulin caused a shift in the amide I band to the low frequency side. The observed changes in the amide I frequency and its fine structure indicate an increase of \( \beta \)-sheet conformation in these proteins at low detergent concentration. An increase of DPB concentration to \( 5 \times 10^{-2} \text{ M} \) caused a shift in the amide I band frequency of trypsin to the high frequency side; however, shoulders at 1633 and 1655 cm\(^{-1} \), which are characteristic for \( \beta \)-sheet and \( \alpha \)-helix form, respectively, were also observed. An increase of DPB concentration in \( \beta \)-lactoglobulin solution did not shift the amide I band at 1632.5 cm\(^{-1} \) but a distinct band at 1639 cm\(^{-1} \) together with shoulders at 1646, 1653 and 1675 cm\(^{-1} \) was observed. These observations can suggest a small increase in \( \alpha \)-helix conformation of these proteins at higher detergent concentration.

An analysis of protein structure based on the amide I band is subject to the same limitations as those found in other spectroscopic techniques, such as ORD and DC, namely due to poor band resolution, overlapping of bands resulting in peak displacements (Timasheff et al., 1967). However, the use of a high-precision Fourier transform infrared technique can be helpful in the characterization of the structural changes of proteins in aqueous solution.

*Laser Raman spectroscopy.* Raman spectroscopy of proteins in aqueous solutions provides information on their conformation, especially by analysis of the amide III frequencies which lie at 1220 - 1300 cm\(^{-1} \). The characteristic amide III lines of the helical geometry are at 1265 - 1300 cm\(^{-1} \), \( \beta \)-sheet conformation gives a sharp line
at 1229 - 1235 cm\(^{-1}\) and conformations intermediate between \(\alpha\) and \(\beta\) structures have amide III frequencies of medium strong intensity in the range 1240 - 1260 cm\(^{-1}\) (Walton & Blackwell, 1973; Chen & Lord, 1976).

Unfortunately, there are difficulties with obtaining satisfactory spectra of most proteins, especially in detergent solution, so attention was focused on trypsin and \(\beta\)-lactoglobulin which, as mentioned above, show an increase in \(\beta\)-sheet conformation in DPB solution.

In the Raman spectrum of 5% trypsin in 0.1 M NaCl solution at room temperature (Fig. 3) strong amide III lines at 1252 cm\(^{-1}\) with shoulders at 1240 and 1280 cm\(^{-1}\) can be seen. Addition of DPB at a concentration of \(5 \times 10^{-2}\) M to trypsin solution caused a shift in the amide III band frequency from 1252 to 1247 cm\(^{-1}\). The observed small shift of the amide III lines may suggest a slight stabilization of the \(\beta\)-sheet conformation of polypeptide chain, and may result from overlapping of amide III vibrations which correspond to the \(\beta\)-sheet and unordered forms of polypeptide chain. The shoulder at 1270 cm\(^{-1}\) indicates that some helical form is still present.

![Fig. 3. Raman spectra of trypsin in aqueous solution containing 0.1 M NaCl: A, native protein; B, in \(5 \times 10^{-2}\) M dodecylpyridinium bromide.](image)

A frequency region sensitive to the backbone conformation is also at 930 cm\(^{-1}\). Frushour & Koenig (1975) have attributed a weak line in the Raman spectrum of poly-l-alanine at 931 cm\(^{-1}\) to the \(\alpha\)-helix conformation. As can be seen in Fig. 3, addition of DPB to trypsin solution did not induce lines at 931 cm\(^{-1}\) indicative
of the \(\alpha\)-helical form. The interaction between the protein and the cationic detergent was quite different from the sodium dodecyl sulphate-trypsin interaction where an increase of \(\alpha\)-helix conformation was observed by circular dichroic studies (Jirgensons, 1973). The analysis of the Raman spectrum for trypsin in native form and in detergent solution showed that, with the exception of lines at 1030 cm\(^{-1}\) which correspond to the pyridinium ring of the detergent, there were no appreciable changes in the spectra.

![Raman spectra](image)

**Fig. 4.** Raman spectra of \(\beta\)-lactoglobulin in aqueous solution containing 0.1 m-\(\text{NaCl}\): \(A\), native protein; \(B\), in \(2 \times 10^{-2}\) m-dodecylpyridinium bromide.

The Raman spectrum of 5% \(\beta\)-lactoglobulin in aqueous solution containing 0.1 m-\(\text{NaCl}\) is presented in Fig. 4. The strong amide III lines indicate a high content of \(\beta\)-sheet conformation, which is in a good agreement with the recently presented data (Frushour & Koenig, 1975). Addition of DPB at a concentration of \(2 \times 10^{-2}\) m caused a shift of the amide III line to a frequency of 1247 cm\(^{-1}\) and appearance of a distinct band at 1230 cm\(^{-1}\). This suggests that the addition of cationic detergent caused stabilization of \(\beta\)-sheet conformation. The observed small shift of the amide III
frequency to the high frequency side may have resulted from overlapping of amide III vibrations which correspond to the unordered and β-sheet conformations of polypeptide chain. The observed changes in the amide III band of β-lactoglobulin in DPB solution are in agreement with the data from infrared spectroscopic studies presented above.

It can be concluded that the addition of cationic detergents to protein solutions decreases α-helix conformation in helical proteins, and, at low detergent concentrations, stabilizes β-sheet conformation in proteins containing this form. The magnitude of these changes are smaller for cationic than for anionic detergents. This is in agreement with spin labelling and hydrodynamic studies (Wasylewski et al., 1979) which indicate much lower ability of cationic detergents, such as alkylpyridinium bromides, to induce conformational changes in the protein molecule.

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REFERENCES


ODDZIAŁYWANIA BIAŁKA - DETERGENTY KATIONOWE

BADANIE ODDZIAŁYWAN BRONKU DODECYLOPIRYDYNIOWEGO Z BIAŁKAMI PRZY POMOCY
FOURIEROWSKIEJ SPEKTROSKOPII W PODCZERWIEŃ ORAZ LASEROWEJ SPEKTROSKOPII RAMANA

Streszczenie

Stosując metodę fourierowskiej spektroskopii w podczerwieni oraz laserowej spektroskopii Ramana, badano wpływ bromku dodecylopirydyniowego na konformację hemoglobiny, mioglobininy, albuminy surowicy wołu, lizozymu, owalbuminy, rybonukleazy, trypsyny i β-laktoglobuliny. Badany detergent kationowy powoduje zniżenie zawartości struktury α-helik w białkach wykazujących wysoką zawartość tej struktury. Dodanie bromku dodecylopirydyniowego do roztworu białek wykazujących wysoką zawartość struktury β powoduje stabilizację tej struktury przy niskich stężeniach badanego detergenu.

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