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FAR INFRARED SPECTRA OF ACID PHOSPHATASE FROM RAT LIVER. SPECTRA OF THE NATIVE AND THE HEAT- AND ACID-DENATURED ISOENZYMES*

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The spectrum of acid phosphatase, as well as that of its two isoenzymes, shows in the region 100 - 500 cm⁻¹ a band at 362 cm⁻¹ which appears to be due to second-order structure (α-helix). The character of these spectra is unchanged by deuteration of the protein.

Heat- and acid-denaturation affected all the spectra studied pointing to the occurrence of conformational changes, but a close similarity between the spectra of the two isoenzymes was consistently observed.

The far infrared spectrum (100 - 500 cm⁻¹) of normal vibrations is largely dependent on the protein structure; it has proved a useful tool for determination of the second-order structure of proteins and for observation of the behaviour of amino acids in the side chains. Earlier studies on polypeptides led to assignment of particular bands to α-helix and pleated-sheet structure of polypeptide chains (Feairheller & Miller, 1971; Itoh & Shimanouchi, 1970; Itoh & Katabuchi, 1972, 1973; Shotts & Sievers, 1974). The far infrared spectra of some globular proteins were studied with the use of the Fourier technique (Buotempo et al., 1971; Chirgadze & Ovsepyan, 1973; Shotts & Sievers, 1974).

The far infrared spectra of proteins in aqueous solutions are unsuitable for studies on protein structure (Buotempo et al., 1971; Shotts & Sievers, 1974); better results were obtained with a mixture of protein with vaseline paste (Chirgadze & Ovsepyan, 1973). It seems that lyophilized protein samples can be effectively used for this purpose.

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In this communication, the far infrared spectra of the purified, freeze-dried samples of two acid phosphatase isoenzymes, which were previously studied by laser Raman spectroscopy (Twardowski, 1978, 1979), are presented and discussed.

MATERIAL AND METHODS

The acid phosphatase isoenzymes I and II from rat liver were extracted and purified by the modified method of Igarashi & Hollander (1968). The basic modification consisted in breaking of lysosomes by freezing the liver homogenate. After this procedure, the activity of the enzyme was twice as high as initially. The molecular weight of both isoenzymes was about 100,000. Other properties of the lyophilized final preparations of these glycoproteins were also in agreement with the results of Igarashi & Hollander (1968).

Acid denaturation was performed by dissolving the isoenzymes in 1 M-HCl at pH 1.2 and 25 ± 1°C; after 0.5, 1.5 and 5 h the samples were rapidly frozen and lyophilized.

Heat denaturation was performed by keeping the 10% protein solution in water at 90 - 100°C for 7 min.

The protein was deuterated twice by dialysis of 1 ml of 10% protein solution against 250 ml of \(^2\text{H}_2\text{O}\) at 5°C for 70 - 80 h. Alternatively, \(^2\text{HCl}\) was used for acid denaturation.

For the infrared measurements, thin films of lyophilized isoenzymes (either native or denatured) were prepared on silicon monocrystal windows.

The far infrared spectra were obtained on a Digilab Fourier Transform Spectrophotometer 14 at a resolution of 2 cm\(^{-1}\). Three hundred scans were done.

After each experiment, a smooth curve was used to approximate “scattering backgrounds”, and smoothing operations were done. From the presented spectra, those spectra have been subtracted. A similar procedure was used by Chirgadze & Ovsepyan (1973) for obtaining the difference spectra of myoglobin.

The laser Raman spectra were recorded on a Cary 82 Raman spectrophotometer with the argon ion laser (Spectra Physics). All the spectra were normalized to the band at 1460 cm\(^{-1}\). Instrumental conditions: excitation 488.0 nm, power 50 mW, slit width 3 cm\(^{-1}\), sensitivity 20,000 counts/s, scan speed 0.5 cm\(^{-1}\)/s, time constant 5 s.

The content of the \(\beta\)-structure of protein was evaluated according to Pezolet et al. (1976).

RESULTS AND DISCUSSION

The Raman spectra of native acid phosphatase in aqueous solution and in solid state showed practically no differences over the frequency region from 10 to 1800 cm\(^{-1}\) (Twardowski, 1978, 1979), and in both isoenzymes of the phosphatase the content of \(\beta\)-structure was less than 10%.

The far infrared spectra both of the native and acid-denatured acid phosphatase isoenzymes are presented in Fig. 1, and the line positions are listed in Table 1.
Fig. 1. Far infrared spectra of the native and acid-denatured acid phosphatase isoenzymes. —, Isoenzyme I; ——, isoenzyme II. The isoenzymes were denatured for the time indicated.

Table 1

The observed frequencies (in cm\(^{-1}\)) of the far infrared absorption bands of native and denatured isoenzymes of acid phosphatase from rat liver

Intensity of the bands is denoted as: s, strong; m, moderate; w, weak; vw, very weak; sh, shoulder.

<table>
<thead>
<tr>
<th>Isoenzymes</th>
<th>native</th>
<th>denatured at pH 1.2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5 h</td>
<td>1.5 h</td>
</tr>
<tr>
<td>460 m</td>
<td>454 w</td>
<td>454 w</td>
</tr>
<tr>
<td>415 s</td>
<td>418 s</td>
<td>422 s</td>
</tr>
<tr>
<td>390 s</td>
<td>400 sh</td>
<td>400 sh</td>
</tr>
<tr>
<td>362 s</td>
<td>320 m</td>
<td>320 m</td>
</tr>
<tr>
<td>286 s</td>
<td>286 s</td>
<td>286 s</td>
</tr>
<tr>
<td>238 w</td>
<td>238 w</td>
<td>238 w</td>
</tr>
<tr>
<td>150 vw</td>
<td>150 vw</td>
<td>150 vw</td>
</tr>
</tbody>
</table>

Assignments

- Side chain vibrations
- \(C_\alpha-C_\gamma\) bending
- \(C_\alpha-C_\beta-C_\gamma\) bending
- \(C_\beta-CN\) deformational, N-C\(_\alpha\) torsion, sugar vibrations
- \(C_\gamma-C_\beta-C_\gamma\) bending
- Methyl torsion
- NH bending, lattice vibrations
The far infrared spectra of the two native isoenzymes are identical and both show a distinct band at 362 cm\(^{-1}\) which seems to correspond to the band at 380 cm\(^{-1}\) in myoglobin, ascribed by Chirgadze & Ovsepyan (1973) to \(\alpha\)-helix structure.

Taking into account the studies on normal vibrations of polypeptides (Itoh & Shimanouchi, 1970; Moore & Krim, 1976a,b), other bands were tentatively assigned to vibrational modes. The broad band at 286 cm\(^{-1}\) seems to be due to the \(\text{C}_\alpha\)-CN deformational mode and the \(\text{N}-\text{C}_\alpha\) torsion mode of polypeptide bonds; its position is practically insensitive to changes in conformation of the isoenzymes. This band is probably associated also with the sugar C-OH vibration band.

The bands in the 400 - 500 cm\(^{-1}\) region arise probably from the side-chain vibrations. In myoglobin, Chirgadze & Ovsepyan (1973) observed disappearance of these bands upon denaturation of the protein, and they suggested that denaturation leads to a complete randomness of the conformational state of the side chains. The acid phosphatase proteins showed two strong bands at 390 and 415 cm\(^{-1}\). The two acid phosphatase isoenzymes are known to differ in the content of heteropolysaccharides (Ostrowski et al., 1976), this, however, has no visible effect on the far infrared spectra.

Acid denaturation for 0.5 and 1.5 h led to distinct changes in the far infrared spectra, but the spectra of the two denatured isoenzymes remained practically identical, except for the samples subjected to 5-h acid treatment which showed different intensity of the bands at 400 and 425 cm\(^{-1}\). These differences could point to beginning of hydrolysis.

The band at 362 cm\(^{-1}\), visible in the spectra of lyophilized acid phosphatase isoenzymes, disappeared on acid denaturation. Its disappearance on unfolding of the protein molecule is consistent with the assignment of this band to \(\alpha\)-helix structure.

The strong bands of native isoenzymes at about 390 and 415 cm\(^{-1}\) were shifted after denaturation to 400 - 405 and 425 cm\(^{-1}\), respectively. This effect was probably due to changes in the interactions of the side chain during unfolding of the protein molecule. New bands appeared at 150, 238 and 320 cm\(^{-1}\).

The bands at 235 - 268 cm\(^{-1}\) and 440 - 446 cm\(^{-1}\) are believed to be characteristic for the \(\beta\)-structure of some polypeptides (Itoh & Shimanouchi, 1970) but this seems not to be the case with acid phosphatase as after acid denaturation the enzyme cannot be expected to possess any \(\beta\)-structure. The assignment should rather be made on the basis of studies on normal vibrations of polypeptides (Itoh & Shimanouchi, 1970; Moore & Krim, 1976a,b). In some sequential polypeptides, vibrations at frequencies similar to those observed for acid phosphatase, were ascribed to the \(\text{C}_\alpha\)-\(\text{C}_\beta\)-\(\text{C}_\gamma\) bending modes, and they were calculated for some amino acids (Itoh & Katabuchi, 1972; Moore & Krim, 1976a,b). Thus, the new bands appearing at 238, 320 and 468 cm\(^{-1}\) after acid denaturation of acid phosphatase, could be assigned to methyl torsion and \(\text{C}_\alpha\)-\(\text{C}_\beta\)-\(\text{C}_\gamma\) bending modes. Weakening of the amino acid interactions could have been caused by unfolding of the main protein chain or breaking of the peptide bonds on hydrolysis. The above interpretations are consistent with the results of laser Raman spectroscopy of denatured acid phosphatase (unpublished). A very broad, weak band at 150 cm\(^{-1}\) of the native acid phosphatase
isoenzymes was increased after denaturation. This band could be assigned to the NH bending mode and lattice vibrations (I'oh & Shimanouchi, 1970; Chi gadze & Ovsepyan, 1973; Shotts & Sievers, 1974).

The same results were obtained on deuteration of the protein studied, and its denaturation with 1 M 2HCl.

![Figure 2](image)

Fig. 2. Far infrared spectra of the acid phosphatase isoenzymes heat-denatured at 90 - 100°C for 7 min. ---, Isoenzyme I; --, isoenzyme II.

The spectra of the heat-denatured acid phosphatase isoenzymes are presented in Fig. 2. The observed small decrease in the intensity of the band at 362 cm⁻¹ could be due to lowering of the amount of the protein second-order structure.

![Figure 3](image)

Fig. 3. Laser Raman spectra of the acid phosphatase isoenzymes in the amide III and backbone vibration regions. a, Native isoenzymes in H₂O solution; b, after acid treatment (1M-HCl, pH 1.2) for 0.5 h; c, after heat treatment (90 - 100°C for 7 min). ---, Isoenzymes I; --, isoenzymes II. For details see Methods.

The far infrared spectra of the native and denatured acid phosphatase isoenzymes were confirmed by the laser Raman technique. The Raman spectra of both isoenzymes in the amide III and backbone vibration regions are presented in Fig. 3. In the amide III region, the bands of native isoenzyme I appear at 1280, 1255 and
1230 cm\(^{-1}\), and of isoenzyme II, at 1270 and 1220 cm\(^{-1}\); in the backbone vibration region, the bands for both isoenzymes appear at about 928 - 925 cm\(^{-1}\). These results show that in both isoenzymes the \(\alpha\)-helix second-order structure is predominant (Twardowski, 1978). The content of \(\beta\)-structure was found to be less than 10%. The observed slight differences between the isoenzymes are probably due to the differences in the heterosaccharide moieties of these glycoproteins (Twardowski, 1978, 1979).

On acid denaturation, after 0.5 h of the HCl treatment, the bands ascribed to \(\alpha\)-helix disappeared (Fig. 3b). The small bands visible in the amide III region seem to be due to some other unidentified effect(s) which are observed in the Raman spectra after acid treatment of the protein. In the spectra of the heat-denatured acid phosphatase (Fig. 3c) the \(\alpha\)-helix bands of both isoenzymes are positioned at 1290 cm\(^{-1}\) in the amide III region, and at about 900 cm\(^{-1}\) in the backbone vibration region. The additional bands at about 960 cm\(^{-1}\) seem to be due to random coil structure.

Thus it appears that the far infrared spectra clearly reflect changes in the content of \(\alpha\)-helix structure occurring on denaturation of acid phosphatase but, at variance with the Raman spectra, do not visualize the differences existing between its two isoenzymes.

The far infrared and Raman spectra were recorded at the Central Laboratory of Physical-Chemical Analysis and Structure Research of the Jagellonian University.

REFERENCES


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WIDMA W DALEKIEJ PODCZERWIENI KWAŚNEJ FOSFATAZY Z WĄTROBY SZCZURA. WIDMA IZOEZYMÓW NATYWNYCH ORAZ PODDANYCH DENATURACJI TERMICZNEJ I KWASOWEJ

Streszczenie

Przedstawione w pracy widma obu izoenzymów kwaśnej fosfatazy wykazują w zakresie 100 - 500 cm\(^{-1}\) pasmo absorpcyjne przy 362 cm\(^{-1}\), które jest związane ze strukturą drugorzędową (helikalną). Charakter tych widm nie ulega zmianom po deuteracji białka.

Termiczna i kwasowa denaturacja izoenzymów powodowała wystąpienie w widmach zmian będących wynikiem zmian konformacyjnych, ale podobieństwo między widmami obu izoenzymów było zachowane.

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