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THE STRUCTURE OF PROTEINS IN AQUEOUS SOLUTIONS: AN ASSESSMENT OF TRIOSE PHOSPHATE ISOMERASE STRUCTURE BY FOURIER-TRANSFORM INFRARED SPECTROSCOPY

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Recent resolution enhancement and curve-fitting techniques have been applied to infrared spectra from triose phosphate isomerase in aqueous solution, in order to obtain quantitative information on its secondary structure. From our results, 57% α-helix, 25% ß-parallel and 10% ß-turns are predicted, in close agreement with the X-ray crystallographic data. On the other hand, measurements of band intensities, both in original and deconvolved spectra are shown to be unreliable for the quantification of secondary structures. The presence of ß-edge structure interacting with the α-helical barrel is described and discussed.

The recent development of FT-IR instrumentation has overcome the problems of using infrared spectroscopy in the study of protein structure; however, the most important conformation-sensitive spectral feature, the so-called Amide I band, is still difficult to analyze, since it contains several overlapping components arising from the different structures present in the protein (1). Recently, resolution enhancement techniques such as Fourier self-deconvolution (2) and Fourier derivative (3) have allowed the precise determination of peak positions. These, together with band-fitting te chniques, allow us the quantitative estimation of each kind of secondary structure for a protein in solution (4). A recent review on protein structure obtained from resolution enhanced infrared spectra has shown that much has

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Abbreviations; FT-IR: Fourier-transform infrared spectroscopy

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been advanced in the use of the FT-IR in this field, although resolution enhancement techniques have to be used carefully (5). In the present work we describe triose phosphate isomerase in aqueous solution as seen by FT-IR techniques . Triose phosphate isomerase, a crucial enzyme in glycolysis, is known to be an α/ß barrel containing an inner cylinder of eight strands of parallel ß-pleated sheet; adjacent strands are connected by α-helix segments that run antiparallel to the strands of ß-sheets and form an outer cylinder co-axial with the inner one. Turns and less ordered structures are also present and a short 3_{10} helix links the eighth ß-strand with the eighth α-helix (6). Most proteins studied up to now by FT-IR do not contain significant amounts of ß-parallel conformation; thus our data allow us to confirm assignments for this sort of structure. We also discuss the relationship between the α and ß structures in proteins.

MATERIALS AND METHODS

Triose phosphate isomerase (D-Glyceraldehyde-3-phosphate ketol isomerase; EC 5.3.1.1) Type IIIS from rabbit muscle was obtained from Sigma (Poole, Dorset, England) as a 3.2 M $(NH_4)_2SO_4$ suspension. The suspension was dialyzed against 10 mM $(NH_4)HCO_3$ pH 7.0 and freeze dried prior to its use. The lyophilized protein was dissolved in a D_2O medium (10 mM Hepes pD 7.0) at a 3% (w:w) concentration and placed between CaF_2 windows with 50 µm spacers in a Harrick cell (Harrick Scientific, Ossining, NY). 512 scans were averaged in a Nicolet 10 DX spectrometer, apodized with a Happ-Genzel function and Fourier transformed to give a nominal resolution of better than 2 cm^{-1}. The spectra were transferred to an IBM personal computer where the resolution enhancement calculations were performed (7). Curve-fitting was achieved by firstly assigning peak positions using Fourier derivative with a power of three and a breakpoint of 0.3. The width at half height was also obtained from the third power derivative spectrum relative to zero (3). The bandwidths were then multiplied by a factor between 2.5 and 3 to account for the narrowing induced by derivation and these values were used as an input to decompose the Amide I band by a least squares procedure (8).

RESULTS

The original (solid line), and Fourier self-deconvolved (broken line) spectra of triose phosphate isomerase Amide I band contour

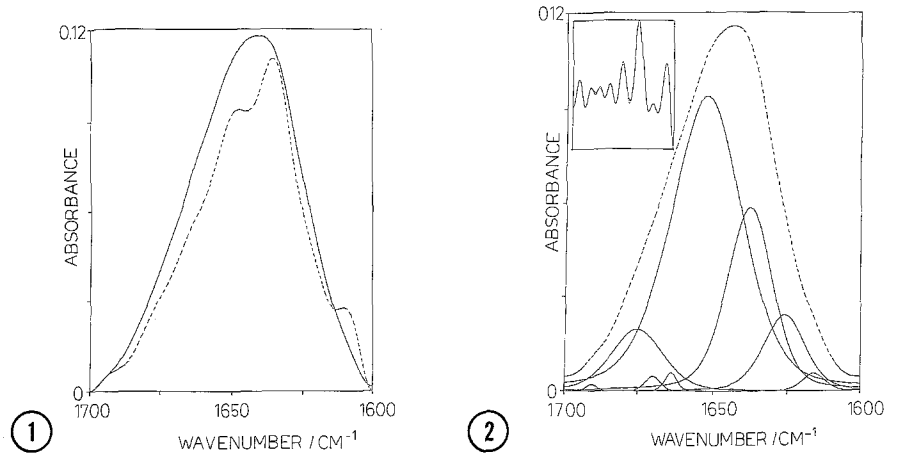

Figure 1. Infrared spectrum of the Amide I band of triose phosphate isomerase: original (solid line) and after Fourier self-deconvolution (broken line) using a bandwidth of 16 cm^{-1} and a resolution enhancement factor of 1.8.

Figure 2. Amide I band contour (broken line) of triose phosphate isomerase and its constituent bands (solid lines). Inset: Fourier derivative (with a power of 3 and a breakpoint of 0.3) of the Amide I band contour. Solid line: original band; broken line: reconstructed from component bands.

are shown in Fig. 1. The peak maximum is at 1643 cm^{-1} in the original spectrum, whereas in the deconvolved one, two peaks are present at 1638 and 1653 cm^{-1}. Also, bands at 1615 cm^{-1} and 1690 cm^{-1} are resolved by Fourier self-deconvolution. Other bands can be resolved using Fourier derivative (9). The 1653/1638 cm^{-1} intensity ratio that, in principle, could be an indication of the relative α/β proportions, is 0.96 in the original spectrum and 0.87 in the deconvolved one. According to X-ray crystallography, the α/β ratio should be about 2.5, thus showing the inherent inaccuracy of direct measurements of band intensities, even more after deconvolution, for structural quantitation.

The result of the band-fitting is shown in Fig. 2, where the component bands (solid line) are drawn together with the original contour (broken line). From the component bands it is evident that, in spite of the appearance of the original and deconvolved spectra, the component with a larger area is the

TABLE I

Frequencies (ν), Bandwidths at Half-Height ($\Delta\nu_{1/2}$), Fractional Band Areas, and Structure Assignment of Triose Phosphate Isomerase

ν (cm^{-1})	$\Delta\nu_{1/2}$ (cm^{-1})	A(%)	Assignment
1616	9	--	Tyrosine
1626	17	9	ß-edge
1638	18	23	ß-parallel + unordered
1653	28	57	α-helix
1664	5	1	3_{10} helix
1670	6	1	ß-turns
1675	20	9	ß-turns
1690	4	1	ß-edge

The crystal structure of triose phosphate isomerase from X-ray diffraction is 55% α-helix, 22% ß-parallel pleated sheet, 2% 3_{10} helix, and 7% unordered structure; the remaining aminoacid residues are involved in ß-turns and loops (6).

1653 cm^{-1} band. Peak maxima positions, bandwidths at half height, percentages of band area and the comparison with the crystal structure are shown in Table I. In order to check the band decomposition procedure, the Amide I contour has been reconstituted by addition of the component bands. The original and reconstituted Amide I bands appear undistinguishable (not shown). In addition, the derivative of both original and reconstructed bands has been calculated and is shown in Fig. 2 (inset). The coincidence of both derivative curves is almost perfect, indicating that, effectively, the Amide I contour has been reproduced.

DISCUSSION

Triose phosphate isomerase is an interesting protein to be used in conformational studies by FT-IR. There are two major structures, α-helix and parallel ß-pleated sheet, that account for 75% of the 248 aminoacid residues of the protein. Whereas α-helical proteins have been widely studied by FT-IR, very few proteins with significant amounts of ß-parallel structure have been characterized (10). Moreover, in triose phosphate isomerase

only 18 residues are in an unordered structure (6), which is not enough for band shifts to be observed when D_2O is used as a solvent (11).

From theorical studies (1) ß-sheet structures are considered to exhibit two vibrational modes, a strong one around 1632 cm^{-1} and a weak one at higher frequencies that, for parallel ß-sheet, is near 1645 cm^{-1}. α-Helix has only one band around 1650 cm^{-1} (1). Figure 1 shows that the two major peaks are at 1638 cm^{-1} and at 1653 cm^{-1}, the former band being stronger than the latter one. From the crystal structure, 54% of α-helical structure and 21% of ß-sheet are predicted, what is very close to our values as shown in Table I. Band intensity has been extensively used in a quantitative way for studying protein conformation (see ref. 5 for a discussion) but, as shown, this procedure can be inaccurate. The difference between intensity and area is not peculiar to triose phosphate isomerase but occurs as well in other proteins with ß-parallel structure (J. L. R. Arrondo, manuscript in preparation). Moreover, since the ß-sheet band is narrower than the α-helix, Fourier self-deconvolution enhances it and thus, in the deconvolved spectrum the intensity ratio of α-helix/ß-sheet decreases. Therefore, band intensity is a very risky parameter for quantifying conformational changes.

The band at 1638 cm^{-1} contains the above mentioned two components of parallel ß-sheet structure, but they cannot be resolved in D_2O medium, probably because of the superposition of an unordered structure component at 1643 cm^{-1} (1). In fact, we have shown previously that in H_2O medium, where the band corresponding to unordered structure is at 1657 cm^{-1}, two ß-structure bands occur at 1633 cm^{-1} and 1641 cm^{-1} as expected (9).

The 1660-1690 cm^{-1} region is usually attributed to ß-turns, but the high frequency component of the antiparallel ß-sheet is also present; triose phosphate isomerase does not have any ß-antiparallel structure (6), therefore such component is not observed in that spectral region. The 1670 cm^{-1} and the 1675 cm^{-1} bands are slightly shifted when the spectra in D_2O and in H_2O are compared, whereas the 1664 cm^{-1} and the 1690 cm^{-1} bands are in the same position in both media (9). The 1664 cm^{-1} band is close to a frequency attributed to ß-turns, or to the 3_{10} helix (10). The fact that the peak position is constant in both media (as happens with the normal α-helix) makes this band attributable to the 3_{10} helix connecting the eighth ß-strand with the eighth α-helix (6).

The bands at 1628 and 1690 cm^{-1}, that do not exchange either upon deuteration, have also described for other proteins with a high ß-sheet structure, such as azurin (12) and concanavalin A (13); in the latter protein, they have been attributed to ß-edge structure arising from the interaction of the edge of the ß-sheet strands with aminoacid side chains (13). From the three dimensional structure (14) it can be inferred that the ß-barrel is not continous but, instead, can be idealized as composed of two planes, each formed of four ß-strands; therefore, four ß-edges can interact with side chains from the α-helical structures surrounding them.

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