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FOURIER TRANSFORM INFRARED STUDIES IN SOLID EGG WHITE LYSOZYME

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ABSTRACT

Fourier Transform Infrared (FTIR) Spectroscopy is the most recent addition to the arsenal of bioanalytical techniques capable of providing information about the secondary structure of proteins in a variety of environments. FTIR spectra have been obtained in solid egg white lysozyme. The spectra display the usual amide I, II and III bands. Secondary structural information obtained from the spectra after applying resolution enhancement techniques to the amide I band has been found consistent with the x-ray crystallographic data of the protein and also to the spectroscopic data of the protein in aqueous solution.

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1 Introduction

FTIR spectroscopy has recently been shown to be a powerful technique to probe secondary structure of a protein molecule [1,2,3]. It provides high quality spectra with relative ease and with very small amount of proteins in a variety of environments, such as aqueous, lipid bilayer, crystalline and organic solvents. There are no problems associated with background fluorescence, background scattering, size of the molecule, the presence of a chromophore or necessary crystal or solution environment which are encountered in most of the other structural techniques usually employed to study protein structure, like x-ray crystallography, nuclear magnetic resonance spectroscopy, circular dichroism, fluorescence and Raman spectroscopy. Apart from extensive computing and data manipulation capabilities, FTIR also has a number of advantages over the usual dispersive IR spectroscopy which include, improved signal to noise ratio, multiplexing, a significant reduction in scan time, high energy throughput and superior wavelength accuracy. The ability of FTIR to measure IR spectra of protein crystals as well as solutions, the intact tissue or even a single cell [4] makes FTIR methods particularly well-suited for comparing the structure of a protein in different environments. A continuing basic question in the study of protein structure is the relationship between solution and crystal structures. FTIR data in aqueous solutions of many proteins is now available where as the solid state IR data is restricted only to the earlier measurements [5] using dispersive techniques, which do not give detailed secondary structural information of the protein molecule due to poor resolution and lack of powerful analytical techniques. This study was undertaken to compare the protein structural information obtained by solid state FTIR to that obtained by other spectroscopic or crystallographic techniques. Lysozyme was chosen for this study because its sequence and three dimensional structure is well known and a host of spectroscopic data in solution is also available for comparison.

Being a newer technique for structural biologists, each step in data acquisition and analysis has been shown comprehensively. Details of the instrument and underlying principle of the technique can be found elsewhere [6].

2 Experimental

The protein sample was obtained from Sigma Chemical Company in polycrystalline form and was used without further purification. KBr pallets of the sample were prepared for IR measurement.

All spectra were obtained using Shimadzu 4300 FTIR spectrometer. In all the cases the instrumental housing was purged with dry air. 100 interferograms were recorded at 4 cm^{-1} resolution and were averaged to obtain a single spectrum of improved signal to noise ratio. An interferogram obtained in this manner with empty sample compartment (see figure 1) was Fourier transformed to give the single channel power spectrum (figure 2), which was used as a reference spectrum. Power spectrum in the protein sample (figure 3) was divided by this reference spectrum to obtain usual transmittance spectrum. The transmittance data was transformed into absorbance data using a routine on the standard software of the spectrometer's data processing system. The spectra were compensated for water vapor and CO_2 bands. Apodization was achieved by using triangular function to suppress the side-lobes in the convolved spectrum. Second derivative routine was used for resolution enhancement, which provided information about the underlying bands in the recorded spectrum. A range of nine points was used to calculate the rate of change of slope along the spectrum. Exact positions of the peaks were determined by using a "peak find" routine available on the standard software used for data analysis. The relative amounts of different protein secondary structures were determined from infrared second derivative amide I spectra by manually computing the areas under the bands assigned to a particular substructure as a fraction of total area under all the assigned bands.

3 Results and Discussion

The finally averaged transmittance FTIR spectrum of the protein is shown in figure 4 from 4000 - 500 cm^{-1} wave number, where as figure 5 shows the expanded absorption spectrum of the protein from 1700 - 1300 cm^{-1} Infrared spectra of proteins and polypeptides exhibit a number of so called amide bands which represent different vibrations of the peptide

moiety that constitute the backbone structure of the protein molecule. An (hypothetical) isolated planar CONH group would give rise to nine amide bands, usually called amide A, amide B and amide I-VII, in order of decreasing frequency [7]. These modes could be described in terms of five in plane :C=O stretching, C-N stretching, N-H stretching, OCN bending, CNH bending and three out of plane: C-N torsion, C=O and N-H out of plane bending displacement coordinates. All the amide bands are visible in the measured spectrum (figure 4) and the three usual amide bands, amide I, II and III are clearly well resolvable in figure 5. Of all the amide modes of the peptide group, the single most widely used one in studies of protein secondary structure is the amide I band from 1700 - 1600 cm^{-1} which originates from C=O stretching vibration of the amide group (coupled to the in-phase bending of the N-H bond and the stretching of the C-N bond of the peptide linkages. The major factors responsible for conformational sensitivity of amide bands include hydrogen bonding and the coupling between transition dipoles which leads to splitting of the amide I mode. The magnitude of this splitting depends on the orientation and distance of interacting dipoles and thus provides information about geometrical arrangements of peptide groups in a polypeptide chain. Proteins usually fold into complex three dimensional structures which consist of a variety of domains containing polypeptide segments folded into different types of secondary structures. Since each of these conformational entities contribute to the infrared spectrum, the observed amide I band contours are complex composites of many overlapping component bands that represent different structural elements such as α-helices, β-sheets, turns and unordered structures. Due to the intrinsic linewidth of these overlapped bands, the component bands can not be instrumentally resolved and different computational techniques are required for resolution enhancement which include band fitting, factor analysis, second-derivative analysis and Fourier self deconvolution [1,2,3]. Second-derivative spectrum of the the amide I band was therefore obtained [see figure 6]. The resultant protein spectra upon second-derivative analysis yielded bands with frequencies characteristic of specific secondary structures as predicted by theory [7] and as observed in many proteins

in aqueous solution [1,2,3]. Band assignments made on the basis of second-derivative infrared spectra are summarized in Table 1.

Table 1

Amide I Band Frequencies and Assignments for Solid Lysozyme

Mean Frequencies (cm^{-1})	Assignments
1677	Turns
1668	Turns
1658	α-helix
1643	Unordered
1635	β-sheet
1627	β-sheet

Percentages of various amounts of different types of secondary structures present in lysozyme as obtained by the assigned band area analysis of second-derivative FTIR spectrum are: α-helix; 35%, β-sheet; 17%, Turn; 26% and random structure 22%. A comparison of these results with similar information obtained by other spectroscopic and crystallographic studies is given in the following table.

Table 2

Comparison of the Results with Lysozyme Secondary Structure as Determined by Other Spectroscopic and Crystallographic Techniques

Method	Secondary Structure (%age)				Reference
—	α-helix	β-sheet	Turn	Random	—
CD spectroscopy	29-45	11-39	8-26	8-60	[10-16]
Solution FTIR	40	19	27	14	[8]
X-ray (1)	45	19	23	13	[9]
X-ray (2)	39	11	32	18	[17]
Solid FTIR	35	17	26	22	This study

Though there is a large variation in the obtained information about the relative amounts of secondary structure present in the protein molecule from different studies, Amide I second-derivative band areas analysis for solid lysozyme has been found to be consistent with X-ray crystallographic data and other spectroscopic studies in aqueous

solution. It may be noted that values for "percentage secondary structures" are always somewhat subjective even when based on accurate bond lengths and angles. Ambiguity arises from the uncertainty in the choice of the exact point along the peptide chain where one segment of secondary structure begins and another ends. This choice depends not only on the manner in which an ideal helix or sheet has been defined by various investigators but also on just how regular any of these regions of protein substructure really are. With these uncertainties in mind, the agreement between the reported solid state FTIR work and other techniques is encouraging. Solid state FTIR second-derivative analysis has therefore been shown to be a convenient technique for secondary structural estimation in proteins. There are no problems of solvent bands subtraction in solid state analysis, which are often encountered in FTIR studies of proteins in solution.

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4 References

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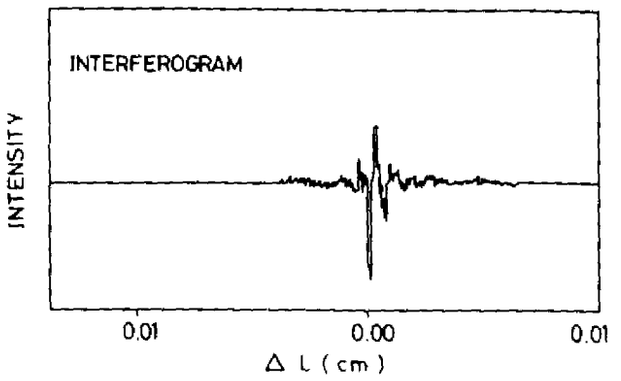

FIG.1. INTERFEROGRAM WITH EMPTY SAMPLE COMPARTMENT

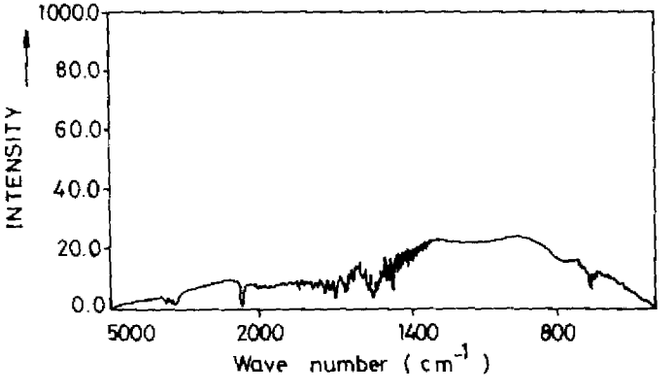

FIG.2. FOURIER TRANSFORM OF THE INTERFEROGRAM SHOWN IN FIG.1; THE SINGLE CHANNEL REFERENCE POWER SPECTRUM MEASURED THROUGH THE EMPTY COMPARTMENT.

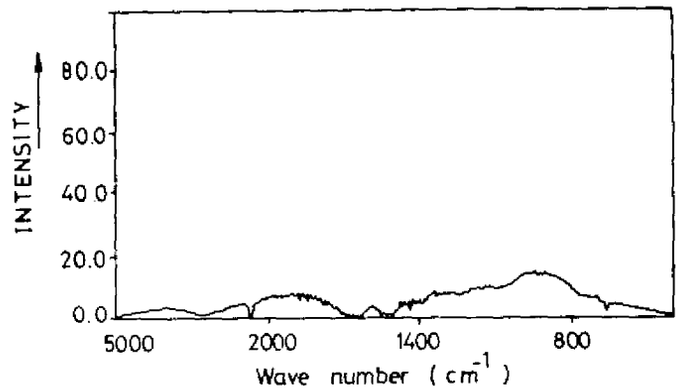

FIG.3. SINGLE CHANNEL POWER SPECTRUM OF LYSOZYME IN KBr. PALLET.

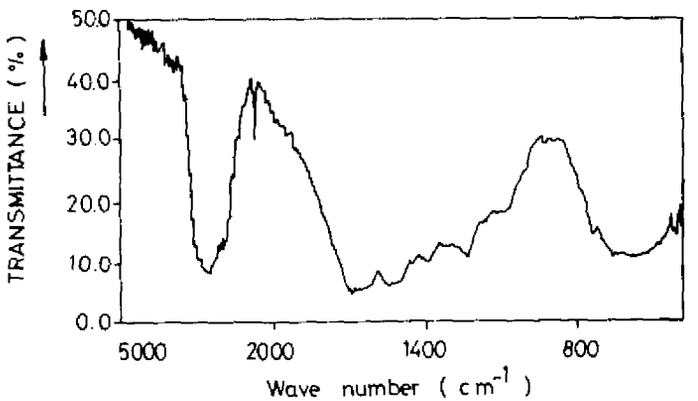

FIG.4. TRANSMITANCE SPECTRUM OF LYZOZYME SAMPLE OBTAINED AFTER DIVIDING SPECTRUM IN FIG.3. BY SPECTRUM IN FIG.2.

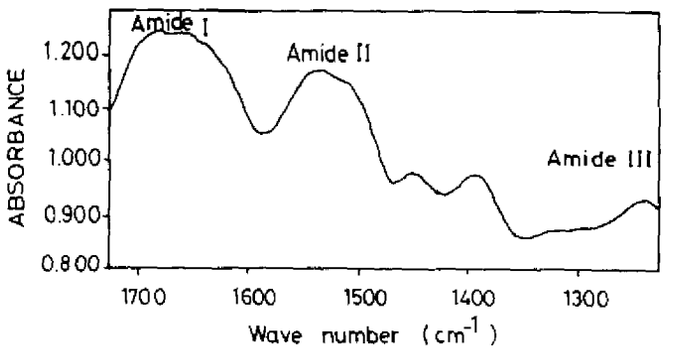

FIG.5. ABSORPTION SPECTRUM OF LYSOZYME SAMPLE FROM 1700 cm^{-1} TO 1300 cm^{-1} SHOWING AMIDE I, AMIDE II AND AMIDE III SPECTRAL REGIONS.

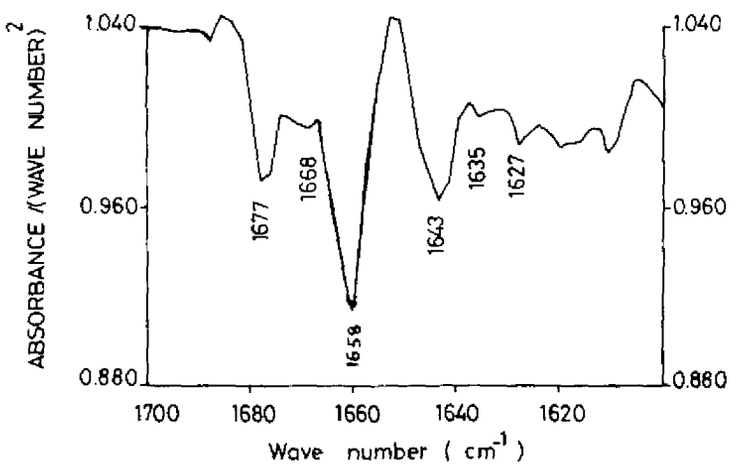

FIG.6. SECOND - DERIVATIVE FTIR SPECTRUM OF LYSOZYME IN THE AMIDE I REGION FROM 1700 cm^{-1} TO 1600 cm^{-1}.