

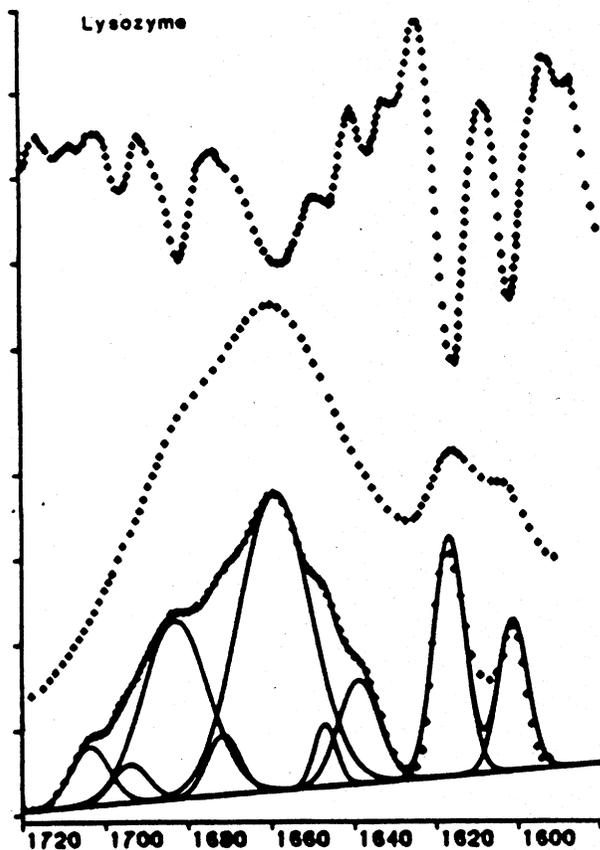
AMIDE I REGION OF RESOLUTION-ENHANCED RAMAN SPECTRA OF PROTEINS

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Fourier deconvolution and second derivative techniques were applied to the 1550-1750 cm^{-1} region of the Raman spectra of the proteins lysozyme, immunoglobulin, insulin, β -lactoglobulin, and α -lactalbumin in the crystalline state. The amide I band was thereby resolved into three to six components. Some of these can be clearly assigned to conformations such as α -helices and β -strands.

The following generalizations appear to hold: helical sections exhibit a band close to 1657 cm^{-1} ; in most proteins β -strands exhibit a band near 1670 cm^{-1} although in insulin the band appears at higher frequency (ca. 1682 cm^{-1}). A weak band close to 1638 cm^{-1} is also frequently observed. Turns show bands above 1680 cm^{-1} ; a weak band sometimes observed near 1645 cm^{-1} is possibly associated with hydrated segments. Lysozyme, which contains a large proportion of unordered regions (ca. 30-40%), shows a strong band at 1683 cm^{-1} which may be associated with these segments. This is quite different from infrared spectra obtained in deuterium oxide solution, where the band for unordered segments is observed close to 1645 cm^{-1} [1]. These assignments must be regarded as tentative until more data are available.



The Figure shows the second derivative spectrum, the original spectrum, and the deconvolved spectrum of lysozyme. (Relative intensity is plotted versus wavenumber in cm^{-1} .) The original spectrum was obtained at ca. 4 cm^{-1} spectral resolution and was treated with a thirteen point Savitsky-Golay smoothing function before resolution enhancement. The deconvolution procedure used is essentially the same as the one described by Kauppinen [2] and by Mantsch et

al. [3]. The line shape function was assumed to be Lorentzian with a full width at half height (FWHH) of 18 cm^{-1} . The resolution enhancement factor K was set to 2.8. The figure demonstrates that resolution-enhanced Raman spectra of proteins in the amide I region can be obtained which are analogous to previously reported infrared results [1,4,5]. Assignments in the two cases differ, however, because (a) the Raman data are for solids while the IR are for D_2O solutions and because (b) selection rules and relative intensities are not the same for the two techniques. (Furthermore, the signal-to-noise ratio of conventional Raman spectra is markedly inferior to that of FTIR spectra.)

It is interesting to speculate about possible quantitative applications of this approach. The important questions concern (a) experimental intensity measurements and (b) the Raman scattering cross-section for segments of the peptide backbone with different conformations. Only a thorough study will provide clear answers. Nevertheless, if the area of the helix band component of lysozyme is measured as indicated in the Figure (the deconvolved spectrum was fitted with Gaussian components), we find that this component accounts for about 46% of the total area of the amide I band. Assuming that this number indicates the fraction of α -helix in the secondary structure of lysozyme, one can compare this number with previously reported values for the helical content of this protein: FTIR, 41% [1]; Raman (statistical correlations), 44-51% [6-7]; and X-ray, 45% [8]. Use of the Raman technique reported here to estimate the β -strand content of proteins is more difficult at present because the bands due to unordered chains, β -strands, and turns cannot yet be assigned with certainty.

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