FTIR ANALYSIS OF PROTEIN SECONDARY STRUCTURE IN SOLID AND SOLUTION STATES

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Currently there are a number of experimental methods for determination of secondary structure of polypeptides and proteins [1, 2]. Of all analytical tools, Fourier transform infrared spectroscopy (FTIR) is recognized as one of the most useful methods allowing characterization of proteins' secondary structure in aqueous solutions [2, 3], as well as lyophilized samples [4]. The determination of secondary structure of proteins using FTIR is based on the analysis of the amide absorption bands [1]-[6].

In this study, the percentage of the secondary structure elements of nine different proteins consisting of mainly α-helical structure (bovine serum albumin (BSA), hen egg white lysozyme (HEWL), xylose isomerase from *Streptomyces rubiginosus*), mixed α/β structure (albumin from chicken egg white, L-asparaginase from *Escherichia coli*, wheat germ agglutinin (WGA)), and β-sheet structure (human α-1-microglobulin (protein HC), β-lactoglobulin from bovine milk and human cystatin C) was estimated for lyophilized samples and in solution. FTIR data were collected in the transmission mode (proteins in KBr pellets) and using Attenuated Total Reflectance (ATR) for protein solutions in H_2O.

KBr pellets were made by grinding ca. 1 mg of protein with 200 mg of KBr and pressing the mixture using hydraulic press at 15 ton load. Protein solutions of at least 20 mg/ml were prepared by dissolving the appropriate amount of protein in H_2O. Infrared spectra were obtained from 500 co-added interferograms run on Bruker Tensor 27 FTIR spectrometer. All spectra were recorded from 400 cm^{-1} to 4000 cm^{-1} at both 2 cm^{-1} and 4 cm^{-1} spectral resolution.

Fourier self-deconvolution (FSD) was applied to amide I region (1705 – 1595 cm^{-1}) of protein spectra assuming an initial Lorentzian line-shape function with a FWHM of 13 cm^{-1} [5]. Composite bands were assigned to helical segments, β-sheets, turns or unordered structures based on previous experimental work [6] or calculated values [7]. The relative areas of the individual peaks were determined by an iterative curve-fitting procedure that assumed Gaussian (KBr samples) or Voigt (proteins in solution) band envelopes for the deconvolved components. Integrated peak areas were used to estimate secondary structure content for each of the nine proteins studied.

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