FOURIER TRANSFORM INFRARED SPECTROSCOPIC ANALYSIS OF PROTEIN STRUCTURE AND ADSORPTION TO SOLID SURFACE

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Fourier transform infrared(FT-IR)Spectroscory can be used analyze proteins at nanomolar concentrations. A comparison of FT-IR sensitivity with other commonly used spectral techniques such as circular dichroism and fluorescence revealed that FT-IR spectra of immunoglobulin G can easily obtained with good signal-to-noise ratio at a concentration of 10nM. No circular dichroism or intrinsic fluorescence signals were observed for the same concentration of the protein. Our results have suggested that less than 15 pmol protein was required to record FT-IR spectra with adequate signal-to-noise ratio for protein structural analysis.

Protein secondary structure has been analyzed using a Fourier transform infrared spectroscopic method in the amide III region. Although extensive work has been done on protein secondary structure using the amide I region, (1700-1600 cm^{-1}), the amide III region has not been utilized in past for its potential in protein structural analysis. One of the major reasons for non-use of the amide III vibrations is perhaps the very weak signal in the amide III frequency region, (1200-1350^{-1}). However, benefits of using the Amide III region are enormous. For example, water vibrations do not interfere with the protein spectrum unlike they do in the Amide I region. This feature allows for a greater ease in peak definement of the protein spectra. In the amide III region, the bands for the individual secondary structures (α-helix, β-sheet and random coils) do not overlap as much as they do in the Amide I region. This lack of overlapping allows for easier and a more reliable means of peak assignment, and secondary structure band positions are easier to determine. For example, a band at 1650-1655 cm^{-1} in the amide I region can be either assigned to α-helix or to random coil. In amide III region, α-helix bands are present in the 1316-1295 cm^{-1} and random coils present in the 1294-1255 cm^{-1} of the Amide III region. The estimation of secondary structure involves using fourier self-deconvolution and second derivitive analysis to determine the position of the individual bands. Using band positions obtained from the deconvolved and second derivative spectra, curve-fitting on the protein spectra is performed to determine individual band strengths in order to estimate secondary structure of proteins. Secondary structure estimation obtained in this manner has provided results that are consistant with x-ray data for model proteins such as α-chymotrypsin. Results obtained from the amide III band analysis parallel those from amide I bands. Thus amide III region of protein IR spectra has shown to be a valuable tool in estimating the amount of secondary structure present in proteins.

Adsorption behavior of proteins such as lysozyme and immunoglobin G onto ZnSe crystal surface has been detected by the FT-IR spectroscopy in combination with attenuate total reflectance (ATR)