Study of protein conformation of red bean (*Phaseolus angulairis*) globulin by FTIR spectroscopy

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Abstract

The conformation of red bean globulin dispersions ($\approx$ 10% in D_2O or deuterated phosphate buffer pD 7.4) under the influence of pH, chaotropic salts, protein structure perturbants, and heating conditions was studied by Fourier-transform infrared (FTIR) spectroscopy. The FTIR spectrum of red bean globulin showed major bands from 1682 to 1637 cm^{-1} in the amide I' region, corresponding to the four types of secondary structures, i.e. β-turns, β-sheets, α-helix and random coils. At extreme pH conditions, there were changes in intensity in bands attributed to β-sheet (1637 and 1618 cm^{-1}) and random coil (1644 cm^{-1}) structures, and shifts of these bands to lower or higher wavenumbers, indicating changes in protein conformation. Chaotropic salts caused progressive increases in random coil structures and concomitant decreases in β-sheet bands, following the lyotrophic series of anions. In the presence of sodium dodecyl sulfate and ethylene glycol, pronounced increases in the random coil band were observed, accompanied by slight shifts of the β-sheet band. Addition of dithiothreitol and *N*-ethylmaleimide did not cause marked changes in the FTIR spectra. Heating at increasing temperature led to progressive decreases in the intensity of the α-helix and β-sheet bands and increases in random coil band intensity, leveling off at around 60 °C. The data suggest that re-organization of protein structure occurred at temperatures well below the denaturation temperature of red bean globulin (86 °C) as determined by differential scanning calorimetry. This was accompanied by pronounced increases in the intensity of the two intermolecular β-sheet bands (1682 and 1619–1620 cm^{-1}) associated with the formation of aggregated strands at higher temperatures (80–90 °C). Increases in intensity of the aggregation bands were also observed in the heat-induced buffer-soluble and insoluble aggregates.

Keywords: Red bean globulin; FTIR spectroscopy; Protein conformation; Aggregation; Denaturation