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Application Note

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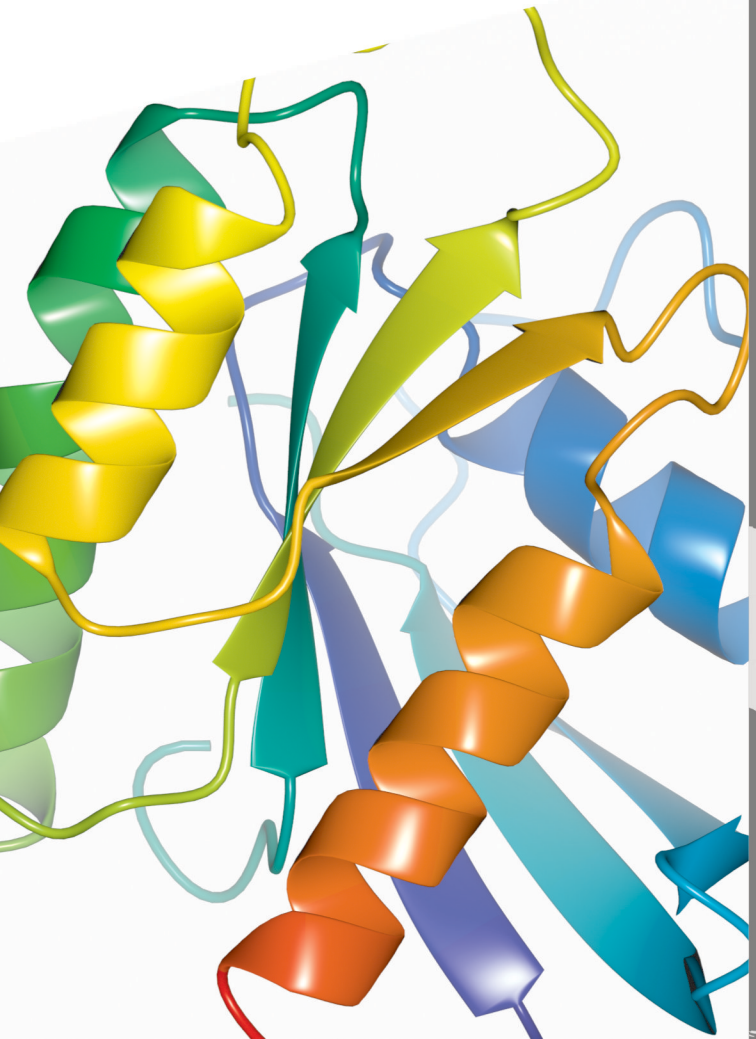
Investigating Protein Conformational and Structural Changes Using Transmission FTIR



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The *in-situ* characterization of protein unfolding, including the intermediate conformations is the holy grail for a host of biological questions.

Proteins have been extensively characterised by FTIR spectroscopy and exhibit many vibrational bands. The most prominent of these is the amide-I vibration between 1700 and 1600 cm^{-1}. This band is often broad and featureless due to many overlapping component bands which precludes a direct evaluation of the secondary structures of proteins without some form of deconvolution. The main application of FTIR to proteins has been the detection of relative conformational and structural changes induced by thermodynamic, chemical or biological perturbations [1-3].

Here variable temperature FTIR spectroscopy was performed on an acylated guanine nucleotide binding protein, Ras, in solution.

Specac Demountable Static Transmission Cell (right) and Water Heating Jacket (left)

Acknowledgement

Dr Shobhna Kapoor, Assistant Professor at the Department of Chemistry, Indian Institute of Technology Bombay kindly provided the research, figures and text that form the basis of this application note.

Experimental

Posttranslationally modified N-Ras proteins were synthesised using maleimidocaproyl (MIC)-controlled ligation (1) as described by Prof. H. Waldmann [4]; First, the N-Ras protein was expressed in a truncated form in E.coli with a free C-terminal cysteine which provides a reactive thiol group for the ligation. The cysteine residue is highly exposed to the solvent making the ligation reaction fast and selective. The truncated protein was then ligated to the C-terminal prenylated peptide sequence, generated via Fmoc chemistry under an argon atmosphere. Purification of the ligated protein was accomplished with Triton X-114 saturated solution.

The protein sample was dissolved in Tris D_2O buffer and loaded into a demountable Specac transmission cell (GS20512) fitted with a water heating jacket (GS20710) that allowed precise control of the protein environmental temperature. Spectra were recorded on a commercial spectrometer.

Results and Discussion

Figure 1 shows the normalized absorbance FTIR spectra of Ras protein in solution in the Amide-I region at various temperatures (top), along with the resultant difference spectra (bottom). No significant changes are observed as the temperature is increased from 25 to 50 °C. As the temperature is further increased, features around 1690 and 1620 cm^{-1} were observed to increase in intensity whilst features around 1650 cm^{-1} decreased. This is suggestive of structural and conformational changes due to initiation of the thermal unfolding process.

Figure 2 shows the temperature-dependent second derivative spectra of the Amide-I band of the protein in solution. The minima observed represent particular secondary structure elements. Above 60 °C, unfolding of the protein sets in, accompanied by a decrease of ordered structure and a concomitant increase in conformations originating from unordered structures and intermolecular β-sheet formation. The shift in the wavenumber for helical secondary structures from 1654 cm^{-1} to 1657 cm^{-1} is indicative of highly solvated and flexible helices. This is probably due to a protein conformation state with a locally unfolded structure and higher hydration induced at higher temperature compared to the native state.

Figure 3 shows the quantitative fit analysis of the amide-I band showing the evolution of different secondary structure elements as a function of temperature. The increase in the relative amount of unordered structures and intermolecular β-sheets at the expense of a decrease in ordered elements such as helices, turns and intramolecular β-sheets argue for unfolding and aggregation of protein with increased temperature.

Conclusions

In summary, the FTIR spectroscopy analysis showed that Ras proteins undergo substantial secondary structure changes such as aggregation following unfolding, leading to the formation of anti-parallel β-sheet aggregates with temperature and a protein state with locally unfolded structure and highly flexible Helices was observed at temperatures above 60 °C.

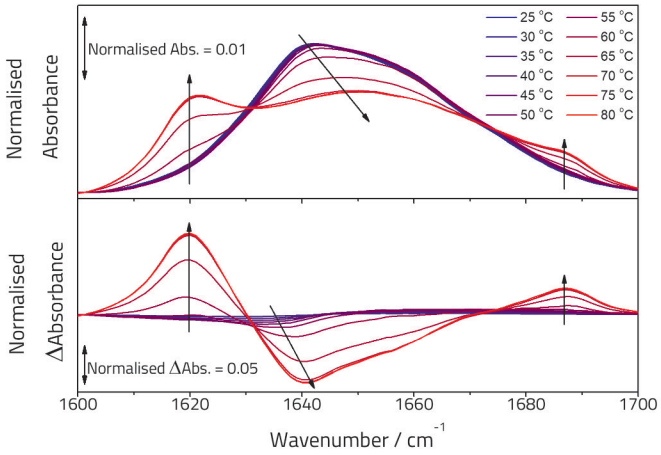

Figure 1: Normalized amide-I FTIR spectra for Ras protein in bulk solution (top), along with the resultant difference spectra (bottom).

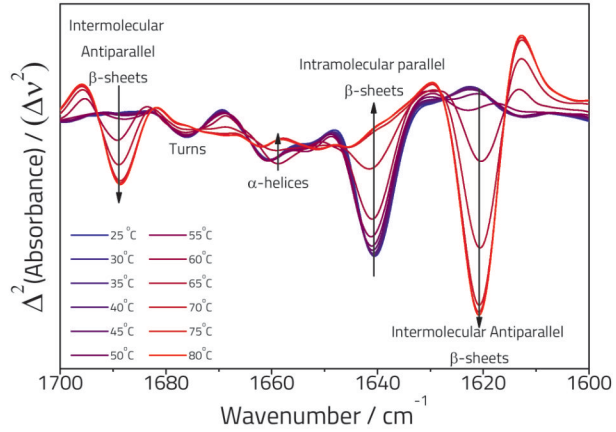

Figure 2: Second derivative amide-I FTIR spectra for Ras protein in bulk solution showing distinct secondary structure minima.

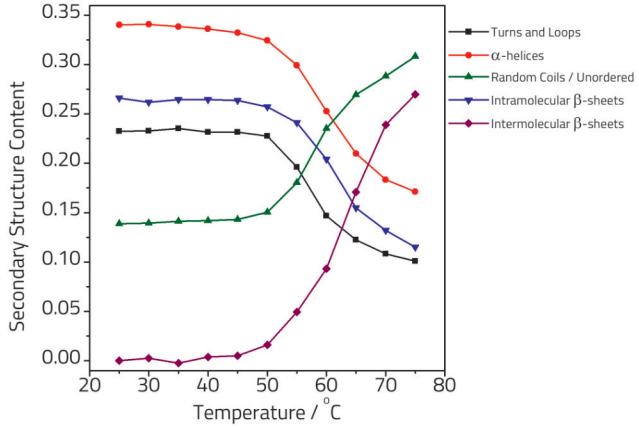

Figure 3: Quantitative temperature-dependence of the secondary structure of Ras protein in solution. These numbers were obtained by curvefitting of the normalised FTIR amide-I spectra.

References

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