Microstructural study of thermal denaturation and gelation of proteins using an Agilent 660 FTIR

Application note

Food science

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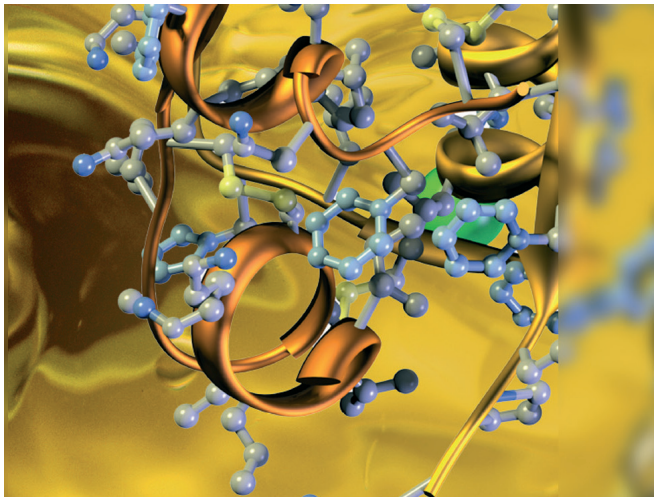

Introduction

FTIR spectroscopy is a powerful tool for the investigation of proteins and for the study of reaction dynamics. It is universally applicable to the analysis of small soluble proteins up to large membrane proteins, providing molecular information with the sensitivity required to detect a change in the environment around a single atom of a large protein. In order to understand the microstructural changes during thermal denaturation and gelation of proteins, the physicochemical properties of β-Lactoglobulin A (β-lg) were investigated, across a broad temperature range, using FTIR spectroscopy.

β-Lactoglobulin is the most abundant globular protein of milk and the major component in the whey fraction of milk. The physicochemical properties of β-lg play a significant role in the overall functionality of whey proteins, particularly the heat-induced gelation process. As commercial food preparations extensively use β-lg in food formulations, a better understanding of its gelation can be used to facilitate product manufacturing.

A raw spectrum of β-lg in deuterium oxide (D_2O) is shown in Figure 1. The protein is made up of 162 amino acid residues and contains two disulfide groups and one free sulfhydryl group. The sensitivity of the amide I vibration to its secondary structure makes it possible to study the folding, unfolding and aggregation of β-lg by FTIR spectroscopy.

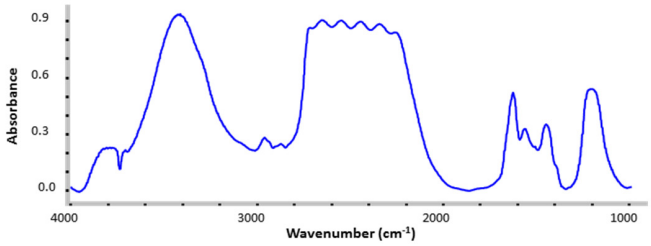

Figure 1. Raw spectrum of β-lactoglobulin in D_2O

Experimental

Instrumentation

An Agilent Cary 660 FTIR spectrometer configured for mid-IR was used for all measurements. All data was collected and processed using Agilent's Resolutions Pro software, which includes as standard, the functionality to monitor reaction dynamic and kinetics experiments such as protein folding or cell membrane transitions, in real time. The Cary 660 was operated in rapid scan mode, so that changes that occur within seconds could be measured. Instrument operating parameters are given in Table 1.

Table 1. Agilent Cary 660 FTIR collection parameters

Parameter	Value
Speed	5 kHz
Scans	128 scans
Spectral range	4000–800 cm^{-1}
Resolution	4 cm^{-1}
Temperature range	40–92 °C

Sample analysis

A solution of β-lactoglobulin B (5% w/v) in deuterated phosphate buffer at pH 7 was prepared. Spectra were collected using Agilent's powerful kinetics software while heating the protein solution from 40 to 92 °C in a temperature controlled transmission cell.

Results and discussion

The spectra shown in Figure 2 were collected rapidly, deconvolved and then compared visually to gain a better understanding of the structural changes that occur in the protein during heating. The deconvoluted spectrum acquired at 40 °C shows seven bands, each of which can be assigned to a secondary structure: 1691 (β-type structure), 1677 (β-sheet), 1664 (turns), 1648 (α-helix), 1634 (β-sheet), 1622 (β-strand), and 1614 cm^{-1} (side-chain vibrations). Heating above 76 °C resulted in the disappearance of the bands at 1677, 1648, 1634, and 1614 cm^{-1} (denaturation) and the rise of the bands at 1682 and 1617 cm^{-1} (aggregation). This information can be correlated to specific bonding processes. For example, the bands at 1682 and 1617 cm^{-1} have been attributed to the formation of intermolecular hydrogen-bonded anti-parallel β-sheet structures associated with aggregate formation.

Conclusions

The study has shown that the Agilent 660 FTIR can be used successfully to monitor the rapid structural changes of an important food protein during heating. This information provides a useful model for studying the relationship between protein structure and function, in order to improve product manufacturing.

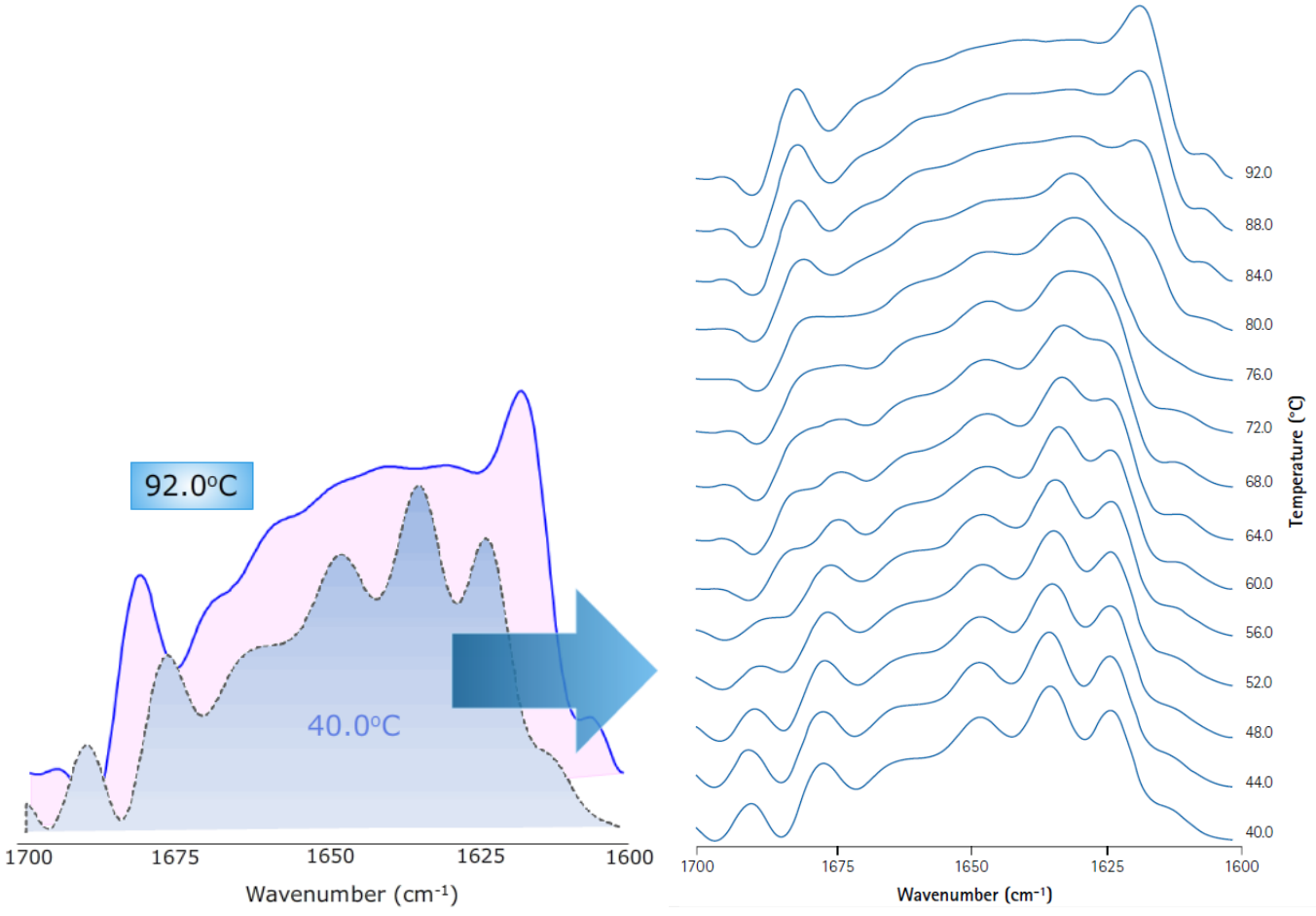

Figure 2. Thermal denaturation of β-Lactoglobulin

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Published March 26, 2012
Publication number: 5991-0019EN

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