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Application News

No. A592

Spectrophotometric Analysis

Protein Analysis Using FTIR
- Analysis on Changes of Secondary Structures in Egg White Proteins Caused by Thermal Denaturation -

Application News No. A585 describes using curve fitting to analyze the secondary structures of bovine serum albumin (BSA). This article describes investigating the changes that occur in the secondary structures of proteins caused by thermal denaturation. The changes were probed based on second derivative and curve fitting of the spectra measured when egg white containing proteins abundantly was heated from 40 to 100 °C using a heatable three-reflection ATR accessory.

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Sample Preparation

Egg white was separated from the yolk, so that only egg white was analyzed. The main protein in egg white is ovalbumin, but various other proteins are also included, such as ovotransferrin and ovomucoid.[1] In this case, the egg white was measured without separation into respective components.

Measurement of Egg White

Egg white was measured with a MicromATR™ ATR measurement accessory equipped with a heatable three-reflection ATR crystal (diamond/ZnSe). Because egg white solidified when heated, a three-reflection ATR crystal that could also be used for measuring solid samples was used. The optical system was purged with dry air to prevent the peaks originating from water vaper from overlapping in the amide I region.

The measurement conditions are listed in Table 1. Using a temperature controller to increase the crystal temperature from 40 to 100 °C in 10 °C increments, adequate heat transfer to the egg white was ensured by waiting for two minutes after placing drops before measuring the samples at respective temperatures. Since egg white contains much water, the absorptions of water must be subtracted from the spectrum of egg white before conducting the secondary structure analysis. Due to the temperature dependence of hydrogen bonds in water, the spectra of water were obtained at each temperature.

Fig. 1 shows the spectra of egg white after subtracting the absorptions of water at each temperature. (The portion that includes the amide I band near 1700 to 1600 cm^{-1} is shown enlarged.)

It has been reported that protein denaturation starts when egg white is heated to 60 °C.[2] Fig. 1 shows that the changes start at the 60 °C with peaks near 1625 cm^{-1} and 1675 cm^{-1} increasing significantly, which confirms a correlation with thermal denaturation.

Table 1 Measurement Conditions

Instrument	: IRTracer™-100 and MicromATR (heatable three-reflection ATR crystal)
Resolution	: 4 cm^{-1}
Accumulation	: 100
Apodization function	: Sqr-Triangle
Zero filling	: 4 times
Detector	: DLATGS

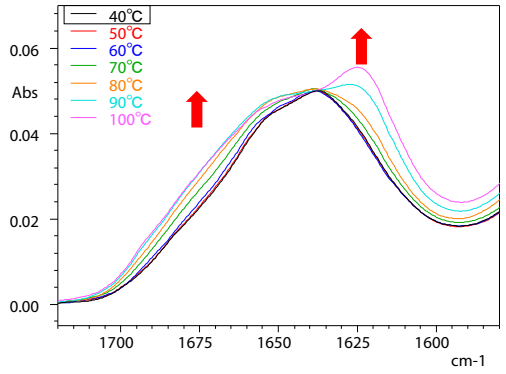

Fig. 1 Infrared Spectra of Amide I Band in Egg White (After Subtracting Absorptions of Water)

Second Derivative Spectral Analysis

Slight variations in the absorption spectrum shape can be shown more clearly by calculating the second derivative spectrum which is ideal for investigating changes in secondary structures of protein (α-helix, β-sheet, β-turn, and random coil structures).

Fig. 2 shows the second derivative spectra for egg white calculated from the spectra in Fig. 1. It shows that β-sheets near 1693 cm^{-1} and 1622 cm^{-1} increase when denaturation occurs in the heated protein, whereas there is a decrease in β-sheet structures near 1637 cm^{-1} and α-helix structures near 1655 cm^{-1}. It also shows that the peaks shifted due to thermal denaturation, which suggests the hydrogen bond status changed.

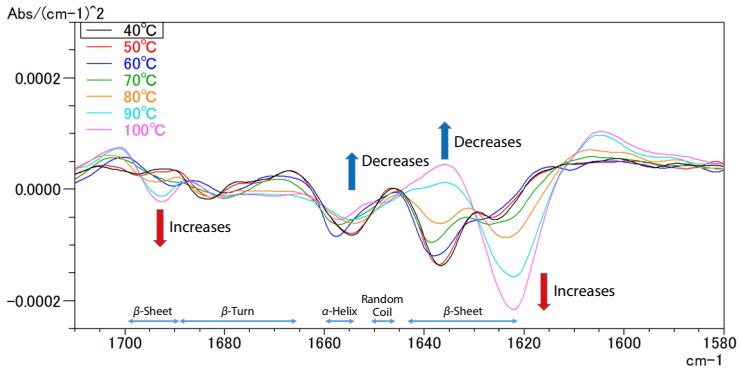

Fig. 2 Second Derivative Spectra of Egg White

Curve Fitting in Amide I Band

Next, curve fitting for the amide I band was conducted to investigate the changes of secondary structures due to thermal denaturation quantitatively. Curve fitting requires first specifying some conditions such as the waveform and the number of absorption bands used for fitting. The number of bands is determined by a second or fourth-derivative spectrum.

Curve fitting was performed for infrared spectra obtained at 40 and 100 °C. Table 2 lists the conditions for curve fitting. The infrared spectrum before curve fitting, the individual peaks determined by curve fitting and the spectrum synthesized from them are shown in Figs. 3 and 4. Checking how closely the spectrum synthesized from separated peaks matches the spectrum from before curve fitting provides an effective way to confirm that curve fitting was performed appropriately. In this case, the black and red lines (original and synthesized spectra) in Figs. 3 and 4 match very closely. Therefore, we concluded that the curve fitting results were good.

Table 2 Conditions for Curve Fitting

Peak curve type	: Gaussian function
Baseline	: Offset 1 Pt
Range	: 1710 to 1580 cm^{-1}
Max. error	: 0.01 %

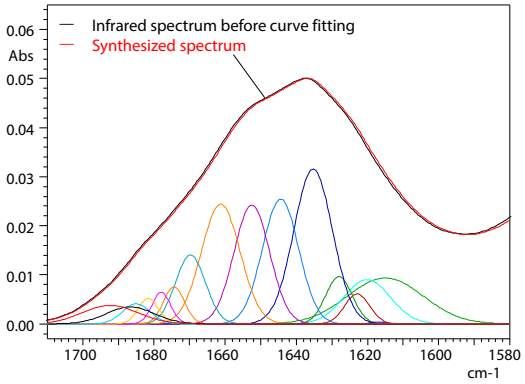

Fig. 3 Individual Peaks and the Synthesized Spectrum Obtained by Curve Fitting (at 40 °C)

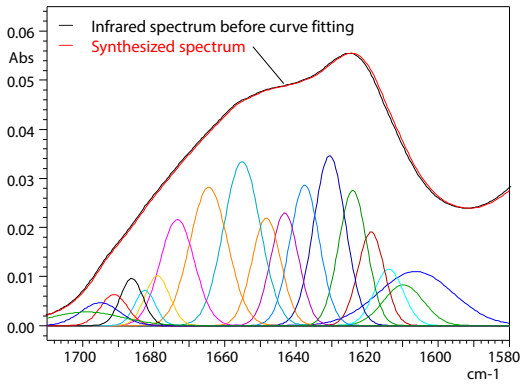

Fig. 4 Individual Peaks and the Synthesized Spectrum Obtained by Curve Fitting (at 100 °C)

Analysis of Individual Peaks Determined by Curve Fitting

The peak detection function was used to determine the wavenumber and area values for each peak determined by curve fitting. After assigning secondary structures to each waveform according to the reference [3], the ratio of them was calculated. The results are shown in Table 3.
A comparison of 40 and 100 °C results shows that the ratio of β-sheets and β-turns increases with increasing temperature. A check of each peak originating from a β-sheet shows that the ratio of β-sheets near 1693 cm^{-1} and 1622 cm^{-1} increased, whereas that near 1637 cm^{-1} decreased. Those results are consistent with the second derivative spectral trends shown in Fig. 2. The decrease in α-helix structures is also consistent with the trend in the second derivative spectrum.

Table 3 Change in Secondary Structures of Protein Due to Temperature

	α-Helices	β-Sheets	β-Turns	Random Coils
40 °C	30.3 %	37.9 %	16.4 %	15.4 %
100 °C	15.1 %	47.6 %	29.7 %	7.7 %

Conclusion

The secondary structures of protein in chicken egg white was analyzed by curve fitting in the amide I band. When egg white was heated from 40 to 100 °C, the proteins began denaturing at 60 °C, which decreased the ratio of α-helices and increased the ratio of β-sheets. It has been reported that thermal denaturation breaks secondary and higher order structures and unfolds part of α-helix structures [4], which is consistent with the curve fitting results shown here. Using FTIR analysis made it easy to measure and review the changes in secondary structures caused by thermal denaturation of proteins.

References:
1) JENNIFER KOVACS-NOLAN. Advances in the Value of Eggs and Egg Components for Human Health. J. Agric. Food Chem. 2005, 53, 8421-8431
2) Yoshinori Mine, Tatsushi Noutomi, and Noriyuki Haga Thermally induced changes in egg white proteins. J. Agric. Food Chem., 1990, 38 (12), pp 2122–2125
3) Jilie KONG, Shaoning YU. Fourier Transform Infrared Spectroscopic Analysis of Protein Secondary Structures. Acta Biochim Biophys Sin 2007, 39(8): 549–559
4) A. Kato and T.Takagi, Formation of intermolecular .beta.-sheet structure during heat denaturation of ovalbumin. J. Agric. Food Chem. 1988, 36, 1156-1159

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