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Secondary structure analysis of barley aleurone holoprotein by FTIR spectroscopy

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Structural information of barley aleurone at various temperatures is obtained by analysis of the conformationally-sensitive amide I band using FT-IR spectroscopy. The second derivative spectrum was performed in order to overcome the bands overlapping due to the different C=O stretching vibrations of each type of secondary structure (i.e. α-helix, β-sheet, turns and unordered). The results of quantitative analysis by curve fitting to the inverted second derivative spectra indicate perturbations of both α-helix and β-sheet structures in thermal unfolding process, depending on the temperature values.

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1. Introduction

Aleurone (from Greek *aleuron*, flour) is an holoprotein (apoprotein combined with its prosthetic group) found in the endosperm of many seeds. It forms the outermost layer of the seed coat in some grains. During seed germination, hydrolysis in the seed causes the aleurone cells to break down into amino acids. Most of the nutritionally important substances of whole grain, such as dietary fiber, vitamins, minerals, antioxidants and phytonutrients, are concentrated in the aleurone layer and in the embryo. Also, the cereal aleurone cell is well established as a model system for studying hormonal regulation of plant cells.

One of the crucial problem of extraction, purification and preservation of proteins is their stability in stress conditions upon irreversibly damage. Also, the utilization of proteins in pharmaceutical and other commercial applications requires close examination of their relative fragility. Because of the resultant enhanced stability, proteins are often formulated in the solid state, even though dehydration tends to alter their structure. Even in the solid form, however, proteins may become inactivated due to various deleterious processes, e.g., aggregation. The damage is manifested as denaturation and aggregation when the protein sample is rehydrated. Denaturation is a process by which hydrogen bonds, hydrophobic interactions and salt linkages are broken and the protein is unfolded. The denaturation of secondary structure involves also changes in ratio among the three common structures: α helix, β sheets and turns [1,2].

In our paper, we employed FT-IR spectroscopy to investigate the thermal unfolding and temperature-induced changes in secondary structure of barley (*Hordeum vulgare* cv. Himalaya) aleurone protein. Infrared spectroscopy is one of the most used techniques for studying alterations in proteins conformation. The change in secondary structure refers to change in the ratio among the secondary structures (i.e. α-helix, β-sheet, turns and random coils). Structural information of proteins was obtained by analyzing the amide I band (1700-1600 cm^{-1}) using FTIR spectroscopy. The secondary structure of a protein is sensible to its denaturing due to some stress conditions (for instance pH or temperature) or due to environment in which the proteins are kept. By applying powerful methods of second derivative and correlation coefficient analysis previous studies reveal that dehydration-induced spectral alterations in the conformation-sensitive amide I region were due to protein unfolding and not simply to the loss of water from the protein. After the Gaussian curve-fitting procedure the resulting component bands are assigned depending on their frequency and, in many cases, the α-helix and β-sheet contents can be calculated with great accuracy from the amide I IR spectra.

2. Experimental

The investigated holoprotein was obtained from barley aleurone cells prepared from mature naked barley cv. Hymalaya [3, 4]. The well-known KBr technique was employed and FT IR measurements were performed in the 4000-350 cm^{-1} domain using a JASCO 6100 spectrometer with a resolution of 2 cm^{-1}. The resultant spectra were smoothed with a 9-point Savitsky–Golay smooth function to remove the white noise. The second derivative spectral analysis was applied to locate positions and assign them to different functional groups [20]. Before starting the fitting procedure, the obtained depths of the minima in the second derivative spectrum and, subsequently, the calculated maximum intensities were corrected for the interference of all neighboring peaks. All second-derivative spectra, calculated with the derivative function of Opus software, were baseline-corrected, based on the method of Dong and Caughey [5], and area-normalized under the second

derivative amide I region, 1700–1600 cm^{-1} and amide III region, 1330-1230 cm^{-1} [6] .

The curve fitting is performed by stepwise iterative adjustment towards a minimum root-mean-square error of the different parameters determining the shape and position of the absorption peaks. The inverted second-derivative spectra were obtained by multiplying by (–1) the second-derivative spectra. Curve fitting was performed by setting the number of component bands found by second-derivative analysis with fixed bandwidth (14 cm^{-1}) and Gaussian profile. The best-average fit gave the intensity of each component band for each spectrum. The area under each peak was used to calculate the percentage of each component and finally used to analyze the percentage of secondary structure components.

3. Results and discussion

Fourier Transform Infrared spectra of barley aleurone between 500 and 4000 cm^{-1} are characterized by three major bands associated with the amide I, amide II and amide III vibrations. Since the position and the shape of these bands are sensitive to the conformation adopted by proteins, they are useful for the evaluation of their secondary structure content. Fig. 1 shows the infrared spectra in this spectral region of barely aleurone at room temperature.

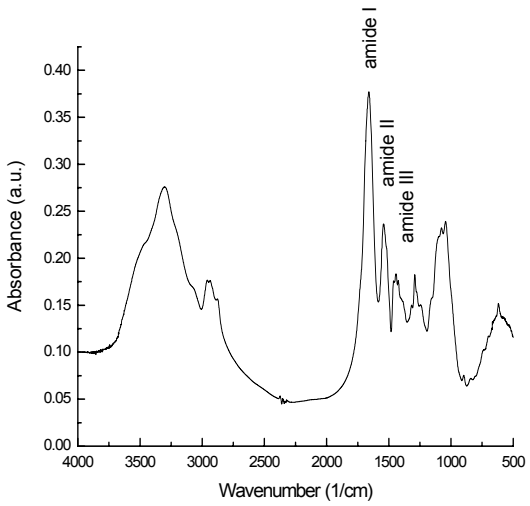

Fig. 1. FT IR spectrum of the barley aleurone protein in the 4000 to 500 cm^{-1} spectral region

Contribution of the water bending vibration at 1650 cm^{-1} was subtracted from these spectra according to the method of Dousseau et al. [7]. The three major bands, observed at 1656, 1547 and 1278 cm^{-1}, are assigned to the amide I, amide II and amide III vibrations, respectively.

The amide I band at 1656 cm^{-1} is mainly due to the α-helical and unordered structures [8-11].

For studies of thermal-induced structural transitions, calculations of the second derivative spectrum are recommended [12]. This method is objective and alterations in component bandwidths, heights and positions, which are due to protein unfolding, are preserved in the second derivative spectrum. Band narrowing techniques, like second derivative, are efficient to decompose the amide I band into its overlapping components. As seen in Fig. 2, the second derivative of the amide I band of barely aleurone is composed of approximately four to four components.

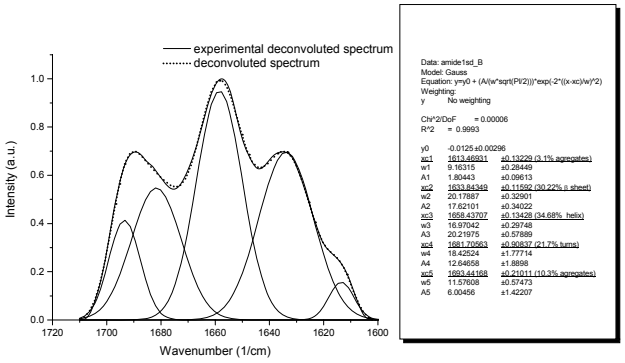

Fig. 2. The fit function of the second derivative in the Amide I spectral region for the barley aleurone at 22 ºC.

The amide I band is due to the in-plane C=O stretching vibration, weakly coupled with C–N stretching and in-plane N–H bending [14-18]. Each type of secondary structure (i.e. α-helix, β-sheet, β-turn and unordered) gives rise to different C=O stretching frequencies [13-18], and, hence, results in characteristic band positions. Band positions are used to determine the secondary structural types present in each protein. The relative band areas (determined by curve fitting) can then be used to quantitative the relative amount of each structural component. Fourier deconvolution, used to determine the component band positions, reveals that both proteins exhibit a major band in range 1650-1653 cm^{-1} characteristics of α-helical structures. The bands in the region 1638-1640 cm^{-1} are expected to be characteristic for native β-sheet structures, while the bands in the ranges 1660-1667 cm^{-1} and 1682-1687 cm^{-1} may possibly be attributed to β-turns. On the basis of earlier IR studies, the bands from 1626-1628 cm^{-1} are indicative of intermolecular (antiparallel) β-sheet and, according with literature, are common IR spectral features for temperature-induced protein aggregation [19].

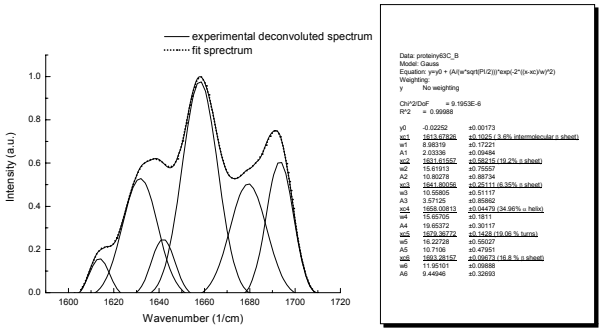

Fig. 3 . The fit function of the second derivative in the Amide I spectral region for barley aleurone at 63ºC.

The effect of temperature on the secondary structure of FT-IR spectra of it is shown by comparison in Figure 2 and 3. It is evident that the IR spectral deconvoluted contour of amide I changed markedly with temperature. With the increase of temperature, the peaks at 1637 and 1670 cm-1 in the amide I band gradually shifted to 1628 and 1674 cm-1. In the thermal process of the peak at 1653 cm-1 assigned to the co-existence of α-helical and random coil structures gradually transformed to 1672 and 1631 cm^{-1} with the increase of temperature. The peak at 1672 cm^{-1} was due to β-turn structure, but the peak at 1631 cm^{-1} was due to β-sheet structure. Furthermore, the peak at 1541 cm^{-1} assigned to the α- helix structure also shifted to 1525 cm^{-1} due to β-sheet with the increase of temperature.

4. Conclusions

Second derivative amide I spectra can be used to investigate changes in secondary structure of proteins during the stress processes which involves their denaturation. Simultaneous qualitative and quantitative analysis of amide I feature by curve fitting to the inverted second derivative spectra reveals a decrease in α-helix content and an increasing β-sheet content with temperature. Obviously, the formation of β-sheet structure increased with temperature. The thermal-dependent of solid barley aleurone was found near 60 ^{o}C. The thermal denaturation plays an important role in the transformation of α-helix/random coil to β-sheet.

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