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ARTICLE

Composition, Secondary Structure, and Self-Assembly of Oat Protein Isolate

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The amino acid compositions, secondary structure, and self-assembly of oat protein isolate (OPI), which was purified from the high-protein Chinese oat, have been investigated by using a combination of amino acid analysis, Fourier transform infrared spectroscopy (FTIR), and tapping mode atomic force microscopy (TP-AFM). OPI, with molecular weights ranging from 14.0 kDa to 66.0 kDa, was rich in essential amino acids and contained 24.7% glutamic acid and 8.1% leucine. The amino acid contents of OPI are 4.5−8.7 times higher than those of oat flour. The secondary structures of OPI have been quantified by the deconvolution of the amide I band of the FTIR spectrum of OPI, which were found to contain approximately 7% β-turn, 19% α-helix, and 74% β-sheet. Tapping mode AFM results further suggest that the oat protein isolate has two major types of shapes, ellipsoidal and disk-like. At protein concentrations below 0.5 mg/mL, most of the OPI molecules are in the isolated form. However, when the concentration of OPI reaches 1.0 mg/mL, some of the OPI molecules self-assembled into large and heterogeneous protein aggregates.

KEYWORDS: Oat protein isolate; amino acid composition; secondary structure; atomic force microscopy; FTIR

INTRODUCTION

Because of the increasing market demands for protein ingredients, many kinds of proteins have been isolated from various sources (*1*−*3*). Oats are potential sources of low-cost proteins with good nutritional values (*4*). Some cultivars of Chinese oats contain approximately 15% crude protein on a dried basis. With a chemical score (*5*) of 72−75, oat protein isolate (OPI) has acceptable nutritional values (*6*). Globally, China is one of the largest oat producers, with an annual yield of approximately 1 million tons, which account for a large percentage of the worldwide oats production. In China, oats are mainly used for the direct production of foods, and the economic value is very low. The extraction of OPI from oats will be of great importance in providing potential value-added products for the food industry. Although the preparation methods for oat protein concentrates (OPC) or oat protein isolate (OPI) by using oat flour or ground oat groats as the major starting raw materials have been reported (*6*−*9*), currently little information is available about the characteristics of their amino acid compositions, secondary structure/conformation, and size and shape, especially for Chinese oats.

Previous reports indicated that OPI possessed good emulsifying activity (*10*) and binding properties (*11*). Very recently, Mirmoghtadaie et al. (*12*) found that deamidated and succinylated oat protein isolate had improved water solubility and emulsifying activity compared with those of their native forms. The emulsifying activity of oat protein isolate may be related to its secondary structure and self-assembly properties of forming the protein adsorption layer at the air/water interface. However, to the best of our knowledge, no research has been carried out regarding the secondary structure and morphology of oat protein isolate. Fourier transform infrared spectroscopy (FTIR) is a powerful tool to determine the secondary structure of a protein (*13*). Through proper fitting of the amide I band of the original FTIR spectrum of a protein as well as analyzing its second-derivative, the conformation of the protein (i.e., helix, expended, or turn) can be obtained (*14*)

The unique secondary structure of OPI results in the distinct self-assembled properties of OPI. One powerful tool to study the conformation or morphology of a protein is through the direct imaging of such a protein using atomic force microscopy (AFM). AFM is the most versatile member of the family of scanning probe microscopy methods and can resolve forces with piconewton sensitivity and has a spatial resolution of nanometer (*15*). These features enable AFM to produce nanometer to micrometer scale images of topography, adhesion, friction, and compliance, and thus make AFM an essential characterization technique for fields from materials science to food science. Extensive research has proved the effectiveness of using AFM to image biopolymers that include proteins, DNA, and polysaccharides (*16*).

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In this article, OPI was first purified from a high-protein oat variety available in China, and the characteristics of amino acid compositions as well as the molecular weights of the protein fractions were determined. The secondary structure of OPI was then analyzed by fitting the amide I and amide II bands of OPI to a series of Gaussian peaks. The size and shape of individual OPI molecules or protein aggregates were finally investigated by tapping mode AFM.

MATERIALS AND METHODS

Materials. Oat flour (40-mesh) of variety BAYOU NO.1 harvested in the fall and grown in Shanxi Province of China was supplied by Shanxi Jin Lvhe Biotechnology Limited Company (Shanxi, China). Tris-alkali, sodium dodecyl sulfate (SDS), *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED), 2-mercaptoethanol, glycin, bromophenol blue, and Coomassie Brilliant Blue G-250 were supplied by Wuhan Hinat Technology Co., Ltd. (Wuhan, China). Precast 4 - 20% polyacrylamide gels were purchased from GenScript Corp. (Piscataway, NJ). Sodium hydroxide, HCl, methanol, acetic acid and sodium citrate were supplied by Wuhan Minghao Biotechnology Co., Ltd. (Wuhan, China). Prestained protein markers were purchased from Fermentas Inc. (Glen Burnie, MD). Milli-Q water was used throughout the experiments.

Isolation of Oat Protein Isolate (OPI). OPI was prepared by isoelectric precipitation. Oat flour (100 g) was mixed with 600 g of water, and the initial pH of the solution was adjusted to 10.0 using 2 M NaOH. The solution was magnetically stirred at 25.0 °C for 2 h. A negligible amount of foaming was observed during the mixing process. The blended slurry was then filtered through wire meshes of 100 μm, 75 μm, and 50 μm. The fine slurry passing through the wire meshes was centrifuged at 3000*g* for 15 min. The supernatant, which mainly contained oat proteins, would precipitate at their isoelectric points after the pH was adjusted to ~5.0 using 0.5 M HCl, followed by centrifugation at 3000*g* for another 15 min at room temperature. The resultant oat protein pellet was washed with 100 mL of DI water 3 times, then the pH was adjusted back to 7.0 and finally freeze-dried using an LG-5 freeze dryer (Shanghai Centrifuge Institute Co., Ltd., China). The protein powder was kept in a desiccator until use.

Proximate Composition. The protein, starch, fat, ash, and water contents of OPI were determined according to AOAC Official Method 2001.11 Protein (Crude) in Animal Feed, Forage (Plant Tissue), Grain, and Oilseeds, AOAC Official Method 996.11 Starch (Total) in Cereal Products, AOAC Official Method 999.02 Oil in Oilseeds, AOAC Official Method 900.02 Ash of Sugars and Syrups, and AOAC Official Method 2001.12 Determination of Water/Dry Matter(Moisture) in Animal Feed, Grain, and Forage (Plant Tissue) (*17*), respectively.

Amino Acid Composition. A 75 mg portion of OPI was placed in a 20 mL ampule and mixed with 10 mL of 6 M HCl. After sealing the ampule, the OPI was hydrolyzed at 110 °C for 22 h under vacuum. After cooling down, the ampule was opened, and the hydrolysate was evaporated to dryness under vacuum at 60 °C. The dried sample was dissolved in 3−5 mL of 0.2 M sodium citrate buffer (pH 2.2) to yield an amino acid concentration of 50−250 nmol/mL. The protein solution was filtered through a 0.2 μm syringe filter and loaded onto a Hitachi L-8800 amino acid analyzer (Tokyo, Japan) equipped with a Hitachi 2622SC ion-exchange column for amino acid composition analysis (*18*). No tryptophan was found under our current experimental conditions.

Sodium Dodecyl Sulfate−Polyacrylamide Gel Electrophoresis (SDS−PAGE). SDS−PAGE was performed using the well-established method (*19*, *20*) with some modifications: the protein components of oat protein isolate were analyzed by SDS−PAGE using precast 4−20% polyacrylamide gels. The oat protein isolate sample was dissolved in 5× sample buffer (GenScript Corp., Catalog Number MB01015, Piscataway, NJ) and heated to 95 °C for 3 min. Ten micrograms of sample was loaded onto the gel. After running, the gel was fixed and stained with Coomassie Blue (10% v/v acetic acid and 0.006% w/v Coomassie brilliant blue G-250, in water). Prestained protein markers were used to identify the molecular weight of each component. The photo of the gel was obtained by scanning the gel using an office scanner and analyzed by ImageJ software.

Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) Spectroscopy. The ATR-FTIR spectra were collected by using a Thermal Nicolet Nexus 670 FT-IR spectrometer (Thermo Fisher Scientific Inc., Waltham, MA) under ambient conditions. Each spectrum was averaged over 512 scans with 4 cm^{-1} resolution. The powder of the oat protein sample was first put between two aluminum foils and pressed into a pea-size small pellet by hand to make it suitable for ATR-FTIR measurement. The small pellet sample was further pressed onto the Ge crystal surface tightly by the ATR accessory to ensure good contact with the ATR crystal. The original FTIR spectrum was smoothed for further deconvolution analysis with the aid of OMNIC software. Spectrum in the wavenumbers ranging from 1580 to 1720 cm^{-1}, which covered the typical amide I peak, was selected for further deconvolution analysis. The smoothing of the typical amide I peak was performed within a certain number of smoothing points (11, 21.213 cm^{-1}). The second derivative of the smoothed amide I absorption peak was conducted after a smoothing process and the positions of individual component peaks were selected for later deconvolution analysis with the aid of OMNIC software.

Atomic Force Microscopy (AFM). Tapping mode AFM images were collected by NanoScope IIIA Multimode AFM (Veeco Instruments Inc., Santa Barbara, CA) equipped with a silicon-etched RTESP7 cantilever (Veeco Nanoprobe, Camarillo, CA) under ambient conditions. Before tip engagement, the drive frequency of the silicon tip was tuned with the aid of Nanoscope 5.30 software and fixed at 260−280 kHz for further scanning. The oat protein isolate was adsorbed onto the pretreated silicon wafers, which were cleansed by piranha solution (7:3 mixture of 98% H_2SO_4 and 30% H_2O_2) and deionized water in a step-wise manner. The protein adsorption procedure was finished through 1-h solution immersion. All of the collected images were flattened before further analysis. Section analysis, which was incorporated in the Nanoscope 5.30 software, was utilized to obtain the quantitative information of oat protein self-assembly morphology.

RESULTS AND DISCUSSION

Proximate Composition. Proximate composition of the oat protein isolate was determined. The oat protein was composed of 87.0% protein, 1.4% fat, 6.1% water, 1.5% ash, and 1.0% starch.

Amino Acid Composition. The amino acid composition of the oat protein is shown in **Table 1**. Tryptophan (Trp) could not be detected as it was hydrolyzed in strong acidic conditions. Glutamic acid (Glu) and leucine (Leu) were the most abundant amino acids found in the oat protein isolate extracted from oat flour, making up about 24.7% and 8.1% of the total amino acids in the oat protein isolate. In comparison, oat flour was found to contain 23.4% and 7.8% of glutamic acid and leucine, as shown in **Table 1**.

The amino acid components in OPI were similar to those in oat flour. However, the contents of isoleucine, methionine, phenylalanine, and arginine as a percentage of the total amino acids were found to be higher in OPI than in oat flour, whereas asparagine, serine, glycine, and cystine were evidently lower in OPI than in oat flour (**Table 1**). These differences may be due to the fact that OPI has different protein compositions from that of oat flour.

The amino acid contents of OPI were found to be 4.52−8.70 times higher than their counterparts in oat flour, as indicated in **Table 2**. Seven of the eight essential amino acids, namely, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, and valine, made up approximately 32.3% and 31.2% of total amino acids for OPI and oat flour, respectively. The percentages of some essential amino acids or amino acid pairs of the OPI fulfilled or exceeded their respective percentages stated in the ideal protein of WHO (*21*). Specifically, phenylalanine, leucine, isoleucine, and valine of oat proteins extracted from the oat flour were 100%, 116%, 100%, and 108% of their counterparts stated in the WHO standard, respectively (*21*). However, the overall quality of OPI was slightly compromised by the low level of lysine and methionine, which accounted

Table 1. Amino Acid Percentages (%) in Total Amino Acids for Oat Protein Isolate (OPI) and Oat Flour (%)

amino acid	OPI	oat flour
threonine (Thr)[a]	3.05	3.16
valine (Val)[a]	5.48	5.26
methionine (Met)[a]	1.78	1.30
isoleucine (Ile)[a]	4.00	3.64
leucine (Leu)[a]	8.13	7.77
phenylalanine (Phe)[a]	6.01	5.67
lysine (Lys)[a]	3.98	4.29
asparagine (Asp)	7.02	7.61
serine (Ser)	5.89	6.80
glycine (Gly)	4.31	5.18
glutamic acid (Glu)	24.66	23.40
histidine (His)	2.44	2.27
arginine (Arg)	7.96	7.29
alanine (Ala)	4.70	5.02
proline (Pro)	4.70	4.94
tyrosine (Tyr)	3.87	3.81
cystine (Cys)	2.03	2.59
total	100.00	100.00

[a] Essential amino acids.

Table 2. Actual Amino Acid Percentages in Oat Protein Isolate (OPI) and Oat Flour as Well as Their Corresponding Ratios

amino acid	A: OPI (%)	B: oat flour (%)	A/B
threonine (Thr)	2.65	0.39	6.80
valine (Val)	4.77	0.65	7.33
methionine (Met)	1.55	0.16	9.70
isoleucine (Ile)	3.48	0.45	7.72
leucine (Leu)	7.07	0.96	7.37
phenylalanine (Phe)	5.23	0.7	7.47
lysine (Lys)	3.46	0.53	6.54
asparagine(Asp)	6.10	0.94	6.49
serine (Ser)	5.12	0.84	6.10
glycine (Gly)	3.75	0.64	5.86
glutamic acid (Glu)	21.45	2.89	7.42
histidine (His)	2.13	0.28	7.59
arginine (Arg)	6.93	0.9	7.70
alanine (Ala)	4.08	0.62	6.59
proline (Pro)	4.08	0.61	6.70
tyrosine (Tyr)	3.37	0.47	7.17
cystine (Cys)	1.77	0.32	5.52
total	87	12.35	7.04

Table 3. Comparison of Amino Acid Compositions in Oat Protein Isolate and Corresponding FAO/WHO Requirements

amino acids	FAO/WHO	oat proteins
lysine	5.5	4.0
threonine	4.0	3.0
tryptophan	1.0	
methionine	3.5	1.8
phenylalanine	6.0	6.0
leucine	7.0	8.1
isoleucine	4.0	4.0
valine	5.0	5.4

for only 72.7% and 51.2% of the lysine and methionine required for children as recommended by FAO/WHO (*21*) (see **Table 3**).

SDS−PAGE. Figure 1a shows the SDS−PAGE results of protein standards and oat protein isolate. Six bands of OPI with protein molecular weights ranging from 14 kDa to 66 kDa were observed in SDS−PAGE. The analysis of the distribution of protein components analyzed from **Figure 1a** was plotted in **Figure 1b**. Our results indicated that the protein compositions (from high molecular weight to low molecular weight) calculated from the areas of **Figure 1b** were approximately: (1) 2%; (2) 3%;

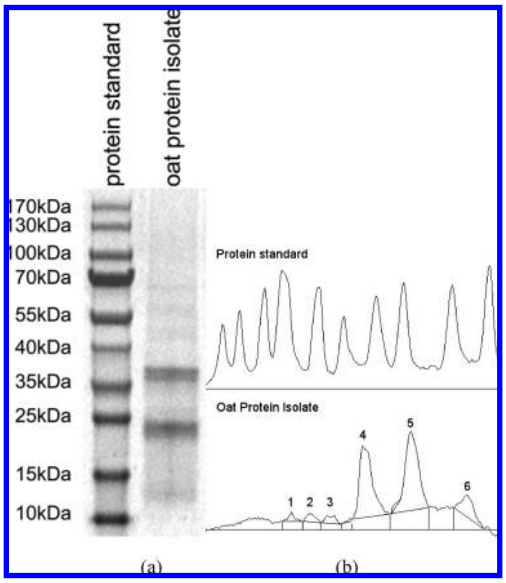

Figure 1. (**a**) SDS−PAGE results of oat protein isolate and protein standard markers and (**b**) distribution of protein components analyzed from a. Protein compositions (from high molecular weight to low molecular weight) calculated from the areas of b are (1) 2%; (2) 3%; (3) 3%; (4) 38%; (5) 43%; and (6) 11%. *x*-Axis and *y*-axis of b are molecular weight and absorbance, respectively.

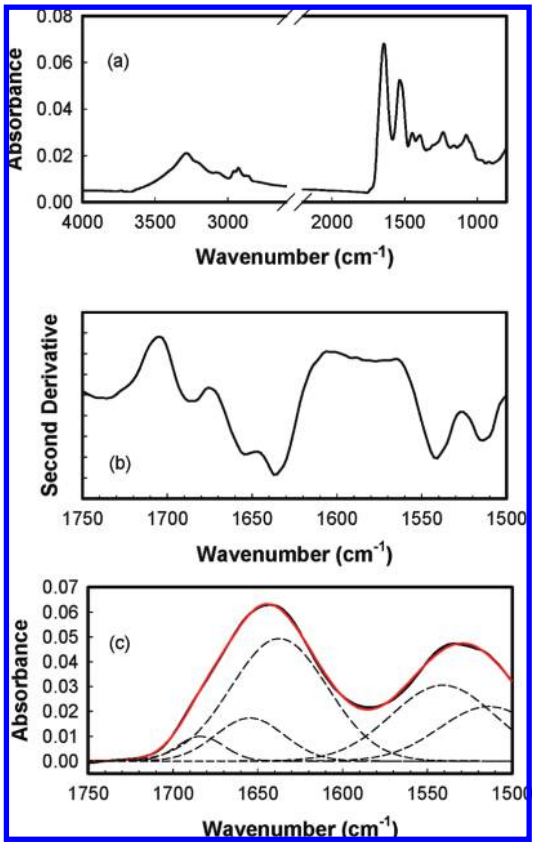

Figure 2. (**a**) FTIR spectrum of oat protein isolate (OPI); (**b**) second derivative FTIR spectrum of the amide I and II bands of OPI; and (**c**) the best fit for the self-deconvoluted FTIR spectrum using nonlinear regression analysis. Amide I and II bands were fitted with Gaussian functions using peak positions obtained from second derivative analysis.

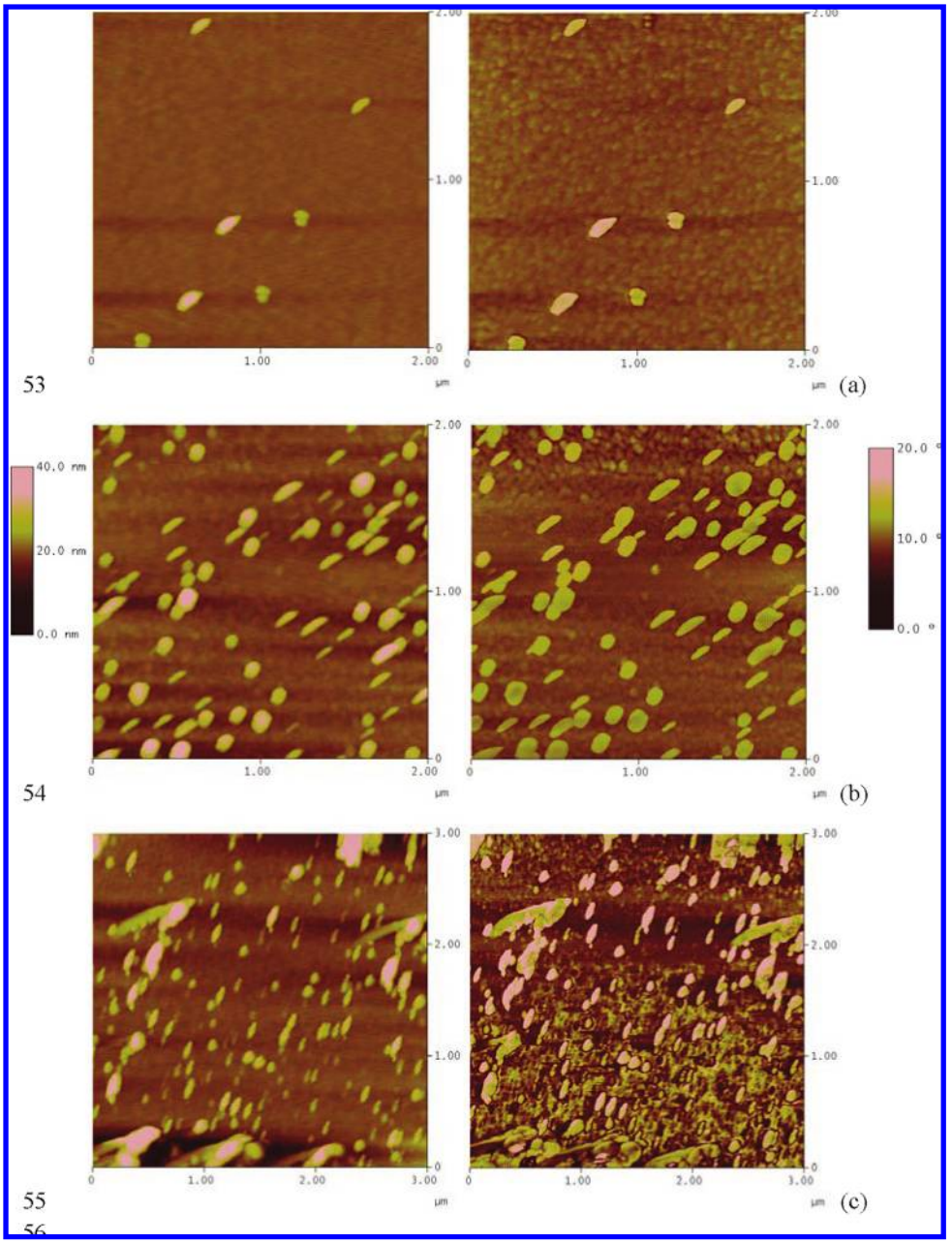

Figure 3. Tapping mode atomic force microscopy (AFM) images of oat protein isolate at different protein concentrations: (**a**) 0.1 mg/mL; (**b**) 0.5 mg/mL; and (**c**) 1.0 mg/mL. On the left are height images, and on the right are phase images. Panels a and b have scan sizes of 2 μm × 2 μm, while c has a scan size of 3 μm × 3 μm.

(3) 3%; (4) 38%; (5) 43%; and (6) 11%. Here, bands 1 to 6 correspond to proteins or peptides with molecular weights (MW) of ~66.2 kDa, ~57.0 kDa, ~45.0, ~36.0 kDa, ~22.0 kDa, and ~14.4 kDa. The dominant two bands were proteins with MWs of ~36.0 kDa (#4) and ~22.0 kDa (#5), which contributed to ~80% of the total protein content. The peptides with a MW lower than 10,000 might also exist. However, they are too small to be detected under the current electrophoresis conditions. Previous research suggested that globulin was the major protein component in oat proteins extracted from oat flour (*20*, *22*, *23*).

Fourier Transform Infrared Spectroscopy. Figure 2a shows the FTIR spectrum of oat protein isolate. The peaks of amide I and amide II, which were located at 1641 and 1532 cm^{-1}, respectively, indicate the typical structure of a protein. The peaks located at ~3300 cm^{-1} and 2930 cm^{-1} correspond to the N−H stretching band and alkyl group stretching bands, respectively. The existence of hydrophobic functional groups such as the alkyl group partially contributed to the associative behavior of OPI.

The amide I band is very important to the high-level structure analysis of a protein. The amide I band itself suggests the stretching vibration of C═O, which belongs to the amide groups weakly coupled with in-plane NH bending and CN stretching (*24*). Many researchers utilized Fourier self-deconvolution to find out the individual component peaks hidden within a broadband (*13*, *14*, *25*). The amide band I is an ideal wavenumber range suitable for protein secondary structure analysis since the amide I band of a protein is a complex composite that consists of a number of components including α-helix, β-sheet, random conformations, and β-turns. **Figure 2b** and **c** exhibit the second derivative plot and the Fourier deconvolution plot of amide I peak of oat protein isolate. Three component bands that belong to the amide I band and are located at 1683.6, 1654.6, and 1637.3 cm^{-1}, respectively, were identified through the

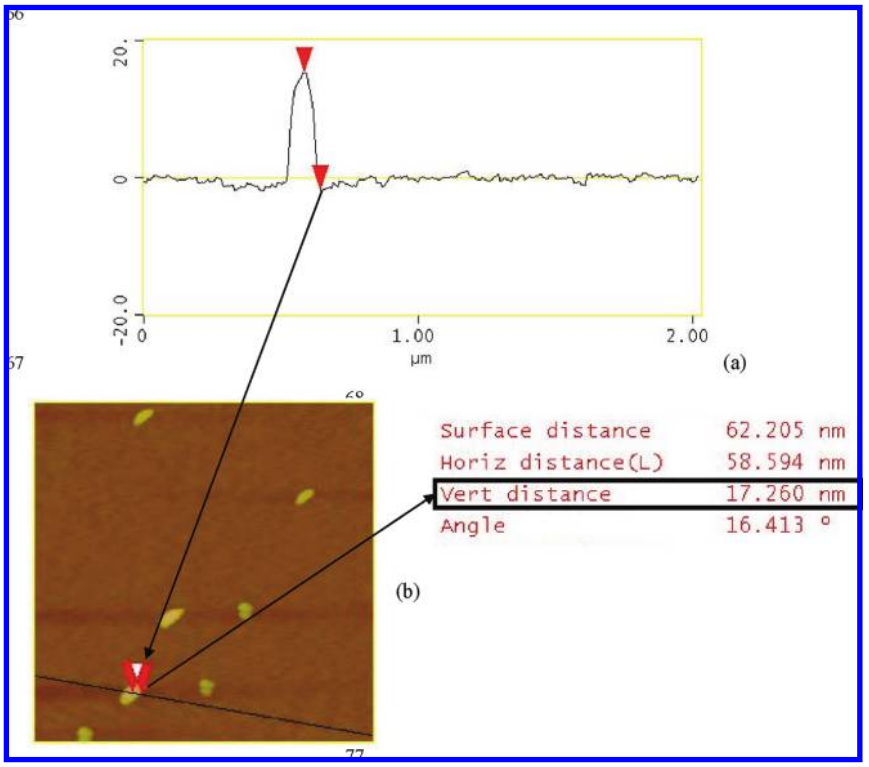

Figure 4. Section analysis of AFM height image of protein particles formed by 0.1 mg/mL OPI water solution: (**a**) section analysis plot of a selected protein; and (**b**) AFM height image. The scan size is 2 μm × 2 μm, and the *z* scale is 40 nm.

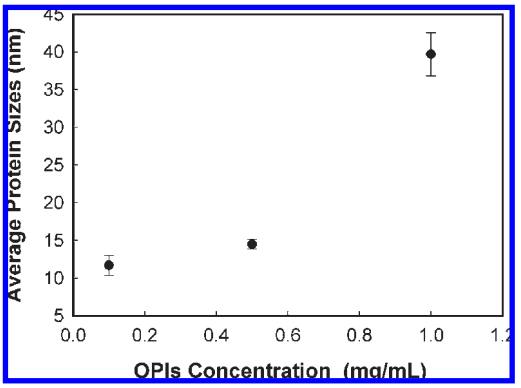

Figure 5. Plot of average protein sizes of oat protein isolate (OPI) versus OPI concentration.

analysis of its second derivative. FTIR is well established as the method for the analysis of protein secondary structure. Previous research suggested the following secondary structures in the amide I region (*13*): β-turn, 1660−1700 cm^{-1}; α-helix, 1650−1656 cm^{-1}; irregular structure, 1640−1644 cm^{-1}; and β-sheet or extended structure, 1620−1640 cm^{-1}. Therefore, the three peaks shown in the amide I region of **Figure 2b** apparently represent the β-turn, α-helix, and β-sheet conformations, respectively (*13*, *14*).

The estimation of the secondary structural elements of OPI can be achieved by the curving fitting of the amide I band in the FTIR spectrum shown in **Figure 2a**. Because of the partial overlap of amide I and amide II bands, we fitted both amide I and II bands with multiple Gaussian peaks, but only the peaks under the amide I band were used in the calculation of protein secondary structure. Two bands that belong to the amide II band were found to be located at 1540.8 and 1513.8 cm^{-1}. **Figure 2c** shows the best fit for the self-deconvoluted FTIR spectrum using nonlinear regression analysis. According to the calculation of the individual component peak area, we can obtain the composition of oat protein secondary structure, which includes approximately 7% β-turn, 19% α-helix, and 74% β-sheet.

Atomic Force Microscopy (AFM). AFM is a powerful tool that can provide topographic images of proteins in ambient atmosphere (*16*). AFM has the unique ability of providing the readers with direct images of OPI at both isolated and aggregated states. **Figure 3** exhibits the height (related to surface morphology) and phase (originated from the difference in mechanical properties) images of oat protein isolate cast from solutions with different protein concentrations. It is found that the self-assembly of individual oat protein isolate could be viewed as either disk-like or ellipsoidal at oat protein concentrations as low as 0.1 mg/mL, as shown in **Figure 3a**. With the increase of protein concentration to 0.5 mg/mL, although the number of protein particles increased significantly as evidenced by the crowdedness of particles in **Figure 3b**, the image is still dominated by individual protein particles with both disk-like and ellipsoidal shapes, as determined by AFM section analysis. For a protein containing three axis lengths, *a* (*x*-axis), *b* (*y*-axis), and *c* (*z*-axis), when $a = b = c$, it is called a spherical protein; when $a = b > c$, it is called disk-like; and when $a > b > c$, it is called ellipsoidal. Certainly, some of these protein particles started to contact each other, which is an indication of protein aggregation. Significant protein aggregation occurs when the oat protein concentration increases to 1 mg/mL, as shown in **Figure 3c**. The increase in oat protein concentration affects the stability of oat protein in aqueous solution and promotes the self-assembly of oat proteins, causing the association of neighboring proteins to form large aggregates.

The sizes of self-assembled protein particles were further analyzed by AFM section analysis. The proteins of different concentrations were spin-coated onto clean silicon wafer surfaces, and dried under nitrogen flow. In this article, the vertical distance from the top of the protein particle to the substrate surface is

regarded as the particle size of oat protein particles in the dried state because it is the value that can well describe the aggregation state of a protein. **Figure 4** shows the image of a typical section analysis of protein particles formed by 0.1 mg/mL oat protein isolate water solution. The size of each individual protein particle self-assembly on the silicon wafer can be selected and measured. The section analysis image shows that the biggest size of individual self-assembled OPI is around 17.3 nm, as shown in **Figure 4**. The sizes of all of the protein particles were selected and measured using the same section analysis method and then averaged to obtain the average protein sizes. **Figure 5** shows the average sizes of self-assembled oat protein isolate as a function of oat protein concentration. One noted that at protein concentrations up to 0.5 mg/mL, the average protein sizes increased slightly from ~12.1 nm at 0.1 mg/mL to ~14.5 nm at 0.5 mg/mL. Further increase of protein concentration to 1.0 mg/mL caused a significant increase in protein sizes. In addition, the standard deviation of average protein sizes is much larger at high concentration (i.e., 1.0 mg/mL) than at concentrations lower than 0.5 mg/mL, suggesting the heterogeneous distribution of protein sizes at high protein concentrations. The larger protein sizes at 1.0 mg/mL protein concentration are mainly due to the formation of large protein aggregates, as clearly evidenced in the AFM height and phase images shown in **Figure 3c**. It should be mentioned that although dynamic light scattering (DLS) has been routinely used to obtain the hydrodynamic radius of a protein, it is mainly limited to spherical proteins. There are several limitations of using DLS to determine the size of OPI: (1) OPI is ellipsoidal rather than spherical; (2) because OPI is an associative protein in aqueous solution, it will be very difficult to use DLS to measure the size of individual OPIs. The other methods that can provide the information of size and shape of a protein include small-angle X-ray scattering (SAXS) and small-angle neutron scattering (SANS) (*26*). Research regarding the use of SAXS to study the structure and conformation of OPI at different OPI concentrations is still ongoing in our laboratory.

Conclusions. In summary, we have determined the amino acid compositions, secondary structure, and self-assembly properties of the oat protein isolate. Results from the present study indicated that oats could be a good nutritional source of protein ingredients for food processing because they contained many essential amino acids needed by human beings. The secondary structure of the oat protein isolate contains approximately 7% β-turn, 19% α-helix, and 74% β-sheet. Furthermore, AFM results provided direct images of oat protein isolate self-assembled at different protein concentrations. At protein concentrations lower than 0.5 mg/mL, the proteins mainly exist in isolate form with either disk-like or ellipsoidal shape. The average individual protein sizes of OPI are between 12 to 15 nm. Further increase of protein concentration to 1.0 mg/mL caused the formation of large and heterogeneous protein aggregates. Detailed studies of the effects of pH values and concentrations on the size and shape of OPI as well as the number of OPI in each aggregate using synchrotron small-angle X-ray scattering are still ongoing. This research provides the general methodology of studying the structure and conformation of new food proteins.

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