Purification and Partial Characterization of Oat Bran Globulin

Ifendu A. Nnanna* and Smiti V. Gupta

Department of Nutrition and Food Science, Room 3009 Science Hall, Wayne State University, Detroit, Michigan 48202

Oat bran protein isolate was fractionated into Osborne fractions: albumin, globulin, prolamin, and glutelin. Globulin and glutelin were the major fractions. The oat bran globulin (OBG) fraction was purified by ammonium sulfate [$(NH_4)_2SO_4$, 30−80% saturation] precipitation. Purification was achieved at 50% saturation of $(NH_4)_2SO_4$. Purified globulin was analyzed by gel filtration, sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE), and circular dichroism (CD) to determine molecular size, subunit molecular weights, and secondary strucure, respectively. Disulfide linkage (−S−S−) and sulfhydryl (SH) contents were also estimated. Apparent molecular weight of globulin was ∼330 kDa. SDS−PAGE showed two broad bands, one in the molecular range between 22 and 31 kDa and the other, between 34 and 42 kDa. CD spectra of OBG in phosphate buffer (0.4 M NaCl, pH 7.6) showed that it conformed to a helical structure of ∼50%, 43% β-structure, and a low percentage of turns and random coil. In the presence of urea (8 M), conformation was predominantly random coil (∼82%) and resembled that of soy 7S globulin. SH content and −S−S− linkages were estimated as 1.0 and 2.1 μmol/g of protein, respectively.

Keywords: *Oat bran globulin; protein purification; secondary structure; circular dichroism*

INTRODUCTION

Our knowledge of oat proteins is substantially less than that of wheat, corn, barley, or rice (Peterson and Brinegar, 1986). Recently, we have focused on isolation and characterization of oat bran macromolecules, gum, and protein (Dawkins and Nnanna, 1993, 1994; Nnanna et al., 1993; Nnanna and Dawkins, 1992, 1994, 1996). Oat bran is essentially the outer covering of the groat (dehulled oat) and is produced by sieving coarsely ground oat flour. Oat bran is used mostly as breakfast cereals and as a rich source of water-soluble dietary fiber (oat gum). The protein content of oat bran is high (∼19−23%), higher than those found in most common cereals, including those of typical oat products (rolled oats, quick oats, baby oat flakes, oat groat flour, etc.).

Several researchers have characterized oat flour proteins concerning concentrates (Cluskey et al., 1973; Ma, 1983), isolates (Ma and Khanzada, 1987), globulin (Peterson, 1978; Brinegar and Peterson, 1982; Ma et al., 1990), and prolamin (Kim et al., 1978). Recently, functional properties of modified oat proteins have also been studied (Ma and Khanzada, 1987; Ma and Wood, 1987). Peterson (1978) was probably the first to extensively characterize oat globulin regarding physical and biochemical properties. By analytical ultracentrifugation techniques, Peterson (1978) estimated a molecular weight of 322 000 and a sedimentation constant of 12.1 for globulin from oat groat flour. The globulin consisted of six each of acidic (α, pI 5.9−7.2) and basic (β, pI 8.7−9.2) polypeptides of subunit molecular weights of 32 500−37 500 and 22 000−24 000, respectively (Brinegar and Peterson, 1982). The two groups of polypeptides showed substantial heterogeneity. Brinegar and Peterson (1982) noted that similarities existed between oat globulin and the legumin (11S) class of storage proteins in certain legumes. The native molecular structure of oat globulin is now thought to be a hexamer of the disulfide linked aβ-species, that is, $(\alpha\beta)_6$ (Peterson and Brinegar, 1986).

It is known that oat globulin fractions have distinctive amino acid composition (Peterson and Brinegar, 1986). The superior amino acid balance of oats results from the fact that the more balanced globulin fraction predominates (Peterson and Brinegar, 1986). These authors previously observed that the globulin β-polypeptides are water-soluble when separated from the α-polypeptides and contain significantly more lysine and methionine (Brinegar and Peterson, 1982). These suggest a potential functional and nutritional application of oat globulin.

Unlike oat flour proteins, oat bran protein and its Osborne fractions, albumin, globulin, prolamin, and glutelin, have received little research attention. More research is needed to increase our knowledge of these protein types. Information from such effort would provide added-value to oat bran, especially now that oat bran is increasingly used in formulation of low-calorie/fat foods. In this present work, we focused on oat bran globulin (OBG) and the following key questions were addressed. First, is the distribution of Osborne fractions in oat bran different from that reported for oat groat flour? Second, is the subunit composition of OBG basically the same as reported for oat globulin? Third, what is the conformation of OBG, and how does it differ from the native conformation of soy 7S globulin? Soy 7S globulin, instead of 11S globulin, was chosen as a reference protein for the following reasons. Glycosylation of soy 7S globulin is 5-fold that of soy 11S, and this is believed to explain why 7S is a better emulsifier than 11S. These properties of soy 7S make it a suitable model to support our on-going protein modification effort, to better understand the protein structure−function relationship, and to clearly observe the effects of denaturants on OBG.

The objective of this paper was to purify and partially characterize oat bran globulin to obtain information

* Author to whom correspondence should be addressed [current address: Monsanto Co., 700 Chesterfield Parkway N., Mail Zone AA2G, St. Louis, MO 63198; telephone, (314) 537-6018; fax, (314) 537-6759; e-mail, iannan@ccmail.monsanto.com].

S0021-8561(96)00269-5 CCC: $12.00

regarding Osborne fractions distribution, subunit composition, molecular size, and secondary structure. Information from this study should enable quantitative and qualitative comparison of oat bran globulin with that from whole oat flour as well as other cereal and legume sources. To our knowledge, the secondary structure of oat globulin has not been reported before.

MATERIALS AND METHODS

Extraction of Oat Bran Protein Isolate (OBPI). Oat bran flour (Quaker Oats Co., Chicago, IL) was ground to pass through 60 mesh (0.5 mm) sieve and stored (4 °C) until use. The ground powder was defatted by intermittent shaking with cold acetone at a 3:1 (v/w) solvent-to-solid ratio for 1 h at room temperature. The suspension was filtered; the solids were washed on a Buchner funnel with cold acetone and dried in vacuo at 60 °C. OBPI was extracted from oat bran by the method of Dawkins and Nnanna (1993). This was briefly (a) alkaline treatment of oat bran flour for removal of starch residue and (b) isoelectric precipitation of protein residue at pH 4.5. The protein content of the OBPI was determined by Kjeldahl's method (N × 6.25).

Fractionation of OBPI. OBPI was subjected to the Osborne fractionation scheme (Osborne, 1910) as modified by Peterson (1978), Kim et al. (1978), and Ma (1983). In the fractionation scheme, OBPI suspension (1:10, w/v, solid-to-solvent ratio) was sequentially extracted with water (pH 7.0), salt solution (1 M NaCl, 0.05 M Tris, pH 8.5), ethanol (52%, v/v), and dilute alkali (0.05 M NaOH) to yield albumin, globulin, prolamin, and glutelin, respectively. Each fraction was extracted at a 1:10 (w/v) solid-to-solvent ratio. The protein content of the fractions was determined by Kjeldahl's method (N × 6.25).

Purification of Oat Bran Globulin (OBG). The globulin residue was subjected to selective purification with ammonium sulfate as described by Wolf et al. (1962). OBG was treated with saturated solutions (pH 8.5) of ammonium sulfate at levels of 30−80%. The suspensions were stirred for 1 h at room temperature followed by centrifugation (15000*g*, 4 °C, 20 min). The precipitated globulin was desalted at room temperature using Sephadex-25 (Sigma Chemical Co., St Louis, MO) packed in a 1.5 × 12 cm column (Bio-Rad Laboratories, Richmond, CA). The desalted globulin samples were freeze-dried and their protein contents determined by Kjeldahl's method (N × 6.25).

Molecular Weight Determination by Gel Filtration Chromatography. Apparent molecular weight of OBG was determined at room temperature using a Pharmacia column (2.5 × 90 cm) packed with Sepharose CL-6B gel (Sigma Chemical Co., St Louis, MO). Two milligrams of purified OBG or standard protein was dissolved in 1 mL of 0.05 M Tris/0.3 M NaCl buffer (pH 8.5), applied onto a pre-equilibrated and calibrated (blue dextran) column, and eluted at 18 mL/h with downward flow. The elution solvent was 0.05 M Tris/0.3 M NaCl buffer (pH 8.5). Five milliliter fractions were collected using an ISCO Retriever II fraction collector (ISCO, Lincoln, NE). The eluates were monitored continuously at 280 nm by an ISCO model UV-5 detector equipped with a flow cell of 2 mm path length. The molecular weight of OBG was estimated using a standard curve (Andrew, 1964). The following reference proteins (Sigma Chemical Co.) were used to establish the standard curve: cytochrome *c* (12.4 kDa), carbonic anhydrase (29 kDa), bovine serum albumin (66 kDa), alcohol dehydrogenase (150 kDa), *β*-amylase (200 kDa), apoferritin (443 kDa), and thyroglobin (669 kDa).

Sodium Dodecyl Sulfate−Polyacrylamide Gel Electrophoresis (SDS−PAGE). OBG was analyzed by SDS−PAGE under reducing conditions (2-mercaptoethanol) using the discontinuous system (12% separating/4% stacking gels) described by Laemmli (1970). Gel slabs were calibrated with the following "low molecular weight" protein standards (Bio-Rad Laboratories, Richmond, CA): phosphorylase *b* (97 kDa), bovine serum albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (31 kDa), soybean trypsin inhibitor (22 kDa), and lysozyme (14 kDa).

Determination of S−S and S−H Content in OBG. Sulfhydryl (SH) groups and disulfide (S−S) linkages in OBG were determined by Ellman's method (Ellman, 1959) as described by Beveridge et al. (1974), for the determination of free SH and S−S groups in skim milk proteins. In general, Ellman's reagent [5,5′-dithiobis(2-nitrobenzoic acid), DTNB] reacts with 1 mol of SH at pH 8.1 to give 1 mol of the colored thio anion (3-carboxylato-4-nitrophenolate, CNT), which has a maximum absorbance at 412 nm. The number of disulfide (S−S) linkages can be determined by incubating the protein solution with 2-mercaptoethanol prior to the treatment with DTNB.

Determination of Secondary Structure by Circular Dichroism (CD). Circular dichroism measurements were carried out with a JASCO 600 recording spectropolarimeter equipped with a data processor for CD (SOFTSEC, JASCO Inc., Easton, MD). The procedure reported by Koshiyama and Fukushima (1973) was followed. The analysis was carried out on purified globulin samples dissolved in a phosphate buffer (pH 7.6, 0.4 M NaCl). A 0.2 mm quartz cell, with 0.1% globulin in the phosphate buffer, was used for obtaining the CD spectrum between 190 and 250 nm, whereas a 10 mm quartz cell (0.15% globulin) was used to obtain a spectrum between the 250 and 320 nm region. The change in secondary structure upon the addition of 8 M urea and 0.25% sodium dodecyl sulfate was also studied. In addition, a purified soy 7S globulin (86.8% 7S and 13.2% 11S; gift from Dr. W. F. Wolf, USDA, Peoria, IL) was analyzed by CD for procedure validation and comparison purposes. The CD spectra were expressed in terms of molecular ellipticity, *q*. The secondary structure fractions (helix, *β*-form, and random coil) of OBG were derived using a linear combination of CD spectra of reference proteins of known secondary structures. The details of the data processing and interpretation are presented elsewhere (Yang et al., 1986).

RESULTS AND DISCUSSION

Fractionation and Purification. Osborne fractionation is used as an initial step in separation of cereal proteins. In the literature, Osborne fractions derived from different cereals are often designated by special names (Belitz and Grosch, 1896). The various designations may result in confusion and incorrect conclusions about protein homogeneity. Therefore, it has been suggested to use the general designations of the Osborne fractions and specify the protein source (Belitz and Grosch, 1896), for example, wheat glutelin instead of glutelin. Accordingly, the protein type we fractionated from oat bran is conveniently designated oat bran globulin (OBG). OBG should not be confused with globulin from oat groat flour; the latter we have referred to simply as oat globulin throughout the text.

Accurate determinations of the quantity of globulin in seed storage proteins require complete and exclusive extraction; the Osborne method does not meet this requirement (Peterson, 1990). This may partly explain why there is a disagreement on the proportion of oat seed protein that is globulin (Peterson, 1990). For this reason, we used combined modified methods of Peterson (1978), Kim et al. (1978), and Ma (1983) with a view to improve extraction efficiency of the Osborne fractions. The modified methods of Peterson (1 M NaCl, 0.05 M Tris, pH 8.5) and Kim [52% (v/v) or 45% (w/w) ethanol] were shown by these authors to maximally extract globulin and prolamin, respectively, from oat flour.

Fractionation of oat bran protein by the modified method yielded glutelin and globulin as the two major Osborne fractions. The water-soluble albumins and the alcohol-soluble prolamins were present in very small

Table 1. Distribution and Purity of Osborne Fractions of OBPI

Osborne fraction	OBPI[a] (%)	protein (N × 6.25)[a] (%)
albumin	2.1 ± 0.5	47.2 ± 0.8
globulin	23.0 ± 1.2	70.0 ± 1.5
prolamin	1.1 ± 0.75	94.0 ± 1.1
glutelin	62.5 ± 2.5	78.5 ± 1.8
residue	11.3 ± 1.7	28.6 ± 1.3

[a] Average of at least two determinations; percentages represent the ratio of the dried (freeze-dried) extracted fractions and original starting OBPI.

Table 2. Protein Content of OBG Treated with Ammonium Sulfate

ammonium sulfate (%)	protein (%)[a]	ammonium sulfate (%)	protein (%)[a]
00	70.0 ± 1.5	60	87.0 ± 2.1
30	88.5 ± 2.5	70	85.5 ± 1.2
50	93.8 ± 1.5	80	81.2 ± 2.0

[a] Average of at least two determinations.

amounts (Table 1). Glutelin was the predominant fraction (63%), whereas globulin was only 23% of oat bran protein. Repeated fractionation of oat bran protein in our laboratory produced the same distribution as seen in Table 1. This is in contrast to earlier reports (Peterson, 1978; Brinegar and Peterson, 1982; Ma, 1983) that showed globulin as the predominant protein fraction in oat seed protein followed by glutelin. Two inferences can be drawn from these results. First, the distribution of globulin and glutelin in oat bran protein may indeed differ from that found in oat groat flour. Second, it is plausible that some globulin escaped fractionation and ended up in the glutelin fraction. The implication of the latter is that the fraction we assigned as glutelin may contain globulin that escaped salt solubilization, thus leading to overestimation.

Table 1 also shows the nitrogen content of the protein fractions. The prolamin fraction was small (1.1%) and relatively pure (~94%). All workers agree that the prolamin (avenin) content of oats is low, with estimates ranging from about 4 to 14% of total protein (Peterson and Brinegar, 1986). Globulin showed a protein content of about 70%. Further purification of the globulin fraction was required for structural analysis. The purification result follows.

Table 2 shows protein concentrations at various levels of ammonium sulfate saturations. Protein purity increased with an increase in the amount of ammonium sulfate up to a 50% saturation level, which yielded globulin of 94% purity. A further increase in the amount of ammonium sulfate showed a decrease in protein percentage.

Molecular Weight and Subunit Composition. Gel filtration chromatograms of the crude and purified globulin are presented in Figure 1. The large major peak for purified OBG corresponds to a molecular weight of 330 kDa. Our molecular weight estimate for oat bran globulin is consistent with that reported by Peterson (1978) and Ma and Khanzada (1987) for globulin from oat flour. By analytical ultracentrifugation, Peterson (1978) reported a molecular weight of 320 kDa for native oat seed globulin. Ma and Khanzada (1987), by gel filtration, estimated a molecular weight of 350 kDa for globulin from oat groats. In our study, a small peak, corresponding to a molecular weight of ~10 kDa, was also observed. We suspect this small

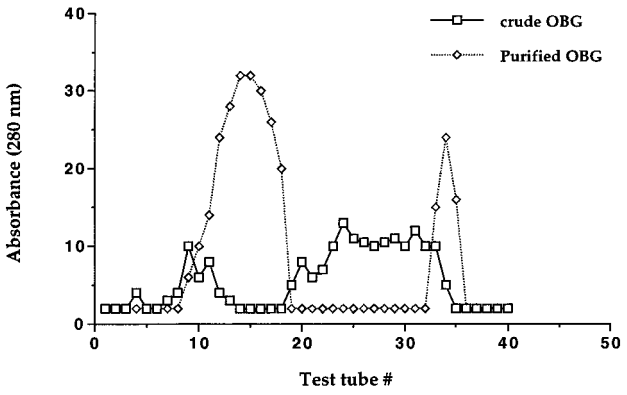

Figure 1. Gel filtration chromatography of crude and purified oat bran globulin (1 mg/mL) on Sepharose CL-6B column (2.5 × 90 cm). Blue dextran (MW = 2×10^6) and cytochrome *c* (MW = 12 400) were used to determine void and total volumes of the column, respectively. Conditions: flow rate, 18 mL/h; temperature, ~25 °C; elution medium, 0.05 M Tris/0.3 M NaCl buffer (pH 8.5) containing 0.02% sodium azide. Data points are means of duplicate runs.

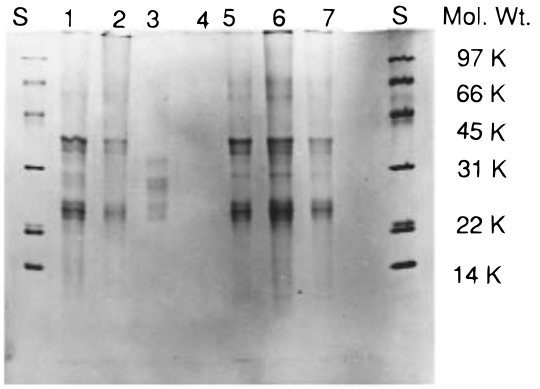

Figure 2. SDS−PAGE patterns of oat bran proteins: S, standard proteins (phosphorylase *b*, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor, and lysozyme); lanes 1−7, respectively, oat bran protein isolate, glutelin, prolamin, albumin, OBG purified by 30% ammonium sulfate, OBG purified by 50% ammonium sulfate and crude OBG fraction.

peak was a contaminant, perhaps a residual albumin fraction.

Figure 2 shows SDS−PAGE patterns of oat bran proteins. Obviously, the majority of the polypeptide groups in oat bran (lanes 1−7) is within the molecular weight range of 22−36 kDa. Protein concentrates from groats of oat cultivars, Hinoat and Sentinel, show major bands in the same molecular weight range (Ma, 1983). This suggests similarity in subunit composition of oat bran proteins and proteins from oat groat flour. As revealed in Figure 2, the glutelin and prolamin fractions showed bands of relatively reduced intensity and were not as prominent as expected, considering that glutelin was the predominant fraction in oat bran and prolamin was in pure form (Table 1). The major bands in lane 2 appear identical with those in lanes 1 and 5−7, making it difficult to conclude that the bands in lane 2 unequivocally belong to glutelin. Globulin polypeptides have been reported to occur in water-soluble (albumin) and residue (glutelin) fractions besides the salt-extracted fraction (Peterson and Brinegar, 1986). Whether this occurred here is uncertain. However, it is certain that the water-soluble fraction was void of globulin. Surprisingly, the albumin fraction showed no bands in the molecular weight range investigated.

Table 3. Sulfhydryl (SH) and Disulfide (−S−S−) Content of OBG

protein sample	−S−S− linkage (mol/g of protein)	SH group (mol/g of protein)
OBG	2.8	0.9
oat globulin[a]	2.1	1.0

[a] Peterson and Brinegar (1982).

The effectiveness of the OBG purification is evident when lane 7 (crude OBG), lane 5 [30% $(NH_4)_2SO_4$ purification], and lane 6 [30% $(NH_4)_2SO_4$ purification] are compared. OBG bands in lane 7 are relatively faint; those in lanes 5 and 6 are very intense as expected. SDS−PAGE showed two distinct polypeptide groups for OBG (lanes 5 and 6), one in the molecular range between 34 and 38 kDa and the other, between 22 and 24 kDa. Each polypeptide group consists of unresolved bands. The OBG patterns have close resemblance to those reported for oat globulin by Brinegar and Peterson (1982) although the molecular weights differ slightly. Polypeptide bands showed by Brinegar and Peterson (1982) were well resolved by gradient gel, a technique that was not applied in our study. In addition, Brinegar and Peterson (1982) characterized the resolved polypeptides by two-dimensional electrophoresis (isoelectric focusing followed by SDS−PAGE) to determine acidic and basic types. By their nomenclature, the two polypeptide groups, 34−38 and 22−24 kDa (Figure 2), we found in OBG correspond to acidic (α) and basic (β) groups, respectively. These authors confirmed that the α- and β-polypeptides were linked by disulfide bonds.

To further verify the similarity between OBG and globulin from oat groat flour, we determined the concentration of disulfide bonds and sulfhydryl groups in purified OBG. As shown in Table 3, approximately 1 free sulfhydryl and 2.8 disulfides per molecule of OBG were determined. This suggests the presence of at least one disulfide bond between the α- and β-subunits. Peterson and Brinegar (1982) reported 2.1 and 1.0 for disulfide bonds and sulfhydryl group, respectively, for oat globulin. The native molecular structure of oat globulin is now thought to be a hexamer of the disulfide linked αβ-species, that is, $(\alpha\beta)_6$ (Peterson and Brinegar, 1986).

On the basis of the −S−S−/SH data and SDS−PAGE patterns, a hexameric structure is likely for OBG, analogous to that proposed for oat globulin by Brinegar and Peterson (1982). However, we found glutelin, instead of globulin, to be the major fraction in oat bran. This requires more research to validate.

Secondary Structure Analysis. Circular dichroic (CD) spectral properties of OBG in the far-ultraviolet (UV; <250 nm) and near-UV (250−320 nm) regions are presented in Figures 3 and 4, respectively. To validate our procedure and for comparison purposes, we also studied the CD of soy 7S globulin, a well-characterized (Koshiyama and Fukushima, 1973) globular protein. As evident from Figure 3, OBG, in phosphate buffer, showed a negative maximum at about 207−208 nm, a spectrum associated with an α-helical conformation. Far-UV CD spectrum of a protein is sensitive to its main-chain conformation and distinguishes between α-helix, β-sheet, and random coil structures. The α-helix makes a dominant contribution with its negative CD bands at 208 and 222 nm and its positive band at 192 nm (Stryer, 1995). The OBG spectrum (Figure 3A) is consistent with this general features of α-helices. Also, the OBG spectrum exhibits a very shallow trough,

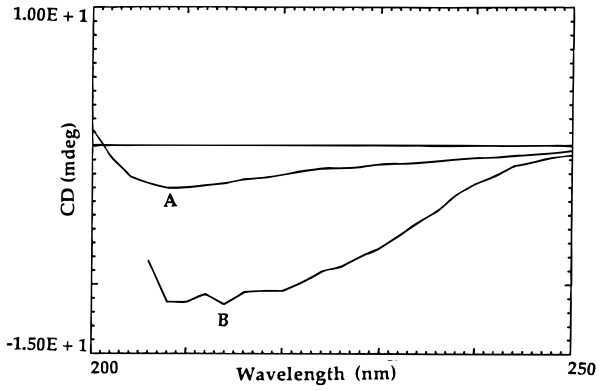

Figure 3. Far-UV CD spectra of OBG (A) and soy 7S (B) at 0.1% concentration in phosphate buffer (pH 7.6, 0.4 M NaCl). A 0.2 mm quartz cell was used.

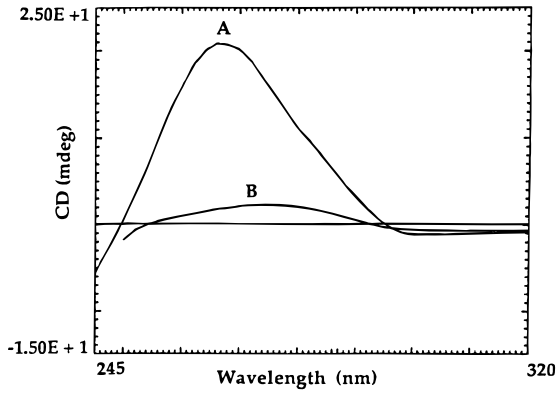

Figure 4. Near-UV CD spectra of OBG (A) and soy 7S (B) at 0.15% concentration in phosphate buffer (pH 7.6, 0.4 M NaCl). A 10 mm quartz cell was used.

whereas the soy 7S globulin spectrum (Figure 3B) depicts a deep trough, indicative of a protein with a large percentage of random coiling. In fact, the soy 7S spectrum remarkably resembles that reported by Koshiyama and Fukushima (1973). The authors reported a large absorption minimum in the CD spectrum near 210 nm, with a small shoulder at 218 nm. Lewis and Chen (1979) also reported a large minimum at 216−217 nm and a shoulder at 222 nm for soy 7S. These authors concluded that soy 7S possessed primarily random coil and β-structure. Indeed, our results (Figure 3 and Table 4) lead to the same conclusion.

The near-UV (250−320 nm) spectrum arises principally from aromatic and disulfide chromophores (Hashizume et al., 1967). Figure 4 shows that OBG absorbed greatly in this region with a maximum between 260 and 270 nm. This perhaps reflects the higher content of aromatic amino acid residues in OBG compared to soy 7S. Soy 7S has been noted to show broad positive bands extending over the wavelengths between 265 and 295 nm, presumably due to tryptophyl and tyrosyl residues (Koshiyama and Fukushima, 1973).

The effect of urea or SDS on the helical structure of OBG is reflected in the respective curve characters (Figure 5). If the curve due to urea (U) is extended manually, a deep trough would result in the lower wavelength region. We were unable to observe the full spectrum due to instrumental constraints. Nevertheless, this tendency of the OBG spectrum toward the lower wavelength region, when in the presence of urea, suggests that hydrogen-bonded structures contribute significantly to the conformation of OBG. Table 4 shows the magnitude of the effect of SDS and urea on OBG

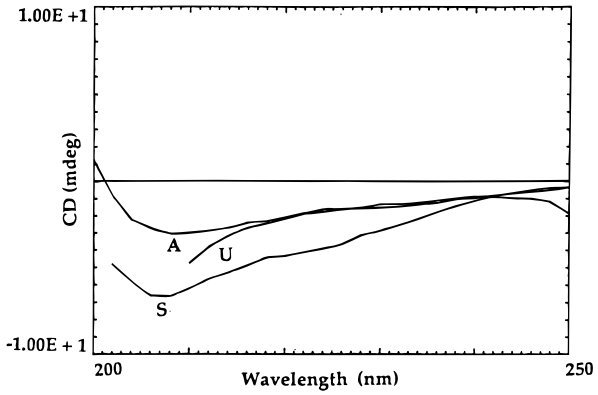

Figure 5. Change in absorption in the far-UV CD spectra of OBG (A) (0.1%) due to the effect of 0.25% SDS (S) or 8 M urea (U). SDS and urea were incorporated into phosphate buffer (pH 7.6, 0.4 M NaCl). A 0.2 mm quartz cell was used.

Table 4. Secondary Structure Components of OBG and Soy 7S Globulin

secondary structure[a]	OBG	OBG/SDS	OBG/urea	soy 7S
α-helix	+49.6	+25.0	−7.9	−14.5
β-sheet	+42.9	+18.3	−14.4	−2.6
turn	+16.1	+30.9	+40.3	+14.2
random	−8.6	+25.9	+82.0	+71.9

[a] Percentage of secondary structure types was estimated using a linear combination of CD spectra of reference proteins (at least five) of known secondary structures. Data were processed by SOFTSEC Program (JASCO, Inc.); details of the program are presented elsewhere (Yang et al., 1986).

secondary structure. SDS decreased the helical content of OBG with a corresponding increase in random coil. Urea denatured OBG and converted it from an α + β-structure to almost a random coil chain, a structure resembling that of soy 7S.

A mathematical computation of the CD spectra provided the data in Table 4. The data can be interpreted as follows. OBG is much more structured with a dominant α-helical component and consists of a high amount of β-sheets and negligible random coil. On the other hand, the large negative CD absorption by soy 7S conforms to a structure that is mainly random coiled. It is important to note that the estimation of β-sheet content is not as strongly reliable as those of α-helix. The reason: β-structures are less regular than the α-helices and contributes less to the CD spectrum (Stryer, 1995). However, if a CD spectrum of a protein shows a well-defined double minimum at 222 and 208 nm or a single minimum between 210 and 220 nm, the CD estimate of the amount of helix or β-sheet may be accepted with some confidence (Yang et al., 1986). The soy 7S spectrum not only satisfies these criteria but coincides with previous reports (Koshiyama and Fukushima, 1973; Lewis and Chen, 1979). For these reasons and the fact that CD measurement is an empirical technique, we feel soy 7S was a good choice to validate our procedure besides lending credence to the OBG spectrum.

Conclusion. Purification of OBG was achieved by 50% ammonium sulfate. The molecular weight estimate was ~330 kDa. The −S−S−/SH data and SDS−PAGE patterns suggest a hexameric structure for OBG, which is analogous to that proposed for oat globulin by Brinegar and Peterson (1982). However, we found glutelin, instead of globulin, to be the major fraction in oat bran. This requires more research to validate. OBG is much more structured with a dominant α-helical component and consists of a high amount of β-sheets and negligible random coil, a structure very distinct from soy 7S (a glycoprotein with a predominantly random coiled secondary structure).

LITERATURE CITED

Andrews, P. Estimation of the molecular weights of proteins by sephadex gel-filtration. *J. Biochem.* **1964**, *91*, 222.

Belitz, H. D.; Grosch, W. Cereals and cereal products. *Food Chemistry*; Springer-Verlag: New York, 1987; pp 499−500.

Beveridge, T.; Toma, S. J.; Nakai, S. Determination of SH and SS groups in some food proteins using Ellman's reagent. *J. Food Sci.* **1974**, *39*, 49.

Brinegar, A. C.; Peterson, D. M. Separation and characterization of oat globulin polypeptides. *Arch. Biochem. Biophys.* **1982**, *219*, 71.

Cluskey, J. E.; Wu, Y. E. V.; Wall, J. S.; Inglet, G. E. Oat bran concentrates from wet-milling process: preparation. *Cereal Chem.* **1973**, *50*, 475.

Dawkins, N.; Nnanna, I. A. Oat gum and β-glucan extraction from oat bran and rolled oats: temperature and pH effects. *J. Food Sci.* **1993**, *58*, 562.

Dawkins, N.; Nnanna, I. A. Studies on oat gum [(1-3, 1-4)-β-D-glucan]: composition, molecular weight estimation, and rheological properties. *Food Hydrocolloids* **1994**, *9*, 1.

Ellman, E. L. Tissue sulfhydryl groups. *Arch. Biochem. Biophys.* **1959**, *82*, 70.

Hashizume, H.; Shiraki, M.; Imahori, K. Study of circular dichroism of proteins and polypeptides in relation to their backbone and side chain conformations. *J. Biochem.* **1967**, *62*, 543.

Kim, S. I.; Charbonnier, L.; Mosse, J. Heterogeneity of avenin, the oat prolamin: fractionation, molecular weight and amino acid composition. *Biochim. Biophys. Acta* **1978**, *537*, 22.

Koshiyama, I.; Fukushima, D. Comparison of conformations of 7S and 11S soybean globulins by optical rotatory dispersion and circular dichroism studies. *Cereal Chem.* **1973**, *50*, 114.

Laemmeli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **1970**, *227*, 680.

Lewis, B. A.; Chen, J. H. Effect of conformation and structure changes induced by solvent and limited enzyme modification on the functionality of soy proteins. In *Functionality and Protein Structure*; Akiva, P.-El., Ed.; American Chemical Society: Washington, DC, 1979; pp 27−38.

Ma, C. Y. Chemical characterization and functionality assessment of protein concentrates from oats. *Cereal Chem.* **1983**, *60*, 36.

Ma, C. Y.; Khanzada, G. Functional properties of deamidated oat protein isolates. *J. Food Sci.* **1987**, *52*, 1583.

Ma, C. Y.; Wood, D. F. Functional prperties of oat proteins modified by acylation, trypsin hydrolysis or linoleate treatment. *J. Am. Oil Chem. Soc.* **1987**, *64*, 1726.

Ma, C. Y.; Harwalkar, V. R.; Paquet, A. Physicochemical properties of alkali-treated oat globulin. *J. Agric. Food Chem.* **1990**, *38*, 1707.

Nnanna, I. A.; Dawkins, N. Electrophoretic and functionality studies of protein isolated from oat bran. *Inst. Food Technol. Book Abstr.* **1992**, 163.

Nnanna, I. A.; Dawkins, N. Interaction and synergism of oat gum with other food gums: effect on viscosity and microstructure of oat gum. International Food Hydrocolloid Conference, Columbus, OH, Sept 6−10, 1994; Abstract B: 01.

Nnanna, I. A.; Dawkins, N. Adsorption-isotherm and effect of food gum blends on viscosity and microstructure of oat gum (β-D-glucan). *J. Food Sci.* **1996**, *61*, 121.

Nnanna, I. A.; Gupta, S.; Tallapaka, L.; Jenniton, E. Isolation and biochemical characterization of oat bran protein: isolate, globulin, and glutelin. *Inst. Food Technol. Book Abstr.* **1993**, 634.

Osborne, T. B. Die Pflanzenproteine Ergeb. *Physiology* **1910**, *10*, 47.
Peterson, D. M. Subunit structure and composition of oat seed globulin. *Plant Physiol.* **1978**, *62*, 506.
Peterson, D. M.; Brinegar, A. C. Oat storage proteins. In *Oats chemistry and technology*; Francis, H. W., Ed.; American Association of Cereal Chemists: St Paul, MN, 1986.
Stryer, L. Protein structure and function. In *Biochemistry*; Freeman: New York, 1995; p 63.
Wolf, W. J.; Babcock, G. E.; Smith, A. K. Purification and stability studies of the 11 S component of soybean proteins. *Arch. Biochem. Biophys.* **1962**, *99*, 265.
Yang, J. T.; Wu, C. C.; Martinez, H. M. Calculation of protein conformation from circular dichroism. *Methods Enzymol.* **1986**, *130*, 208.

Received for review April 18, 1996. Revised manuscript received August 20, 1996. Accepted August 20, 1996.[⊗]

JF960269V

[⊗] Abstract published in *Advance ACS Abstracts,* October 1, 1996.