Utilization of an Improved Methodology To Isolate *Lupinus albus* Conglutins in the Study of Their Sedimentation Coefficients

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Methods utilized frequently for the extraction of globulins from legume seeds lead to a considerable loss of these proteins in the albumin fraction due to a partial solubilization of globulins in the albumin extraction medium. The standard procedure to isolate legume seed globulins was modified by (i) including calcium and magnesium in the albumin extraction solution, which completely solubilizes the albumins free of contaminating globulins, and (ii) adding EDTA and EGTA to the globulin extraction solution, which efficiently extract the globulins. Using this modified methodology, *Lupinus albus* globulins were isolated and subsequently purified, and the main individual globulins, namely α-, *β*-, and *γ*-conglutins, were characterized. α- and *β*-conglutins have been routinely considered as the 11S (or legumin-like) and 7S (or vicilin-like), respectively, protein components of *L. albus* seeds. Using the total globulin fraction or the purified conglutins on isopycnic sucrose or glycerol density gradient centrifugation performed under low or high ionic strength and on gel filtration techniques, it is proposed that *β*-conglutin (with a sedimentation coefficient of approximately 11 S) is considerably heavier than α-conglutin (with a sedimentation coefficient of approximately 7 S).

Keywords: *Extraction; globulins; legume seeds; Lupinus albus; sedimentation coefficients*

INTRODUCTION

Three main storage, glycosylated globulins have been characterized in lupins—*γ*-conglutin, *β*-conglutin, and α-conglutin (Mossé and Pernollet, 1983). *γ*-Conglutin, a minor globulin component, is composed of one main type of subunit (40 kDa) containing two polypeptide chains (18 and 26 kDa) linked by disulfide bonds (Blagrove and Gillespie, 1975; Melo et al., 1994). It occurs *in vitro* in oligomeric form, with a sedimentation coefficient of 10 S and an estimated molecular mass of 280 kDa (Blagrove et al., 1980). *β*-Conglutin, the major globulin component, is composed of 10−12 major types of subunits (17−72 kDa) and a considerable number of minor subunits, with no disulfide bonds (Melo et al., 1994). Finally, α-conglutin contains four main types of subunits (53, 60, 66, and 70 kDa) as well as a number of minor subunits. Upon reduction, each of these main subunits is split into a heavier polypeptide chain (31, 36, 42, and 46 kDa, respectively) and a lighter polypeptide chain (19 kDa), which is common to all four subunits (Blagrove and Gillespie, 1975; Melo et al., 1994).

No clear experiments have been published about the sedimentation coefficients of the two *Lupinus* main globulins, i.e. α- and *β*-conglutins. The first experiments performed with *Lupinus* globulins, involving ultracentrifugation techniques, have shown that these proteins are composed of two main components with sedimentation coefficients of 7 and 11 S (Danielsson, 1949; Joubert, 1955a−c, 1957; Naismith, 1955; Gerritsen, 1956). Indeed, many legume seeds contain two major globulin components with sedimentation coefficients of approximately 7 and 11 S, which, on the sole basis of their sedimentation values, were named vicilin and legumin, respectively (Derbyshire et al., 1976). However, as far as we are aware, the sedimentation coefficients of α- and *β*-conglutins have never been clearly established. Nevertheless, α-conglutin is generally considered as the 11S or legumin-like globulin, whereas *β*-conglutin is regarded as the 7S or vicilin-like globulin.

In this paper we have developed an improved methodology to efficiently isolate the globulins from *Lupinus albus* cotyledons. The sedimentation behavior of the total globulin fraction or of the individual conglutins was subsequently studied by isopycnic sucrose or glycerol density gradient centrifugation performed under low or high ionic strength.

MATERIALS AND METHODS

Plant Materials. Dry seeds of white lupin (*L. albus* L.) cultivar Multolupa, kindly supplied by Dr. J. N. Martins, were hand-sorted, under UV light, to certify their sweet character (Ferreira et al., 1995). Dry seeds of pea (*Pisum sativum* L.), soybean [*Glycine max* (L.) Merr.], and broad bean (*Vicia faba* L.) were obtained in a local market. Duckweed (*Lemna minor* L.) was grown as described before (Ferreira and Teixeira, 1992).

Isolation of the Total Globulin Fraction. Total globulins from legume seeds were extracted and isolated according to two different procedures: a standard procedure, described by Melo et al. (1994), which is a modified version of the method of Blagrove and Gillespie (1978), and an improved procedure based on a modification of the standard procedure. In the standard procedure, the dry cotyledons were milled (0.2 mm sieve) and the resulting meal was defatted with *n*-hexane (34 mL/g of flour) for 4 h with agitation and air-dried after decantation of the hexane. The albumin fraction of the proteins was extracted by stirring with water containing 1 mM phenylmethanesulfonyl fluoride (PMSF) (34 mL/g of flour) for 4 h. The suspension was centrifuged for 1 h at 30000*g*, and the supernatant was used as the total albumin fraction. The total globulin fraction of the proteins was extracted by stirring

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S0021-8561(97)00281-1 CCC: $14.00

with 0.1 M Tris-HCl buffer, pH 8.0, containing 10% (w/v) NaCl and 0.05% (w/v) NaN_3 (34 mL/g of flour), for 12 h. The globulin-containing solution was centrifuged for 1 h at 30000*g* and the supernatant desalted on a PD-10 prepacked Sephadex G-25 column (Pharmacia/LKB), previously equilibrated in 50 mM Tris-HCl buffer, pH 7.5, and used as the source of total globulins. All operations were performed at 4 °C. In the improved procedure, two modifications were introduced: (1) the albumin fraction of the proteins was extracted by stirring with water (adjusted to pH 8.0) containing 10 mM $CaCl_2$ and 10 mM $MgCl_2$; (2) the total globulin fraction of the proteins was extracted by stirring with 0.1 M Tris-HCl buffer, pH 8.0, containing 10% (w/v) NaCl, 0.05% (w/v) NaN_3, 1 mM PMSF, 10 mM ethylenediaminetetraacetic acid (EDTA), and 10 mM ethylene glycol bis(*β*-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA).

Purification of the Individual Globulins from *L. albus* by FPLC Anion Exchange Chromatography. α-Conglutin, *β*-conglutin, and *γ*-conglutin were purified by anion exchange chromatography of the total globulin fraction (ca. 5 mg) in the Mono Q HR5/5 column of the FPLC (Pharmacia/LKB) as described by Melo et al. (1994).

Gel Filtration of the Individual Globulins from *L. albus*. Samples (1.5 mg) of each of the three individual globulins previously isolated by anion exchange chromatography were loaded into the Superose 12 HR10/30 gel filtration column of the FPLC equilibrated with 0.1 M Tris-HCl buffer, pH 7.5. The flow rate was 0.5 mL min^{-1}.

Isopycnic Sucrose or Glycerol Density Gradient Centrifugation. The sucrose gradients were composed of 30% (7.2 mL), 25% (7.2 mL), 20% (7.2 mL), 15% (7.2 mL), and 10% (7.2 mL) sucrose (w/v) made up in 35 mM phosphate buffer, pH 7.6, containing 10 mM 2-mercaptoethanol and 0.4 M NaCl (Hill and Breidenbach, 1974). The glycerol gradients were composed of 45% (4 mL), 40% (4 mL), 35% (4 mL), 30% (4 mL), 25% (4 mL), 20% (4 mL), 15% (4 mL), 10% (4 mL), and 5% (4 mL) glycerol (v/v) made up in 50 mM Tris-HCl buffer, pH 7.5. Both types of gradients were prepared using Ultraclear 38 mL centrifuge tubes. Starting with the highest concentrations, each solution was layered in the centrifuge tube and immediately frozen in liquid nitrogen. After preparation, the 36 mL gradients were stored at −70 °C until needed.

The step sucrose or glycerol gradients prepared were converted into continuous gradients by thawing followed by incubation at 4 °C during 24 h. This procedure to linearize step gradients was reported before for sucrose gradients (Luthe, 1983). Identical results were achieved for glycerol gradients (data not shown). In addition, our results indicate that a centrifugation at 110000*g* for 24 h at 4 °C does not alter in a significant way the shape of the gradients.

Sucrose concentrations were determined by measuring the refractive index at 20 °C using an ABBE refractometer, Model 60/EA. Glycerol concentrations were determined spectrophotometrically at 230 nm.

Ultracentrifugation of Globulins. A sample (2 mL; 1.5 mg of protein) containing total globulins from *L. albus* or *G. max* or the individual globulins from *L. albus* was layered on the top of the gradient and centrifuged at 110000*g* for 24 h at 4 °C in a Beckman XL-90 analytical ultracentrifuge. Immediately after ultracentrifugation, the gradients were collected (1.5 mL fractions) using a peristaltic pump and the A_{280} was continuously monitored. The precipitate was suspended in 200 *μ*L of 50 mM Tris-HCl buffer, pH 7.5, and divided in two aliquots: one aliquot (100 *μ*L) was added to sample buffer and subjected to sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE); the other aliquot (100 *μ*L) was added to 900 *μ*L of 50 mM Tris-HCl buffer, pH 7.5, containing 10% (w/v) NaCl to solubilize the suspended globulins, and used to measure A_{280}.

Sedimentation coefficients of protein fractions obtained after ultracentrifugation were determined according to the method of Martin and Ames (1961), using horse heart cytochrome *c* (1.8 S; peak centered at $V = 2$ mL from the meniscus of the gradient; 12.4 kDa), bovine serum albumin (4.4 S; $V = 5$ mL; 66 kDa), goat immunoglobulin G (6.6 S; $V = 7.4$ mL; 150 kDa), bovine liver catalase (11.3 S; $V = 12.7$ mL; 232 kDa), *Lemna* ribulose bisphosphate carboxylase (15 S; $V = 17$ mL; 530 kDa), and rabbit muscle phosphorylase kinase (22 S; $V = 25$ mL; 1.28 MDa) as standards [data not shown and Martin and Ames (1961), Tominatsu (1980), Ferreira and Davies (1987), and Gueguen and Barbot (1988)]. The coefficient of determination obtained for the equation $S = f(V)$ was $r^2 = 0.99996$.

General Assays. Protein concentration was measured according to a modification of the Lowry method (Bensadoun and Weinstein, 1976). *Lemna* ribulose bisphosphate carboxylase was purified by anion exchange chromatography on the FPLC (Pharmacia/LKB) Mono Q column and by gel filtration on the FPLC Superose 12 column as previously described (Ferreira and Teixeira, 1992). Immunoglobulins G were purified from goat serum by affinity chromatography on the protein G−Superose column of the FPLC (Ferreira et al., 1996). Electrophoresis was performed as previously described (Ferreira et al., 1996).

RESULTS AND DISCUSSION

Isolation of Globulins from Legume Seeds. The seed storage proteins have been studied in detail from the turn of the century, when Osborne (1924) classified them into four groups on the basis of their extraction and solubility characteristics: albumins, soluble in water; globulins, insoluble in water but soluble in dilute salt solutions; glutelins, insoluble in the above solutions but soluble in weak acidic or basic solutions; and prolamins, insoluble in the above solutions but soluble in alcohol/water mixtures (Ashton, 1976; Shewry et al., 1995). This classification is still widely used. However, it is well-known that a clear-cut distinction between these groups of proteins is not always possible, with many proteins showing intermediate solubility behaviors. In this respect, some seed albumins have been identified from several plant species that behave like globulins as far as solubility is concerned (Bollini and Chrispeels, 1978; Youle and Huang, 1978).

We have utilized a procedure to isolate the total globulin fraction of *L. albus* cotyledons (Melo et al., 1994), which is a modification of the method described by Blagrove and Gillespie (1978). In this method, the dry cotyledons were detached from the seeds and milled, and the resulting meal was defatted with hexane. The total albumin fraction of the proteins was extracted by two consecutive treatments of the defatted meal with water containing 1 mM PMSF. The total globulin fraction was finally dissolved by stirring the water-treated meal with buffer containing 10% (w/v) NaCl. The gel presented in Figure 1A shows the polypeptide composition of the albumin and globulin fractions and indicates that a large fraction of the globulins is solubilized during the water treatment. Blagrove and Gillespie (1975) have also noted that *L. angustifolius* globulins are to some extent soluble in deionized water. We have therefore developed an improved methodology that efficiently extracts the total globulin fraction by introducing two principal modifications.

(1) The total albumin fraction was extracted with water containing 10 mM $CaCl_2$ and 10 mM $MgCl_2$, pH adjusted to 8.0. The adjustment of the water pH to 8.0 is important to maintain the pH values between 6.5 and 7.5 during the albumin extraction. Lower pH values promote protein aggregation and precipitation, protein−protein interactions, formation of phytin−protein complexes, and proteolytic activities (Derbyshire et al., 1976; Okubo et al., 1976; Shutov and Vaintraub, 1987). Indeed, the pH drops to approximately 4 when pure water is mixed with the deffated meal. On the other hand, it has been reported that the total protein from

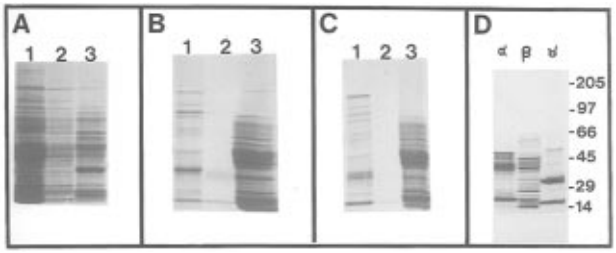

Figure 1. Extraction of albumins and globulins from *L. albus* seeds. Hexane-treated meal (corresponding to 0.125 g of fresh weight of cotyledons) was subjected to two consecutive albumin extractions (lanes 1 and 2, respectively) followed by a globulin extraction (lane 3), and the resulting protein fractions (10 μL) were analyzed by SDS−PAGE. (A) Lanes 1 and 2, albumins extracted with water (130 and 30 μg of protein, respectively); lane 3, globulins extracted with buffer containing 10% (w/v) NaCl (50 μg of protein). (B) Lanes 1 and 2, albumins extracted with water containing calcium and magnesium (10 and 5 μg of protein, respectively); lane 3, globulins extracted with buffer containing 10% (w/v) NaCl (120 μg of protein). (C) Lanes 1 and 2, albumins extracted with water containing calcium and magnesium (17 and 3 μg of protein, respectively); lane 3, globulins extracted with buffer containing 10% (w/v) NaCl, EDTA, and EGTA (150 μg of protein). (D) Conglutins α, β, and γ (40 μg) purified on the Mono Q column of the FPLC. Molecular masses of standards are indicated in kDa.

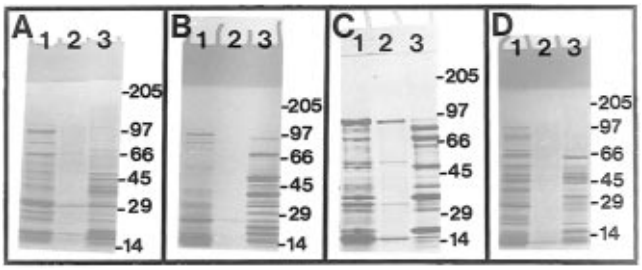

Figure 2. Extraction of albumins and globulins from *L. albus* (A), *P. sativum* (B), *G. max* (C), and *V. faba* (D) seeds. Hexane-treated meal (corresponding to 0.125 g of fresh weight of cotyledons) was subjected to two consecutive albumin extractions with water containing calcium and magnesium (lanes 1 and 2) followed by a globulin extraction with buffer containing 10% (w/v) NaCl, EDTA, and EGTA (lane 3), and the resulting protein fractions were analyzed by SDS−PAGE. Lanes 1 and 2 (40 μL): 60 and 5 μg (A), 68 and 2 μg (B), 80 and 20 μg (C), and 74 and 4 μg (D) of protein. Lane 3 (6 μL): 75 μg (A), 91 μg (B), 115 μg (C), and 75 μg (D) of protein. Molecular masses of standards are indicated in kDa.

L. albus seeds shows a 55% (Sgarbieri and Galeazzi, 1978) or a 75% (Oomah and Bushuk, 1983) solubility in water, implying that the extraction of albumins in water alone may lead to a considerable loss in globulins (see also Figure 1A). However, Cerletti et al. (1978), who also extracted the pentane-treated flour with water, reported that *L. albus* albumins comprise 12.8% of the total seed protein. The inclusion of Ca and Mg in the albumin extraction medium, and the adjustment of its pH to 8.0, allowed a very efficient extraction of the albumins, free of contaminating globulins. This result is evidenced in Figure 1B by the almost total absence of polypeptides in the second albumin extraction (Figure 1B,C, lane 2) and by the totally different polypeptide patterns present in the albumin and globulin fractions (Figure 1B, lanes 1 and 3). Indeed, the incubation of the hexane-treated flour in water solubilized 76% of *L. albus* seed protein, but this value decreased to 13% when calcium and magnesium were included. This result clearly indicates the ready solubility of albumins and the great insolubility of globulins in water containing Ca and Mg (pH 8.0). Indeed, the two major *L. albus* conglutins, i.e. α- and β-conglutins, are highly insoluble in water containing Ca and Mg. However, a fraction of γ-conglutin is lost in the albumin fraction, even when the divalent cations are present (Figure 1B,C, lane 2). Calcium and magnesium have not been utilized, as far as we are aware, in the extraction of storage proteins. However, they have been used to selectively precipitate soybean globulins, glycinin and β-conglycinin (Wolf and Sly, 1965; Fukushima, 1968; Saio et al., 1968; Saio and Watanabe, 1973; Rao and Rao, 1975). These ions bind to the carboxyl groups of aspartic and glutamic acid residues and to the imidazole groups of histidine residues in the polypeptide chains of soya globulins (Kroll, 1984; Peng et al., 1984).

(2) The total globulin fraction was extracted with 0.1 M Tris-HCl buffer, pH 8.0, containing 10% (w/v) NaCl, 0.05% (w/v) NaN_3, 1 mM PMSF, 10 mM EDTA, and 10 mM EGTA. It has been reported that interactions between calcium and proteins are dramatically reduced in the presence of 0.5 M NaCl or 10 mM EDTA (Peng et al., 1984). The use of EDTA and EGTA increased the efficiency of globulin extraction, as shown by comparing Figure 1C, lane 3, with Figure 1B, lane 3.

The procedure optimized in Figure 1 for *L. albus* was used to extract the albumin and globulin fractions from other legume species—*L. albus* (control, Figure 2A), *P. sativum* (Figure 2B), *G. max* (Figure 2C), and *V. faba* (Figure 2D). The results presented in Figure 2 indicate that this is a good method to isolate albumins and globulins from legume seeds as evidenced by the different polypeptide patterns of these fractions in all species examined. The albumins comprise 13.4% of the total seed proteins in *L. albus*, 12% in *P. sativum*, 13% in *G. max*, and 17% in *V. faba*. This is in agreement with the values reported in the literature (Bewley and Black, 1978). However, higher values (30−40%) have also been reported for plants from the genera *Pisum* and *Acacia* (Murray, 1979).

Sedimentation Coefficients of *L. albus* Individual Globulins. The classical studies of Osborne (1924) and Danielsson (1949) involving a large number of species lead to the conclusion that, with very few exceptions, legume seeds contain two major types of globulins with sedimentation coefficients of approximately 7 S (7−9 S) and 11 S (10−11 S) and molecular masses of 150−200 and 300−450 kDa, respectively (Derbyshire et al., 1976). These were named vicilin-type and legumin-type globulins, respectively, after *P. sativum* vicilin and legumin (Osborne and Campbell, 1898).

We have isolated the total globulin fraction from *L. albus* cotyledons and purified the individual conglutins by anion exchange chromatography on the Mono Q column of the FPLC (Melo et al., 1994). Total globulins and each of the individual conglutins were subsequently analyzed by isopycnic glycerol density gradient centrifugation and SDS−PAGE. The results of this experiment are illustrated in Figure 3. The main features are as follows: (i) *L. albus* total globulin is composed by four fractions, corrresponding to four protein peaks (Figure 3A). The SDS−PAGE analysis of each of the protein peaks (Figure 3B) revealed that the two heavier protein peaks contain each α- and β-conglutins, the third peak consists mainly of α-conglutin, with traces of γ-conglutin, and the lighter protein peak contains α- and γ-conglutins. Despite the large number of authors who have used density gradient ultracentrifugation to study and fractionate *Lupinus* total globulins, Sgarbieri and

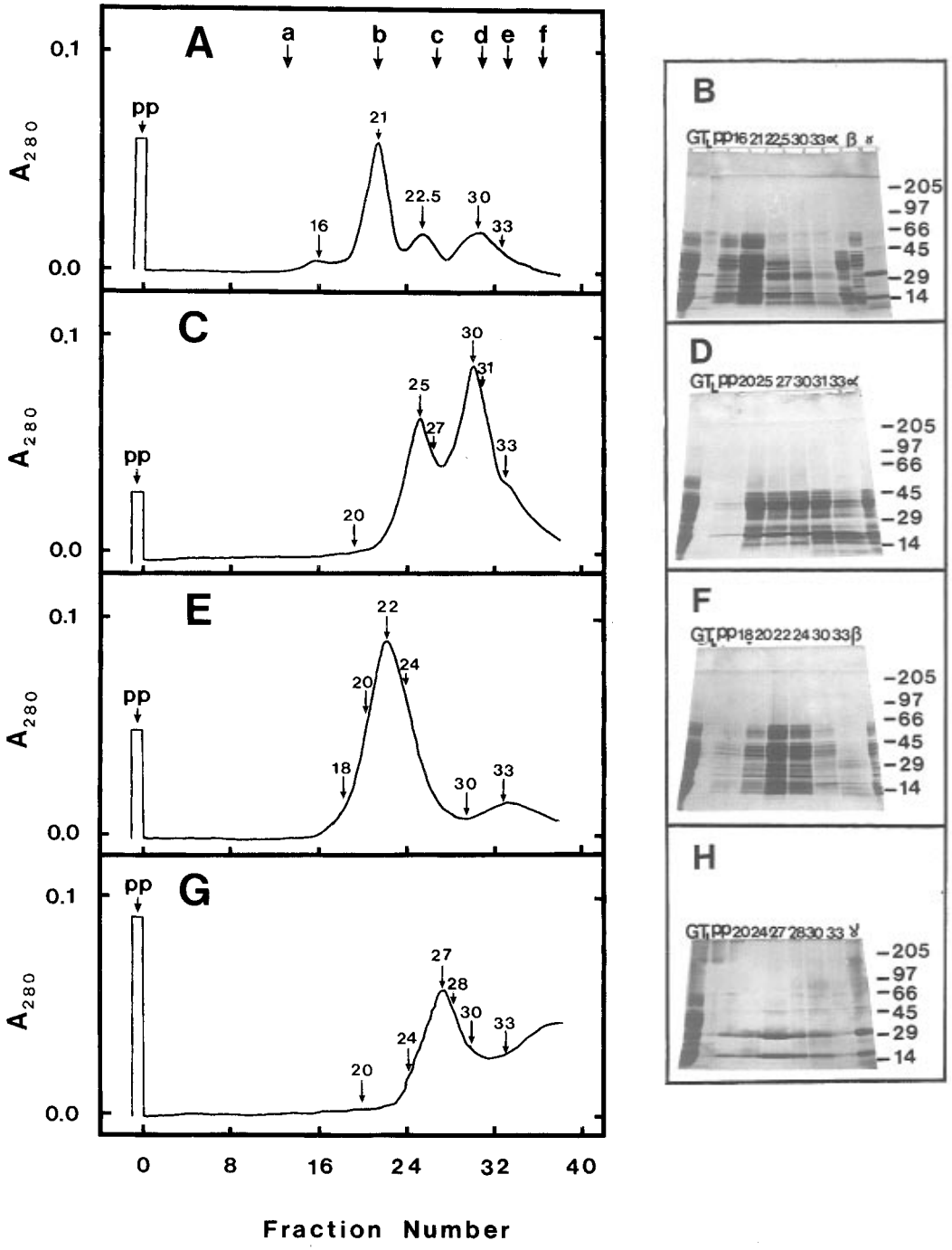

Figure 3. Characterization of *L. albus* globulins by isopycnic centrifugation on glycerol gradients. Total globulins (A, B), α-conglutin (C, D), *β*-conglutin (E, F) and *γ*-conglutin (G, H) were isolated and subjected to isopycnic glycerol density gradient centrifugation as described under Materials and Methods. The gradients were collected (A, C, E, G) and selected fractions analyzed by SDS−PAGE (B, D, F, H). The markers used in the ultracentrifugation studies were as follows: a, phosphorylase kinase (22 S); b, ribulose bisphosphate carboxylase (15 S); c, catalase (11.3 S); d, immunoglobulin G (6.6 S); e, serum albumin (4.4 S); f, cytochrome *c* (1.8 S). Molecular masses of standards are indicated in kDa in panels B, D, F, and H. GT_L, total globulins; pp, precipitate; α, *β*, and *γ*, α-, *β*-, and *γ*-conglutins, respectively; numbers on top of the gels correspond to the gradient fractions.

Galeazzi (1978) have noted that this technique does not offer a good resolution and that the molecular masses of the resulting protein peaks must be interpreted as average molecular masses of heterogeneous material. (ii) α-Conglutin is composed of two fractions (Figure 3C), with identical polypeptide patterns (Figure 3D) and with sedimentation coefficients of 11.5 and 6.2 S. This is in agreement with the work of Duranti et al. (1988), who reported that α-conglutin consists of 12 and 7 S species which are involved in an association−dissociation equilibrium that is shifted toward association by increasing the ionic strength of the medium. (iii) *β*-Conglutin consists of a single protein peak with a sedimentation coefficient of 14.2 S (Figure 3E,F). (iv) *γ*-Conglutin originated a main protein peak with a sedimentation coefficient of 9.8 S (Figure 3G,H). Blagrove and Gillespie (1975) and Blagrove et al. (1980) reported sedimentation coefficients of up to 10 S for *γ*-conglutin. However, *γ*-conglutin was also found in the precipitate in the bottom of the centrifuge tube, probably representing high-order aggregates of this globulin. A similar situation was observed during the analysis of the total globulin fraction (Figure 3A,B). This hypothesis is supported by the observation that pelleted *γ*-conglutin

is totally solubilized in high ionic strength solutions. The data presented in Figure 3 clearly indicate that, under the conditions of study, β-conglutin is heavier than α- and γ-conglutins. This result was confirmed by gel filtration of the individual conglutins on the Superose 12 column of the FPLC (results not shown). These proteins were eluted as single protein peaks with estimated native molecular masses of 150 kDa for α-conglutin, 200 kDa for β-conglutin, and 115 kDa for γ-conglutin. When the total globulin fraction was analyzed by gel filtration, several protein peaks were obtained. However, each protein peak was always composed of a mixture of conglutins, highlighting the importance of using purified proteins, free of cross-contaminations, to study molecular properties of the individual conglutins.

The high sedimentation coefficient obtained for β-conglutin, particularly when compared with that of α-conglutin, is clearly in contrast with the values found in the literature. One possible explanation for this discrepancy could lie in the low ionic strength used in the glycerol gradients utilized in Figure 3. Under these circumstances, association of β-conglutin and/or dissociation of α-conglutin could occur, as was reported for the 12 S protein component of *L. angustifolius* and *L. luteus* (Joubert, 1955a−c). Hill and Breidenbach (1974) have characterized the sedimentation coefficients of the storage proteins from *G. max*, the legume species of which the seed proteins have been most intensively studied. These authors extracted the globulins from defatted *G. max* meal with 35 mM phosphate buffer, pH 7.6, containing 0.4 M NaCl and 10 mM 2-mercaptoethanol and subjected the total globulin fraction to sucrose density gradient centrifugation under high ionic strength (0.4 M NaCl) (10−30% w/v; 24 h at 105000*g*). Using this methodology, Hill and Breidenbach found three sedimenting classes of proteins, with sedimentation coefficients of 2.2, 7.5, and 11.8 S. In an attempt to avoid any artifact occurring during the extraction, fractionation, or ultracentrifugation and to perform density gradient centrifugation under high ionic strength conditions, we have applied exactly the methodology of Hill and Breidenbach (1974) to extract and ultracentrifuge the total globulin fraction from *G. max* (control, Figure 4A) and *L. albus* (Figure 4B). As expected, the protein profile presented in Figure 4A is virtually identical to the one reported by Hill and Breidenbach. An identical protein profile was obtained for *L. albus* total globulins (Figure 4B), including three protein peaks with approximate sedimentation coefficients of 12, 7.7, and 2.5 S and a precipitate in the bottom of the centrifuge tube. When we analyzed each of these protein fractions by SDS−PAGE (Figure 4C), we reached the following conclusions: the 12 S protein peak is composed exclusively of β-conglutin, the 7.7 and 2.5 S protein peaks consist of α-conglutin, and the precipitate contains γ-conglutin.

Our results indicate that β-conglutin is considerably heavier than α-conglutin and that γ-conglutin may be present in a form with a very high sedimentation coefficient. These results are in disagreement with the data reported in the literature. In an attempt to explain this discrepancy we propose the following explanation: *Lupinus* total globulins were initially studied and characterized by ultracentrifugation techniques and were shown to be composed essentially by three components with sedimentation coefficients of approximately 11 S (legumin-like), 7 S (vicilin-like), and 2 S,

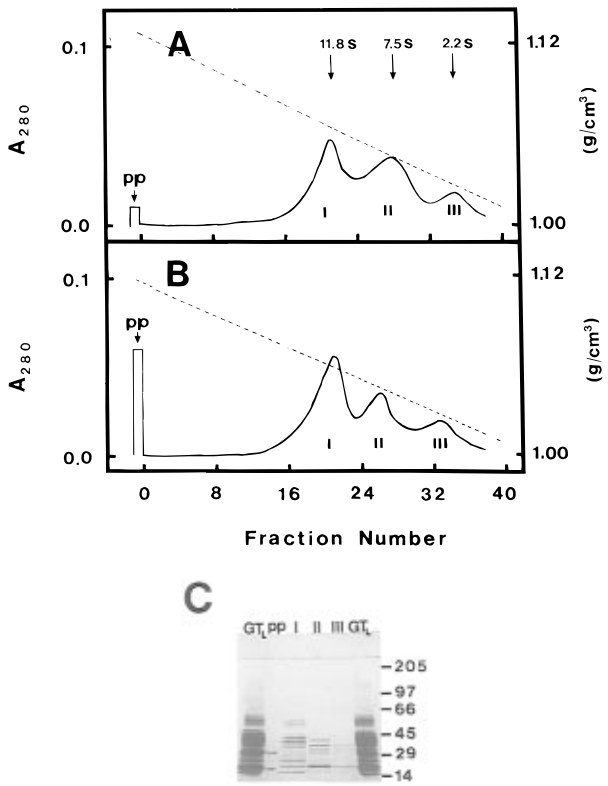

Figure 4. Characterization of *G. max* and *L. albus* total globulins by isopycnic centrifugation on sucrose gradients. Total globulins (1.5 mg) from *G. max* (A) or *L. albus* (B) were extracted and subjected to isopycnic sucrose density gradient centrifugation (10−30% w/v sucrose), performed under high ionic strength, as described by Hill and Breidenbach (1974). Samples from the three protein peaks and the precipitate shown in panel B were analyzed by SDS−PAGE (C). Molecular masses of standards are indicated in kDa. GT_L, total globulins; pp, precipitate; I, II, and III, protein peaks I, II, and III, respectively.

in a similar manner with the storage proteins of most legume seeds (Danielsson, 1949; Joubert, 1955a−c, 1957; Naismith, 1955; Gerritsen, 1956; Derbyshire et al., 1976; Mossé and Pernollet, 1983). Several years later, *Lupinus* total globulins were fractionated by electrophoresis on cellulose acetate membranes into conglutins α, β, and γ (Blagrove and Gillespie, 1975). Subsequently, the conglutins were isolated by selective precipitation, by diethylaminoethyl-cellulose or FPLC/Mono Q anion exchange chromatography, and by agarose or polyacrylamide gel electrophoresis (Duranti et al., 1981; Bush and Tai, 1994; Melo et al., 1994). However, as far as we are aware, no direct correlation has ever been made between the 11, 7, and 2 S protein components and the α-, β-, and γ-conglutins. In summary, the results reported in this paper indicate that α-conglutin and β-conglutin do not correspond, respectively, to the 11S and 7S protein components of *Lupinus* seeds.

ABBREVIATIONS USED

EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; FPLC, fast protein liquid chromatography system; PAGE, polyacrylamide gel electrophoresis; PMSF, phenylmethanesulfonyl fluoride; SDS, sodium dodecyl sulfate; Tris, tris(hydroxymethyl)aminomethane.

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Received for review April 11, 1997. Accepted July 14, 1997.[⊗] This research was financially supported by the Junta Nacional de Investigação Científica e Tecnológica under Project PMCT/C/BIO/886/90.

JF9702813

[⊗] Abstract published in *Advance ACS Abstracts,* September 1, 1997.