ELECTROPHORETIC STUDIES OF RICE PROTEINS AND CHARACTERIZATION OF RICE ENDOSPERM α-GLOBULIN

by

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INTRODUCTION

Among the major grain crops, the only one that is grown almost exclusively as human food is rice. Rice constitutes about one half of the diet of 1.6 billion people, and another 400 million rely on it for between a fourth and one half of their diet (1).

Rice is an annual grass belonging to the same family as barley, oats, rye and wheat. The rice genus is Oryza. The two cultivated rice species, each with a great number of varieties, are O. sativa of Asia and O. glaberrima of West Africa (1). Oryza also includes 20 wild species which are scattered in Asia, Africa, Australia and Central and South America. The Asian rices (O. sativa) are much more important than the African (O. glaberrima) when considering the amount of land planted to each group.

Over the millenniums the cultivated species of Asia differentiated into three subspecies based on geographic conditions. They are indica, japonica (also called sinica) and javanica (1). Indica rices were originally confined to the humid regions of the Asian tropics and subtropical areas. Japonicas were cultivated in subtropical temperate zone regions. Javanicas flourished in the equatorial region of Indonesia (1). In addition to their adaptation to climate, the three races differ in amylose content, the elongation of the grain, the temperature at which the grain becomes gelatinous, and the aroma in cooking. Japonica cultivars are generally short-grain whereas indica cultivars are usually long- or medium grain.

The rice grain (or rough rice) consists of an edible portion, the

rice caryopsis, and its covering structures, the hull or husk (Fig. 1). As layers are successively removed in milling, the proportion of protein in the remaining kernel decreases and the proportion of carbohydrate increases. As percentage of dry weight, the protein contents of brown rice (dehulled), of bran (aleurone layer plus germ), and of polish (the inner bran layers with part of the germ and a small portion of the starchy endosperm) are higher than that of milled rice (endosperm only), particularly in the low-protein samples (2). Although this may be partly explained by the higher degree of milling noted for the low-protein samples, the protein-rich grain may have a more even protein distribution. Thus, with increasing overall protein content, the protein content of milled rice increases more rapidly than the protein content of bran (2). Table 1 (3) shows the nutrient content of brown and milled rices of two samples differing in protein content.

RICE PROTEIN

Osborne (4) classified plant protein on the basis of solubilities in several solvents. Albumin is the water-soluble fraction, globulin is the salt-soluble fraction, prolamin is the alcohol-soluble fraction, and glutelin is the fraction soluble in dilute acid or alkali. Solubility fractionation of the proteins of milled rice, bran and rice polish indicated that glutelin was the predominant fraction in the whole grain; albumin and globulin were concentrated in the bran and rice polish; and prolamin was the most evenly distributed protein fraction (2). Differences in the total protein content of the whole grain are due mainly to differences in glutelin content of the starchy endosperm (2).

AMINO ACID COMPOSITION

Based on the provisional amino acid scoring pattern reported by World Health Organization of the United Nations in 1973 (5), rice protein is deficient in lysine, the sulfur-containing amino acids, leucine and threonine (6) (Table 2).

The amino acid composition is not uniform within the rice grain (Table 3). The decrease of lysine on milling is disproportionately large, while there are relative increases of isoleucine, leucine and valine (7). The embryo, aleurone cells, and grain coat together amount to about 9% of the grain by weight. Yet they contain a relatively large proportion of the total protein, 17%, and account for 23% of the lysine and 19% of the threonine (8). Because these histological components are removed during milling, the promotion of increased consumption of brown rice rather than milled rice seems desirable.

Comparison of amino acid compositions of the four Osborne protein fractions (Table 4) indicates that the albumin fraction contains the highest proportions of the uncharged polar amino acids (27.8 mole%) and the lowest amounts of acidic amino acids (21.6 mole%). The content of basic amino acids (15.4 mole%) is the highest in globulin fraction which register low in hydrophobic amino acids (37.1 mole%). Values for glutelin are intermediate for all classes of amino acids. The amino acid composition of rice glutelin is similar to that of soybean glycinin (9). This is shown by classes of amino acids as follows:

Amino acid (mole %)	Rice glutelin	Soybean glycinin
Hydrophobic	38.6	35.4
Uncharged polar	22.7	21.9
Basic	12.2	12.1
Acidic	26.6	29.7

They both are high in glutamic acid and low in methionine, cystine and

histidine but glycinin has somewhat higher lysine content (4.4 mole%) than rice glutelin (2.3 mole%). The most striking rice fraction in composition is prolamin. Among the four Osborne fractions, prolamin has the highest amounts of hydrophobic (45.4 mole%) and acidic (28.2 mole%) amino acids, whereas the contents of basic (7.3 mole%) and uncharged polar (19.0 mole%) amino acids are the lowest (6). Wheat gliadin (10) is also low in basic amino acids (4.0 mole%) and high in hydrophobic (43.3 mole%) and acidic (39.4 mole%) amino acids.

PROTEIN BODY

Three types of rice endosperm protein bodies have been reported, namely: Ls (large spherical), Ss (small sphericel) and Cr (crystalline) (11,12). The Ls is lamellar with a concentric ring and dense center. The predominant Ls protein is prolamin (13). Cr bodies stain homogeneously and are not lamellar (13,14). It was shown to be digested by pepsin more rapidly than the spherical protein bodies (15). Cr bodies are rich in glutelin and globulin. The Ss is thought to form during endosperm development but later than Ls (11). In addition to differences in size and time of formation, the Ss also lacks concentric rings and dense center. Consequently, on the basis of morphology and mechanism of secretion, the Ss forms a third class of protein bodies.

While there is general agreement as to when protein body deposition begins (about 7 days after flowering) (11), there is a lack of aggrement as to how the deposition occurs. Oparka and Harris (16) have confirmed that Ls and Ss are derived from localized dilations within cisternae of rough ER but they found multiple Ls development within a single cisterna. This is contrary to the results previously reported by Bechtel and Juliano

(11). Also, the discussion as to whether the Ls is bounded by a single or double membrane remains unresolved.

Oparka and Harris have demonstrated that the Cr is also initiated by dilations of the cisternal ER, but these dilations differ from those forming Ls in that the Cr bounding membrane is free of ribosomes (16). It is proposed by Bechtel and Juliano (11) that Cr is secreted via the Golgi apparatus and is a derivative of dictyosome. Whereas judging from their thick section study (thick sections of zinc iodide-osmium tetroxide post-fixed tissues of developing rice grains), Oparka and Harris suggested that the dictyosomes may act in transporting protein by accumulating it, initially in small peripheral vesicles which then aggregate around and discharge into a large vesicle at the distal face of the dictyosome. The vesicle may in turn discharge into the Cr protein body (16).

GLUTELIN

The major storage proteins of most cereal grains are glutelin and prolamin. In rice, glutelin is the major protein of the starchy endosperm, constituting at least 80% of the total protein whereas prolamin accounts for less than 5% (17).

Rice glutelin is composed of two principal types of subunits, the MW 22,000-23,000 and MW 37,000-39,000 polypeptides (18). Analysis by gel electrophoresis (with and without reducing agent) (19) showed that these two polypeptides are linked together by disulfide bonds and the bigger one (MW 37,000-39,000) was shown to be homologous to the β-subunit of pea legumin. This is not the first cereal storage protein to be identified as homologous to legumin, but the identification of "rice legumin" is novel in that the differences in sequence between pea legumin

and rice legumin affect the solubility properties of the proteins, so pea legumin is salt soluble but the rice glutelin is not soluble under nondenaturing conditions. Although rice legumin is the major protein in rice glutelin, other proteins, such as 14,000 MW polypeptide in this fraction may prove to be more related to the proteins of the "classical" glutelin fraction of those other cereals (19).

Several storage proteins of cereals and legumes are known to be synthesized as short-lived precursor polypeptides (18) which undergo modification during or after translation. Using in vitro protein synthesis systems, Croy et al. (20) and Tumer et al. (21) showed that two major subunits of pea legumin and soybean glycinin are formed from a high molecular weight precursor by a post-translational cleavage. Recently, Yamagata et al. (18) presented evidence for the existence of a precursor (a polypeptide of MW 57,000) of the two major subunits of glutelin. They suggested that the system for the biosynthesis and accumulation of rice glutelin is very similar to that for legumin or glycinin synthesis in peas or soybeans.

PROLAMIN

Rice prolamin is composed mainly of a 13,000 MW polypeptide (18). It is usually contaminated with a glucan which may be complexed with the protein, as shown by disc electrophoresis (22). Further studies showed the presence of polyphenolic contaminants, which may be separated by gel filtration in Sephadex G-200 using 0.01 N NaOH as solvent (23). Removing the ethanol and freeze-drying the sample results in a prolamin preparation which is no longer soluble in 70% ethanol but only in dilute alkali (23). This may be due to protein denaturation or uncomplexing with polyphenol.

ALBUMIN

The albumin fraction constitute about 5% (by weight) of milled rice protein. This set of proteins can not be exhaustively extracted from milled-rice flour, even after five consecutive extractions. These albumins are present in the water extract but require some salt (e.g. 0.1 M $(NH_4)_2SO_4$) for complete solubilization (24).

Silaev et al. reported 9 to 11 albumin bands by polyacrylamide gel electrophoresis at pH 8.65. Mitsuda reported 2 major and 5 or 6 minor bands by Cellogel electrophoresis in 0.025 M glycine buffer at pH 10.5 or 11.5 (17). Villareal and Juliano (24) reported three major subfractions---basic albumin, albumin I and albumin II. Isoelectric focusing showed that albumin I contained neutral proteins (pI's 6.8-7.7), albumin II had the most acidic components, while basic albumin proteins had pI 8.3 and 8.7.

Iwasaki et al. (25) have also reported a investigation of rice albumins. The albumins were extracted from milled rice of long, medium and short grain varieties with 5% NaCl and were separated from globulins by ammonium sulfate precipitation. The gel filtration (Sephadex G-100) of the three rice varieties showed similar patterns, each of them gave four fractions with molecular weights ranging from 10,000 to 200,000. By starch-gel electrophoresis with urea, these albumins were separated into about 20 bands. Although patterns of the three rice varieties were similar, clear differences existed. Thus, the authors suggested classifying rice varieties into groups by differences in electrophoretic patterns of albumin proteins.

GLOBULIN

Quensel (26) and Danielsson (27) classified seed globulins into α, β, and γ components in order of increasing rate of sedimentation in the ultracentrifuge. Morita and Yoshida (28) also showed that rice globulins could be classified into four components. Among them, α and β can be found in bran, embryo, outer and inner endosperm; γ-globulin is the major globulin of embryo but occurs also in bran and outer endosperm; whereas β-globulin is almost limited to the endosperm.

γ-globulin emerged at the void volume in gel filtration (Sephadex G-200) and, thus, might have a molecular weight of 200,000 (28). γ-globulin was found to occur in high concentration in embryo and bran, which are the most active parts in biological functions of rice grain. Morita et al. (28-33) systematically investigated the nature of this rice γ-globulin. There were three major components of γ-globulin: γ_1, γ_2 and γ_3 (29). These components were revealed to appear homogeneous in electrophoresis as well as sedimentation (29). The molecular dimensions and chemical compositions of γ_1 and γ_3 have been described (30,33). Morita et al also studied the subunit and secondary structures of γ_1 component (31,32).

Available data on globulin components of milled rice are meager. About the only point of agreement among these reports is the presence of two fractions in the milled-rice globulin (34,35). One is α-globulin and the other is a more soluble globulin with lower molecular weight and high sulfur content (36). According to Houston and Mohammad (34), α-globulin accounts for 40% of the total globulin of rice endosperm. Their preparation showed a single component by free-film electrophoresis, Sephadex G-100 chromatography and ultracentrifugation (S value of 1.6) but

dissociated into two components in 7.5 M urea. Dissociation was not found with urea solutions less than 5 M, nor were cyanate ion effects noted. Gel filtration on a calibrated Sephadex G-100 column provided an estimated molecular weight of this α-globulin of 25,500. Thus, Houston and Mohammad concluded that there is only one component in the α-globulin. As to the dissociation caused by urea, they suggested that either the urea could differentially denature two proteins having equal charges and molecular weights or there could be an equilibrium between native and urea-denatured protein of a single species.

Perdon and Juliano (37) found a pH dependent aggregation phenomenon of α-globulin. In their studies, SDS-PAGE of α-globulin revealed one subunit of α-globulin with molecular weight of 18,000; gel filtration at pH 6.5 exhibited two peaks with molecular weights of 20,000 and 98,000. α-globulin gave one band by electrophoresis at pH 4.5 but two bands at pH 8.3; one sharp peak was shown by sedimentation in acetic acid (pH 2) and NaOH (pH 11.7) but a broad asymmetric peak was obtained at pH 6.7, 8.3 and 8.9. The two proteins could not be separated by DEAE-cellulose chromatography. They suggested these data could be explained by a pH dependent partial aggregation of α-globulin to a pentamer between pH 6 and 9.

WORK DESCRIBED IN THIS STUDY

The traditional method of preparing α-globulin from milled rice is by $(NH_4)_2SO_4$ precipitation at 30% saturation from a 5% NaCl extract, followed by repeated precipitation at pH 4.5. Basically, α-globulin preparations isolated this way by different groups have similar properties, which include molecular weight and amino acid composition

(34,35,37). Since it has been exposed to an extreme pH (i.e. pH 2) and then been precipitated at pH 4.5, important questions are : is this α-globulin damaged during isolation ? Is it denatured ? In this study, I have devised a milder isolation scheme for this α-globulin and compare α-globulin so isolated with that purified with the traditional method. Also, two-dimensional gel electrophoresis will be applied to the Osborne solubility fractions of milled rice flour to assess their levels of complexity.

MATERIAL

RICE

Newbonnet variety (long grain) from USDA, Arkansas.

REAGENTS

Acrylamide, ultrapure electrophoresis grade: Sigma chemical company.

Ammonium persulfate: Sigma chemical company.

Ammonium sulfate: MCB manufacturing chemists, Inc.

Ampholine pH 3.5-10 (40%), pH 5-7 (40%), pH 4-6 (40%): LKB.

Bis-acrylamide: Eastman Kodak company.

Coommassie brillant blue R: Sigma chemical company.

Dibasic sodium phosphate: Fisher scientific company.

ß-mercaptoethanol: Sigma chemical company.

Monobasic sodium phosphate: Fisher scientific company.

Nonidet P-40 (NP-40): BRL.

Sodium dodecyl sulfate (SDS), electrophoresis grade: polysciences, Inc. or Bio-Rad Laboratory.

N,N,N',N',-tetramethylethylenediamine (TEMED): Sigma chemical company.

Trichloro-acetic acid: Fisher scientific company.

Trishydroxymethylamino methane (TRIS), ultrapure grade: Sigma chemical company.

Urea, ultrapure grade: Schwarz-Mann.

COLUMN MEDIA

Hydroxylapatite (Bio-gel HTP): Bio-Rad Laboratory.

Sephadex G-75: Sigma chemical company.

Sephadex G-100: Pharmacia.

Sepharose 6B-100: Pharmacia.

MEMBRANE

Dialysis tubing, Spectrapor 3,500 and 6,000-8,000 molecular weight cut off: Spectrum Medical Industries, Inc.

METHODS

EXTRACTIONS

Rice seeds were milled, ground to 68-mesh, defatted with four volumes of acetone at room temperature for 1 hour, and air-dried.

a) SEQUENTIAL EXTRACTION

The defatted milled rice flour was sequentially extracted with deionized water, 0.5 M NaCl, 70% ethanol and 0.5% SDS/0.6% β-ME. The sample was stirred with two volumes of solvent at room temperature for 20 minutes three times. Extracts were separated from residues by centrifugation at 3,500g for 10 minutes. These extracts were stored in the freezer.

b) DIRECT EXTRACTION OF TOTAL RICE PROETIN

Defatted milled rice flour was extracted with two volumes of 0.5% SDS/0.6% β-ME three times (20 min. each) at room temperature. The residue was removed by centrifugation at 3,500g for 10 min. The extract was stored in freezer.

PRECIPITATION

a) AMMONIUM SULFATE PRECIPITATION

This was performed at 4°C. $(NH_4)_2SO_4$ was added slowly to the amount needed. The solution was stirred at least 2 hours and then centrifuged at 16,300g for 20 min. to remove the supernatant.

b) REDUCING SALT CONCENTRATION

Some salt-soluble proteins were precipitated by diluting the protein solution with deionized water. Precipitated proteins were

separated from supernatant by 10 min. centrifugation at 3,500g.

c) pH 4.5 PRECIPITATION

Portions of defatted milled rice flour were extracted with 4 volumes of 5% NaCl by stirring for 2 hours at room temperature. Solids were removed by centrifugation and the supernatant was decanted and filtered. The filtrate was subjected to $(NH_4)_2SO_4$ precipitation at 30% saturation. The precipitate was separated by centrifugation, dissolved in 2.5% NaCl, filtered and again precipitated with ammonium sulfate (30% saturation). The precipitate was dissolved in 2.5% NaCl, dialyzed against deionized water overnight at 4°C and freeze-dried. The dry powder protein sample was then dissolved in 1% acetic acid at pH 2.5. Gradual addition, with vigorous stirring, of 0.5 N NaOH to bring the pH to 4.5 produced a precipitate. This was separated by centrifugation and dissolved in 1% acetic acid. The pH 4.5 precipitation was performed a total of three times. Finally, the precipitate was dialyzed against deionized water and freeze-dried.

CHROMATOGRAPHY

a) GEL PERMEATION

The NaCl extract was subjected to $(NH_4)_2SO_4$ precipitation at 30% saturation. The precipitate was dissolved in 0.5 M NaCl/0.05 M Tris-Cl (pH 8.2) and fractionated on a Sepharose 6B-100 column (0.9 x 102 cm) in the same solvent, at a flowrate of 21 ml/hr.

b) ADSORPTION CHROMATOGRAPHY

This was performed on a Hydroxyapatite column (4.6 x 9 cm) equilibrated with 0.5 M NaCl/0.05 M Tris-Cl (pH 8.2). After the flowthough had completely eluted, 0.05 M sodium phosphate (pH 7) was

applied. The flowrate was 83 ml/hr.

c) SEPHADEX G-25

A column (4.1 x 33 cm) was equilibrated and eluted with 0.5 M NaCl/0.05 M Tris-Cl (pH 8.2). The flowrate was 82 ml/hr.

d) SEPHADEX G-75

A column (1.4 x 82 cm) was equilibrated and eluted with 0.5 M NaCl/0.05 M Tris-Cl (pH 8.2). The flowrate was 23 ml/hr.

TWO-DIMENSIONAL GEL ELECTROPHORESIS

The first dimension was either isoelectric focusing (IEF) (38) or non-equilibrium pH gradient electrophoresis (NEPHGE) (39). The second dimension was discontinuous SDS slab gel (210 x 150 x 0.8 mm) electrophoresis.

The first dimension gel was a 6-inch tube gel (2.5 mm inner diameter) composed of 9.2 M urea, 2% Nonidet P-40, 4% acrylamide/bisacrylamide (from a 30% stock solution comprised of 28.4% acrylamide and 1.6% bisacrylamide) and 2% Ampholines. The anode (upper) solution was 0.01 M H_3PO_4 and cathode (lower) solution was 0.02 M NaOH. Electrophoresis was usually carried out at an initial voltage of 300, the voltage was then increased in several steps over an one hour period to 1,000. The voltage was maintained at 1,000 until a total of 2,000 to 4,000 volt-hour had elapsed.

For IEF, gels were run at least 20,000 volt-hour (usually 20 hours after voltage reaches 1,000). At the end of the run, the gel was removed with a syringe. The gel was then placed for one hour in equilibration buffer (10% glycerol/5% βME/2.3% SDS/0.0625 M Tris-HCl (pH 6.8) and small amount of bromphenol blue) and either run immediately in the second

dimension or frozen in dry ice/alcohol bath and stored at -20°C.

The second dimension, the gel was polymerized from 12% acrylamide/ 0.2% bisacrylamide. The gel was run at room temperature at 5-7 milliamp until the dye front reached the bottom of the gel. The staining solution was 0.25% Coomassie brilliant blue R/10% acetic acid/5% ethanol. The destaining solution was 10% acetic acid/20% ethanol.

SDS POLYACRYLAMIDE GEL ELECTROPHORESIS

This was the same as the second dimension in the two dimensional gel system described above, except that up to 18 samples can be loaded to the gel. A comb was used while making stacking gel to produce wells where the protein samples could be applied into. The slabs were electrophoresed until the dye front reaches the bottom of the gel. Gels were stained and destained in the same manner as described above for two dimensional gels.

ULTRACENTRIFUGAL ANALYSIS

Ultracentrifugation was carried out in a Beckman Model E analytical ultracentrifuge equipped with Schlieren and interference optics and an RTIC temperature control unit.

a) SEDIMENTATION COEFFICIENT

Sedimentation velocity measurements were carried out at 20°C at a speed of 60,000 rpm using Schlieren optics. A purified protein solution (about 5.5 mg/ml) in 0.5 M NaCl/0.05 M Tris-Cl (pH 8.2) was examined after dialysis against the same buffer overnight at 4°C. The protein sedimentation coefficient in solvent at 20°C ($S_{20,B}$) was calculated from a linear least squares analysis of the sedimentation velocity of the protein (dlnr/dt), and $S_{20,B}$ for both HA-prep and pH 4.5-prep α-globulin is 2.005.

The values were corrected to $S_{20,W}$ (at 20°C in water) from the following formular:

$$S_{20,W} = S_{20,B} \times \eta_{rel,20} \times \frac{(1 - \bar{v} \times \rho_{20,W})}{(1 - \bar{v} \times \rho_{20,B})}$$

The following values have been used (for α-globulin):

$\eta_{rel,20}$, solvent viscosity relative to water viscosity at 20°C, 1.09011.

$\rho_{20,B}$, solvent density at 20°C, 1.0224 ml^{-1}.

$\bar{v}$, partial specific volume, 0.7065 cm^3/g, this value is calculated from amino acid composition of HA-prep α-globulin determined in this study.

b) MOLECULAR WEIGHT DETERMINATION

Molecular weight was determined by the sedimentation equilibrium method using interference optics. Centrifugation was carried out at a rotor speed of 39,894 rpm at 22.1°C and the molecular weight was calculated as follows:

$$M = \left(\frac{d\ln C}{dr^2}\right) \left(\frac{2RT}{1 - \bar{v}\rho_{20,B}}\right) \left(\frac{1}{w^2 r}\right)$$

Same $\rho_{20,B}$ and $\bar{v}$ values as in $S_{20,W}$ determination were used here. $(d\ln C/dr^2)$ was obtained through linear least squares analysis of the concentration distribution in the ultracentrifuge cell. It is 1.3126 for HA-prep α-globulin.

AMINO ACID COMPOSITION

Purified protein sample was dialyzed against deionized water in the cold room overnight and the protein suspension was freeze-dried. Then, 0.5 ml 6 N HCl was added to the lyophilized sample. Hydrolysis was done under vacuum at 120°C for 24 hours after HCl was removed. Samples were

dissolved in 0.22 N sodium formate/3.3 N para-toluene sulfonic acid with Norleucine included as an internal standard. An aliquot was applied to a ion exchange column on a Dionex D-300 Kit single column accelerated analyzer. The system was calibrated using a Beckman standard mixture of amino acids (with equimolar concentrations of all amino acids). Tryptophan was not determined due to its decomposition during acid hydrolysis. Results were calculated from dividing the peak area of each amino acid by that of its corresponding peak in the standard and recorded as mole%.

CIRCULAR DICHROISM MEASUREMENTS

All circular dichroism (CD) spectra were obtained on a Cary 60 spectropolarimeter with a Model 6001 CD attachment, using a 1 mm cell for far U.V. region and 1 cm cell for near U.V. region. Ellipticities are reported in deg. cm^2/dmol of amino acid residures and were calculated using 110 as the mean molecular weight of the amino acid residure. For the spectrum of α-globulin, the protein concentration was calculated from its extinction coefficient (i.e. 1.12, experimentally determined value in this study) and A_{280}. Those values are:

	near V.U.	far U.V.	
HA-prep	0.69	0.421	
pH 4.5-prep	1.29	0.531	(units: mg/ml)

Protein samples were dissolved in 0.05 M Tris-Cl (pH 8.2)/0.5 M NaCl.

RESULTS AND DISCUSSION

PART A: ELECTROPHORETOGRAMS AND NOMENCLAMATURE OF RICE PROTEIN

Figure 2 shows one-dimensional electrophoretic patterns of the four Osborne solubility fractions of rice flour and that of the total rice protein (obtained by direct extraction of rice flour with 0.5% SDS/0.6% ß-ME). Although the predominant bands differ for each fraction, the protein fractions are not entirely distinct.

Several different electrophoresis procedures have been applied to rice proteins (8,19,25,40-43). These are all one-dimensional procedures. Because of the considerable complexity, including various pI's, molecular weights and abundances, rice proteins can not be well separated by a method that involves one parameter only (that is, for instance, either pI or molecular weight). Thus, the two-dimensional gel electrophoresis techniques reported by O'Farrell (IEF-SDS procedure) (38) and O'Farrell et al. (NEPHGE-SDS procedure) (39) were used in this study to analyze rice proteins. These systems have been widely employed to study other cereal proteins (44-51).

In order to get the best resolution, several different conditions have been used for the first dimension electrophoresis. In the NEPHGE system, pH 3.5-10 ampholine and 3,000 volt-hours turned out to be the best combination for all samples I studied.

Figure 3 shows the electrophoresis pattern of water extracted proteins. A considerable complexity of proteins in this fraction is indicated: approximately 40 spots can be seen. Thus, this method reveals at least 20 more polypeptides than any other procedures reported before

(17,24,25).

There are fewer proteins in the salt-extracted fraction. As shown in Figure 4, the salt extracted protein fraction seems to comprise largely two subfractions with approximately equal amount (Figure 2, track 2). The one with higher molecular weight, α-globulin, was identified by its molecular weight and abundance using data reported previously by other researchers (34,37). The other subfraction I suppose to be the more soluble globulins with higher sulfur content reported by Hernandez (36). The low molecular weight globulins seems to be composed of two groups of polypeptides which are heterogeneous (Figure 4, left lower corner).

There is hardly any ethanol extracted protein (prolamin) in rice endosperm (Figure 2, track 3). According to the literature, rice prolamin is composed mainly of a 13,000 MW polypeptide (18). As shown in figure 5, the electrophoretogram of proteins extracted by 70% ethanol after H_2O and 0.5 N NaCl extraction, there are some other proteins also extractable by 70% ethanol. The two faint groups (arrow heads) appear to be the major groups (group A and B) seen in SDS extracted protein fraction (Figure 6). My prolamin fraction contains 2 polypeptides in the MW 13,000 region. Further studies would be needed to confirm which component (or components) is the prolamin reported by other researchers.

The residual protein after successive extractions of milled rice flour with H_2O, NaCl solution and 70% ethanol is principally glutelin (2). In fact, Lozsa (52) defined glutelin in this way rather than as the alkali-soluble extract. Since most proteins can be solubilized by SDS solution in the presence of β-mercaptoethanol (53,54), 0.5% SDS/0.6% β-ME was the reagent used in my study for extracting glutelin as well as total protein from milled rice flour. Since up to 80% of rice endosperm protein

is glutelin, the gel pattern of the glutelin fraction (Figure 6) is similar to that of total rice protein (Figure 7), obtained by direct extraction with 0.5% SDS/0.6% β-ME from milled rice flour.

Figure 2 shows that there are two major polypeptides of MW 37,000 and 22,000 in the glutelin fraction (track 4). This is consistent with what has been reported by other researchers (18,19). According to Zhao et al. (19), two polypeptides (one from each group) link together by disulfide bond (or bonds) and these disulfide-linked subunit pairs are thought to be homologous to pea legumin. Moreover, the MW 22,000 polypeptide has been shown to have amino acid sequence homology to pea legumin β-subunits.

It was reported that for legumin synthesis in pea cotyledon a precursor 60,000 polypeptide is split into MW 20,000 and MW 40,000 polypeptides (20). The system in rice glutelin biosynthesis is found very similar to that for pea legumin. Based on their pulse-chase experiment and SDS-PAGE, Yamagata et al. (18) concluded that the MW 22,000 and 37,000 polypeptides of rice glutelin are formed by splitting of the MW 57,000 precursor. Casey et al. (55) have also observed the same post-translational cleavage of this precursor. Note that there is a polypeptide band around MW 57,000 region of SDS-extracted protein fraction on SDS gel (Figure 2, track 4). More evidence would be needed to identify this polypeptide as the precursor mentioned before.

In soybean, it has been demonstrated that both the acidic and basic subunits of glycinin are heterogeneous (9,56). Evidence of sequence heterogeneity in the rice glutelin MW 22,000 polypeptide was also reported by Zhao et al. (by showing the isolated peptides varying in sequence at single position) (19), however, they were not able to show it on their two-dimensional gel in which the separation of polypeptides is based on

molecular weight in both dimensions. In this study, I have worked out the conditions for best resolutions for group A (MW 22,000) and group B (MW 37,000) separately. For group A, it is a combination of ampholine with pH range of 7-9 and 3,500 V-hr(for the first dimension). This is shown in figure 8, four spots (one with slight different MW with the other three) are seen in this group. For group B, isoelectric focusing between pH 4.5-8.2 (ampholines: pH 3.5-10) was applied in the first dimension. Two sub-groups with slight different molecular weights appeared. They correspond to the two bands with MW around 35,000 in track 6 Figure 2. Each of these sub-groups has a broad pI range, 5.9 to 6.5 and 5.7 to 8.1, but all group B polypeptides are relatively acidic compared with group A polypeptides. In Figure 6, the polypeptide group pointed by arrow head corresponds to the MW 57,000 band in Figure 2 track 4. If this polypeptide group is the precursor for MW 22,000 and 37,000 rice glutelins, its heterogeneity indicated by the two-dimentional gel pattern may imply the existence of a family of homologous genes.

Judging from the electrophoretogram of total rice protein (Figure 7), α-globulin (the very intense spot under group B) may be the most abundant product of a single gene among the total rice endosperm protein.

The terms "albumin", "globulin", "prolamin" and "glutelin" from the Osborne classification scheme refer to solubility fractions, and not single protein species. Therefore the use of these terms implies a mixture of proteins. Some proteins can be extracted by more than one class of solvents (57,58). In other words, these proteins are not entirely group-specific concerning with Osborne solubility classification. The electrophoretogram presented here obviously agrees with these observations. For instance, besides the NaCl extracted fraction,

α-globulin exists in water (the most intense spot in Figure 2) and SDS-extracted fractions, too (a very clear spot under group B). Moreover, groups A and B in SDS extracted fraction can also be extracted a little with 70% ethanol (Figure 5, arrow head pointed). Thus, it is recommended here in accord with a suggestion of Dr. J. Gatehouse (personal communication) where homology among some seed and/or cereal proteins is established, those proteins should be named by which can indicate their homology, such as legumin, vicilin, lectin and trypsin inhibitor.

PART B: STUDIES ON α-GLOBULIN

Precipitation is a valuable method of protein purification, especially as an initial step during an isolation process. The types of reagents important for these precipitations are inorganic salts such as $(NH_4)_2SO_4$), organic solvents (acetone or alcohols), and nonionic hydrophilic polymers. In general, organic solvents show marked tendencies to denature proteins, thus, $(NH_4)_2SO_4$ and PEG (polyethylene glycol) 6000 were tried in this study following extraction. Both of them precipitated globulins from a salt extracts but the former has advantages over the latter, particularly in removing nucleic acids indicated by the increasing A_{280}/A_{260} ratio from 0.82 to 1.30. Ammonium sulfate precipitation can also partially separate the low molecular weight globulin from α-globulin. Almost all of the α-globulin, with some of the low molecular weight polypeptides, was precipitated within the 0-30% $(NH_4)_2SO_4$ saturation region. So, after salt extraction, $(NH_4)_2SO_4$ precipitation at 0-30% saturation was included in α-globulin preparation.

Subsequent to this point, I tried several methods to completely separate α-globulin from low molecular weight contaminants. Judging from SDS gel analysis (Figure 2), there is approximately 11,000 apparent difference in molecular weight between α-globulin and the contaminants. Thus, gel filtration on Sepharose 6B-100 column was tried first. But it did not work; α-globulin and the contaminants eluted together.

Removal of the contaminants from α-globulin was ultimately accomplished by hydroxyapatite (HA) chromatography. Buffer (0.05 M Tris-Cl, pH8.2/0.5 M NaCl) was used to equilibrate the column, to introduce sample and to wash out the unbound protein contaminants. A linear phosphate gradient from 0 to 0.3 M sodium phosphate buffer (pH 7)

was applied. α-globulin was eluted around 0.05 M, which was the elution condition used thereafter.

It is not fully understood how hydroxyapatite works. It is a general rule among the proposed mechanisms (59-62) that protein binding to HA occurs both by nonspecific attraction between protein positive charges and HA or by specific complexing of protein carboxyls with calcium loci on the mineral. Elution can take place either as the result of the nonspecific ion screening of charges or by the specific displacement of protein groups from sites on the column with which they had complexed. Basic proteins having pI above 8 can be eluted with Cl^- whereas the majority of acidic proteins can not.

Figure 10a shows the sequential products during α-globulin preparation. After HA chromatography, α-globulin was subjected to Sephadex G-25 column for buffer exchange back to 0.05 M Tris-Cl (pH 8.2)/0.5 M NaCl. Then, it was concentrated by ammonium sulfate precipitation at 0-30% saturation and I call this concentrated material "HA-preparation" (HA-prep). The disadvantage of this method is that a small fraction of α-globulin eluted from HA column is degraded into two smaller polypeptides. I believed that this fraction has been cleaved without disruption of the tertiary structure, because the denaturing conditions of SDS-PAGE show two more bands (Figure 10a, track 6) while ultracentrifugation yield a single symmetric peak (Figure 11) (which corresponding to 2.32S ($S_{20,w}$)). The reason for this degradation problem is unclear. PMSF has been included in the column buffer to inhibit possible enzyme action but it showed no effect.

Gel filtration on a calibrated Sephadex G-75 column (in 0.5 M NaCl/0.05 M Tris-Cl, pH 8.2) provided an estimated molecular weight for

HA-prep α-globulin of 17,400 (Figure 12). SDS-PAGE gave a rough estimation of 26,000 (Figure 2), whereas it is 13,300 by ultracentrifuge study (sedimentation equilibrium) (Figure 13). Sedimentation equilibrium is the most accurate physical method now available for the determination of macromolecular weights. Various factors have been mentioned as possible reasons for anomalous behavior of proteins in SDS-PAGE. These are many positive (63,64) or negative (65) charges, the presence of oligo or polysaccharide chains (66), high proline content (67) and residual crosslinkages (68). Among the possible reasons mentioned above, probably the many negative or positive charges, due to the abundance of glx and arg (Table 5), can explain why the MW estimated by SDS-PAGE for α-globulin reported here is much higher than that by equilibrium sedimentation.

The pH-dependent aggregation phenomenon (between pH 6 and 9) suggested by Perdon et al. (37) was not found in my study. In contrast to what Perdon et al. have reported, there is only one symmetric peak, for both HA-prep and pH 4.5-prep α-globulin, obtained at pH 8.2 by sedimentation velocity measurement (Figure 11).

Figure 10b is the α-globulin isolated and purified by the procedures commonly used by other groups of researchers (34,37) and I call the protein so obtained "pH 4.5-preparation" (pH 4.5-prep). I have found that the yield of this preparation method is about one third of that of HA method. The sedimentation coefficient of pH 4.5-prep was measured same way as of HA-prep and values was the same (2.005S, uncorrected).

Table 5 shows the amino acid compositions of HA-prep and pH 4.5-prep α-globulins. The composition of HA-prep is similar to that of α-globulin reported by other researchers (34,37,42) (see Table 5). Whereas the composition of pH 4.5-prep is not quite the same as either the

published data (34,37,42) or that of the HA-prep. However, this difference may be contributed more by experimental error than by the variation among the proteins.

The relatively high level of arginine in this protein probably can help in improving rice protein quality from viewpoint of nutrition. The genetic codes of arginine and lysine are very similar. In fact, some of them are different in one base only. Theoretically by single-base pair substitution, arginine codon can be changed into that of lysine and result in the substituting arginine by lysine during synthesis of this α-globulin. My data (Table 5) indicate that lysine content of α-globulin can be theoretically raised from trace amount to about 10 mole% by lysine-for-arginine substitutions. Assuming the mass percentage of α-globulin to the total is 5% and there is no lysine in α-globulin before substitution (0 x 0.05 + ? x 0.95 = 3.4, ? = 3.6), this conversion will give a 15% increase of lysine in total protein (10 x 0.05 + 3.6 x 0.95 = 3.92, (3.92 - 3.4) / 3.4 = 0.15). This conversion, if it is successfully done, will nearly bring up lysine content in the rice (53 mg/g) to the suggested level (55 mg/g, Table 2).

In Figures 14 and 15 are shown the CD spectra of both HA-prep and pH 4.5-prep in the far UV and near UV regions respectively. The α-helical contents, calculated from far UV CD spectra, are 44.4% for HA-prep and 29.1% for pH 4.5-prep. The difference in the near UV region is dramatic. Since HA-prep α-globulin has never been exposed to denaturing condition during preparation, the difference in CD spectra of pH 4.5-prep from that of HA-prep may imply that pH 4.5-prep α-globulin was denatured to some extent. The denaturation of pH 4.5-prep apparently did not severely change its secondary structure (α-helical content). But the conformation

of this protein in some area was apparently altered to produce change in environment of tyrosine or tryptophan residues. This might explain the difference in near UV CD spectra between pH 4.5-prep and HA-prep (Figure 15).

HA-prep is better than pH 4.5-prep when we are concerned with the yield and undesired denaturation. Thus, hydroxyapatite chromatography is recommended for α-globulin preparation. More study is needed, however, to solve the degradation problem.

CONCLUSION

Because of the active synthesis and accumulation of relatively few molecular species over a short, well-defined period, the biosynthesis of storage protein in seeds provides an excellent system for studies on the molecular basis of the regulation of specific gene expression. Investigation of the grain proteins of several cereals now show that valuable information about the genotype, pedigree and genetic origins can be decoded by protein analysis using techniques such as gel electrophoresis and isoelectric focusing (69). My study is the first that combines these two techniques to provide a clear picture of rice endosperm proteins. For each of the four Osborne protein fractions NEPHGE with pH 3.5-10 ampholytes for 3,000 volt-hours followed by 12% SDS-PAGE is the best combination. For better resolution of the two major groups in SDS extractable protein fraction, pH 7-9 ampholytes should be used for the relatively basic group (group A); and IEF with pH 3.5-10 ampholytes for the relatively acidic group (group B).

Hydroxyapatite chromatography is a better method for α-globulin isolation than traditional method. The HA method gives higher yield and keeps the protein under nondenatured condition. The α-globulin so obtained has a molecular weight of 13,300 estimated by sedimentation equilibrium method, and it gives a single symmetric peak ($S_{20,w}$ = 2.32) in sedimentation velocity determination. Amino acid analysis shows that Glx, Ser, Gly and Arg are the four most abundant amino acids in this α-globulin.

TABLE 1

NUTRIENT CONTENT OF BROWN AND MILLED RICES

	IR8		IR480-5-9	
	Brown rice	Milled rice	Brown rice	Milled rice
Moisture (%)	10.1	9.9	11.2	10.7
Protein (%N x 5.95)	8.5	7.6	11.6	10.8
Crude fat (%)	2.3	0.3	3.1	0.5
Crude fiber (%)	1.1	0.6	2.2	1.1
Dietary fiber (%)	1.8	1.3	2.7	1.5
Crude ash (%)	1.36	0.36	1.74	0.55
Total P (%)	0.30	0.08	0.30	0.13
Phytin P (%)	0.21	0.03	0.25	0.06
Total Zn (parts 10^{-6})	20	10	25	16
Thiamin ($\mu g\ g^{-1}$)	3.97	0.20	3.61	0.82
Riboflavin ($\mu g\ g^{-1}$)	0.48	0.19	0.50	0.23

IR8 and IR480-5-9 (O. sativa) were obtained from 1974 dry season crop of the International Rice Research Institute (IRRI) farm.

This table comes from reference #2.

TABLE 2

AMINO ACID COMPOSITION IN THE HISTOLOGICAL COMPONENTS OF RICE

	Embryo (A)	Aleurone layer plus grain coat (B)	Starchy endosperm (C)	Total (A+B+C)	Suggested[a] level
ALa	67.5	64.9	57.4	58.7	
Arg	103.3	86.3	77.0	79.6	
Asp	102.8	108.5	98.4	99.8	
Cys	11.0	11.8	8.3	8.8	35b
Met	16.5	13.7	16.4	16.0	
Glu	169.4	146.1	203.2	194.9	
Gly	58.7	51.2	44.3	45.8	
His	38.6	33.4	29.5	30.4	17c
Ile	42.1	48.8	49.2	48.7	40
Leu	68.3	89.8	93.4	91.7	70
Lys	72.5	59.0	42.6	46.1	55
Phe	51.0	66.6	60.7	60.8	60d
Tyr	26.2	35.1	30.8	31.0	
Pro	40.5	59.0	60.7	59.3	
Ser	27.0	30.0	36.4	35.2	
Thr	42.1	35.1	33.1	33.9	40
Val	64.7	75.2	67.2	68.0	50

The amino acid compositions are given in mg/g of protein. Data come from reference 8.

[a] Provisional amino acid scoring pattern (5). These values are the suggested levels of those amino acids that should be present in a protein in order to have a biological value approaching 100.

[b] Value for Met + Cys.

c Suggested level for infant.

[d] Value for Phe + Tyr.

TABLE 3

AMINO ACID ANALYSES OF RICE[a] AND ITS COMPONENTS

AMINO ACIDS	WHOLE GRAIN	STARCHY ENDOSPERM	EMBRYO	ALEURONE LAYER PLUS GRAIN COAT	GRAIN COAT
Ala	8.6	8.9	9.8	10.2	11.5
Arg	6.1	5.5	8.2	5.8	4.4
Asp	9.2	9.2	8.9	10.5	11.7
Cys	0.47	0.44	0.43	0.44	0.25
Glu	16.4	17.1	13.8	13.2	8.5
Gly	9.0	8.4	11.4	10.4	10.8
His	2.5	2.3	3.0	2.7	1.9
Ile	4.6	4.8	4.1	4.5	4.9
Leu	8.7	9.0	6.8	8.3	9.2
Lys	4.1	3.7	6.4	4.9	4.8
Met	1.6	1.4	1.3	0.85	0.70
Phe	4.5	4.5	3.8	4.5	5.1
Pro	6.3	6.9	4.9	6.1	7.8
Ser	4.5	4.7	3.6	3.9	4.5
Thr	3.7	3.5	4.6	3.9	4.3
Tyr	2.3	2.3	1.7	2.2	1.9
Val	7.4	7.4	7.3	7.8	7.6
% protein[b]	6.9	6.1	14.5	11.7	7.8

Given in mole%.

This table comes from reference 8.

Tryptophan was not determined due to its decomposition during acid hydrolysis.

a The brown rice (O. sativa) used in this work was IRRI variety IR32.

b %protein was calculated from the known amount of material loaded and recovery of amino acids from the analyzer.

TABLE 4

AMINO ACID COMPOSITION[a] OF RICE[b] PROTEINS AND THEIR FRACTIONS

AMINO ACID[c]	OSBORNE FRACTION				
	ALBUMIN	GLOBULIN	PROLAMIN	GLUTELIN	TOTAL
Asp (A)	9.6	8.2	8.3	9.7	9.1
Thr (U)	5.1	2.7	1.3	3.0	3.5
Ser (U)	4.7	7.1	5.1	5.4	4.8
Glu (A)	12.0	16.4	19.9	16.9	16.9
Pro (H)	5.9	4.2	5.5	6.0	5.4
Gly (U)	13.0	9.8	6.2	8.9	8.9
Ala (H)	11.1	8.1	9.5	7.9	8.6
Cys (U)	2.2	0.9	trace	1.7	0.9
Val (H)	6.8	7.1	7.0	6.8	6.7
Met (H)	1.5	2.6	0.8	1.7	1.3
Leu (H)	3.3	4.0	4.4	4.1	4.6
Ile (H)	5.9	6.5	12.3	7.0	8.4
Tyr (U)	2.8	2.4	6.4	3.7	3.7
Phe (H)	2.6	3.7	4.4	4.1	4.0
Lys (B)	5.1	3.2	1.0	2.3	3.4
His (B)	2.2	2.2	1.7	2.1	2.2
Arg (B)	5.3	10.0	4.6	7.8	6.6
Trp (H)	1.0	0.9	1.6	1.0	1.1
Distribution of amino acid categories					
Hydrophobic (H)	38.1	37.1	45.5	38.6	40.1
Uncharged polar (U)	27.8	22.9	19.0	22.7	21.8
Basic (B)	12.6	15.4	7.3	12.2	12.2
Acidic (A)	21.6	24.6	28.2	26.6	26.0

a Given in mole%.

b Polished rice (*O. sativa*), Texas long grain.

c Letters in the parentheses represent the classes the amino acids belong to.

This table comes from reference 6.

TABLE 5

AMINO ACID COMPOSITION OF α-GLOBULIN

Amino Acid	HA-prep	pH4.5-prep	(A)	(B)	(C)
Asp	3.3	4.3	3.8	3.3	4.7
Thr	1.9	2.1	2.0	1.9	2.3
Ser	10.4	9.8	9.7	10.9	10.6
Glu	21.8	18.4	25.0	22.9	23.9
Gly	10.0	11.1	8.5	8.6	8.5
Pro	5.9	7.1	5.1	4.9	5.4
Ala	7.1	8.6	5.7	5.6	5.0
Cys	4.0	3.3	3.4	4.5	3.1
Val	2.9	3.5	3.5	3.8	4.4
Met	5.4	4.7	3.6	4.6	3.4
Ile	1.0	1.3	1.3	1.3	1.8
Leu	6.7	7.2	6.2	6.1	6.3
Tyr	5.3	5.7	5.5	5.4	4.7
Phe	2.5	2.9	2.6	2.4	2.6
His	1.2	3.3	0.1	trace	0.3
Lys	trace	trace	0.5	0.1	0.9
Arg	9.9	5.6	13.6	13.9	11.0

Data are given in mole%.

α-globulin was prepared from rice variety Newbonnet and samples were hydrolyzed for 24 hours before being analyzed (see Methods).

Amino acid composition of HA-prep presented here is the average of three analyses. Tryptophan was not determined.

A, B and C are the amino acid compositions of α-globulin reported by Pascual et al. (42), by Houston and Mohammad (34) and by Perdon and Juliano (37) respectively.

Fig. 1. Diagrammatic representation of the structure of a paddy grain in longitudinal section.

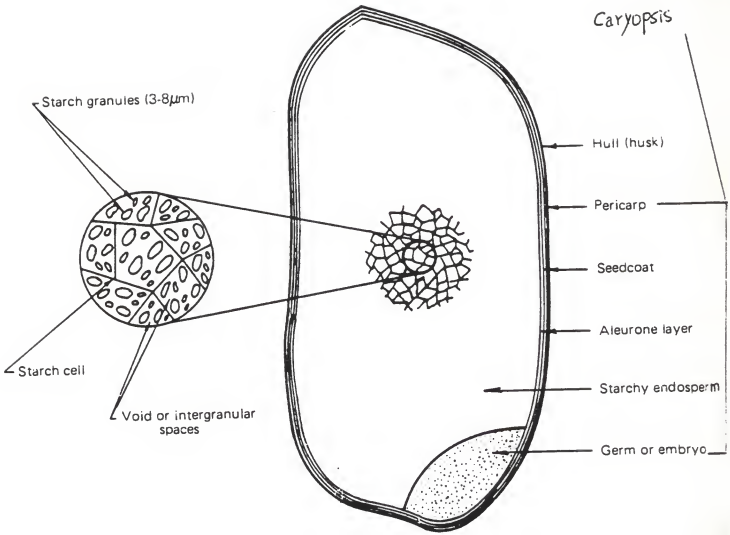

Caryopsis
Starch granules (3-8μm)
Hull (husk)
Pericarp
Seedcoat
Aleurone layer
Starchy endosperm
Germ or embryo
Starch cell
Void or intergranular spaces

Fig. 2. SDS-PAGE of total rice protein and the four Osbrone solubility fractions.

1st track: Total protein (direct SDS extract of rice flour)

2nd track: SDS extracted proteins (after water, NaCl, EtOH extracts)

3rd track: EtOH extracted proteins

4th track: NaCl extracted proteins

5th track: H_2O extracted proteins

6th track: Molecular weight marker.

Phosphorylase B (92,000)

BSA (66,000)

Ovalbumin (45,000)

Carbonic anhydrase (31,000)

Soybean trypsin inhibitor (21,000)

Lysozyme (14,000)

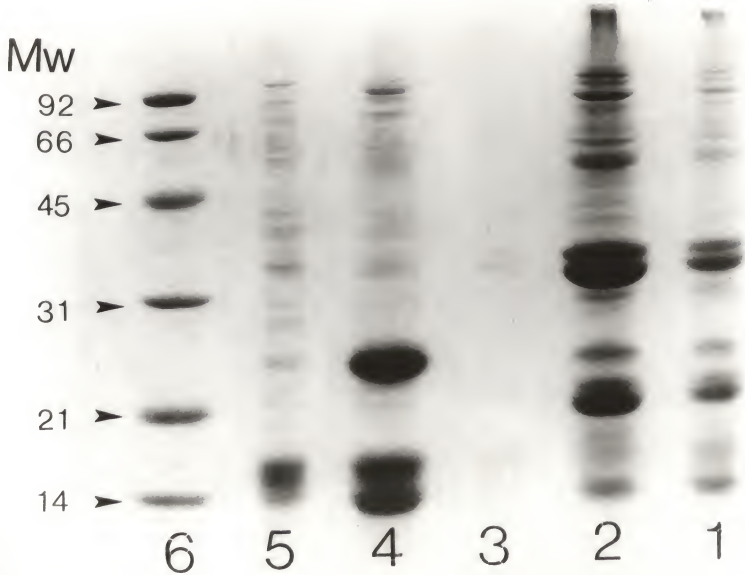

Mw
92
66
45
31
21
14
6
5
4
3
2
1

Fig. 3. Two-dimensional gel electrophoresis of rice water-extractable proteins. Ampholines: pH 3.5-10. Electrophoresis (NEPHGE) was carried out for 3,000 volt-hours.

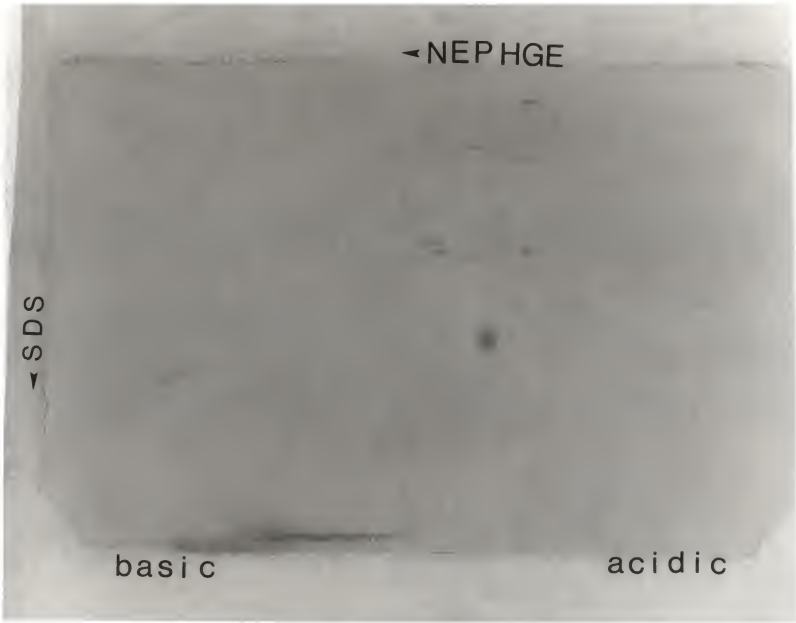

NEPHGE
SDS
basic
acidic

Fig. 4. Two-dimensional gel electrophoresis of rice NaCl-extractable proteins. Ampholines: pH 3.5-10. Electrophoresis (NEPHGE) was carried out for 3,000 volt-hours.


NEPHGE
SDS
basic
acidic

Fig. 5. Two-dimensional gel electrophoresis of rice EtOH-extractable proteins. Ampholines: pH 3.5-10. Electrophoresis (NEPHGE) was carried for 3,000 volt-hours.

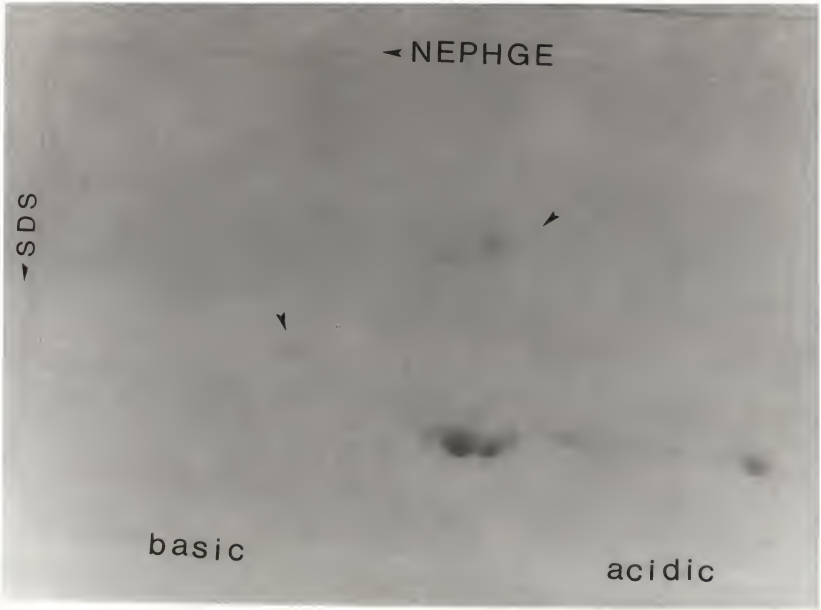

NEPHGE
SDS
basic
acidic

Fig. 6. Two-dimensional gel electrophoresis of rice SDS-extractable proteins. Ampholines: pH 3.5-10. Electrophoresis (NEPHGE) was carried out for 3,000 volt-hours.

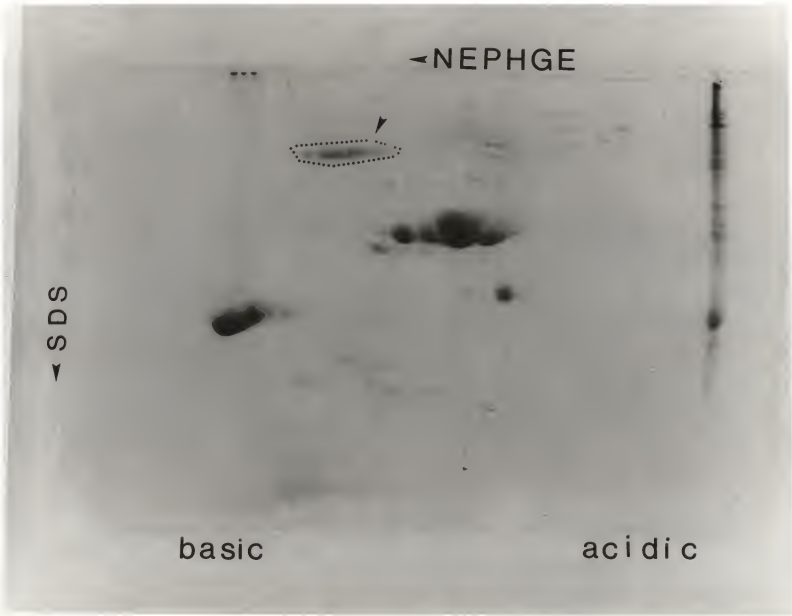

NEPHGE
SDS
basic
acidic

Fig. 7. Two-dimensional gel electrophoresis of rice total proteins. Ampholines: pH 3.5-10. Electrophoresis (NEPHGE) was carried out for 3,000 volt-hours.

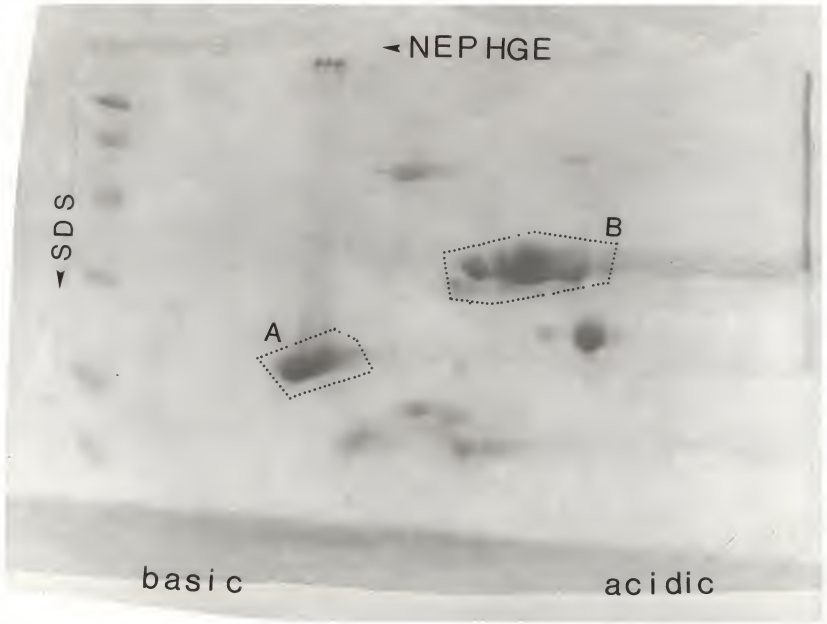

NEPHGE
SDS
A
B
basic
acidic

Fig. 8. Two-dimensional gel electrophoresis of rice SDS-extractable proteins. Ampholines: pH 7-9. Electrophoresis (NEPHGE) was carried out for 3,500 volt-hours.

NEPHGE
SDS
basic
acidic

Fig. 9. Two-dimensional gel electrophoresis of rice SDS-extractable proteins. Ampholines: pH 3.5-10. Electrophoresis was carried out to equilibrium (IEF).

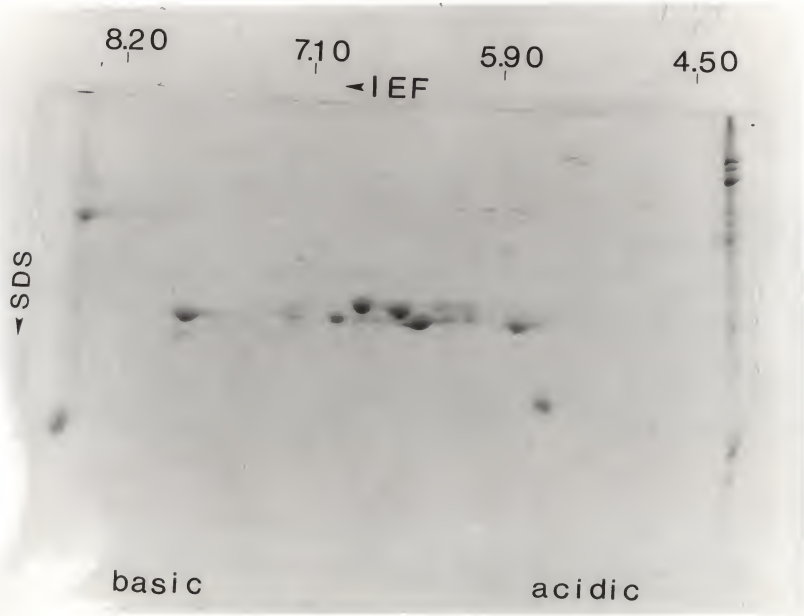

8.20
7.10
5.90
4.50
IEF
SDS
basic
acidic

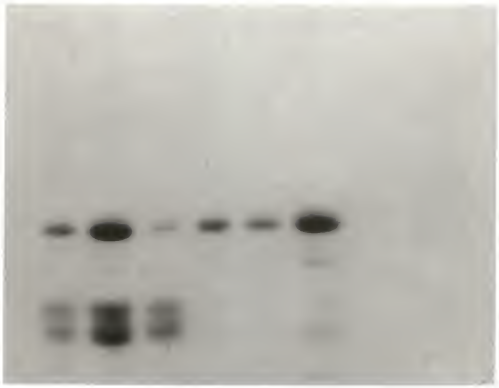
Fig. 10. SDS-PAGE of sequential products during α-globulin preparation.

10a. (UPPER PANEL): HA-prep.

From right to left, the first track is the NaCl extracts followed by 0-30% $(NH_4)_2SO_4$ precipitate (2nd track), the 3rd track is the flowthrough (LMWG) of HA column and the 4th track is the isolated α-globulin which was then buffer exchanged (5th track) and concentrated (6 track).

10b. (LOWER PANEL): pH4.5-prep.

From right to left, the first track is the NaCl extracts followed by 0-30% $(NH_4)_2SO_4$ precipitate which was repeated twice (2nd and 3rd tracks). And then it was dissolved in acetic acid and precipitated again at pH4.5 for three times (track 4-6). The last track is the lyophilyzed pH4.5-prep.



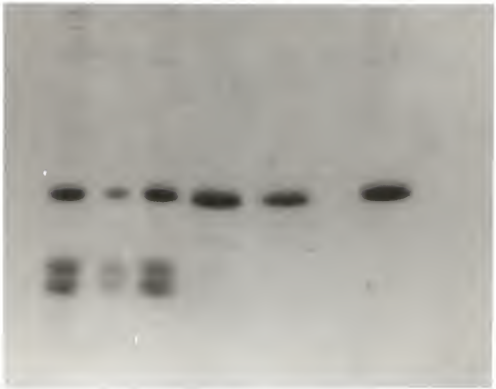
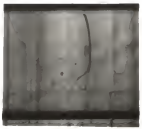
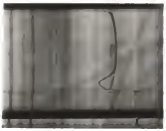


Fig. 11. Sedimentation pattern of α-globulin (HA-prep) at 60,000 rpm, 20°C. Times after the rotor attains speeds, from left to right, are 10 min., 18 min., 28 min. and 38 min.





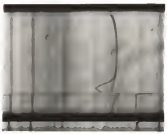


Fig.12. Molecular weight estimation by gel filtration (Sephadex G-75). Protein standard used:

protein	MW	logMW
Cytochrome-c	12,384	4.09
Lysozyme	14,600	4.16
Myoglobin	17,800	4.25
Soybean trypsin inhibitor	21,500	4.33
α-chymotrypsinogen-A	25,000	4.40
Ovalbumin	43,000	4.63
Bovine serum albumin	67,000	4.83

"X" is α-globulin (HA-prep) with Kav: 0.446.

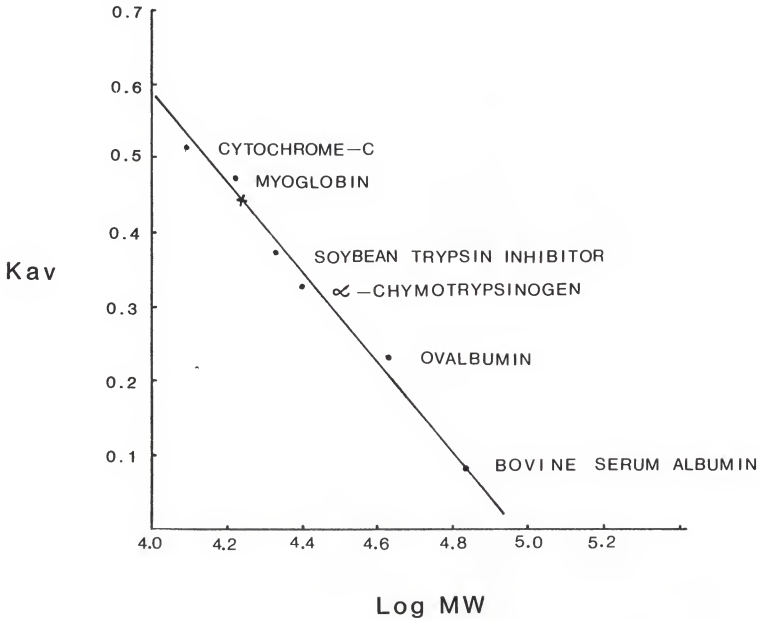

0.7
0.6
0.5
0.4
0.3
0.2
0.1
Kav
CYTOCHROME—C
MYOGLOBIN
SOYBEAN TRYPSIN INHIBITOR
∝ —CHYMOTRYPSINOGEN
OVALBUMIN
BOVINE SERUM ALBUMIN
4.0
4.2
4.4
4.6
4.8
5.0
5.2
Log MW

Fig. 13. A sedimentation equilibrium experiment with α-globulin (HA-prep). X-axis (R^2) is the square of the distance from meniscus to the center of the sample solution. C in the Y-axis (ln C) is the concentration of the sample solution.

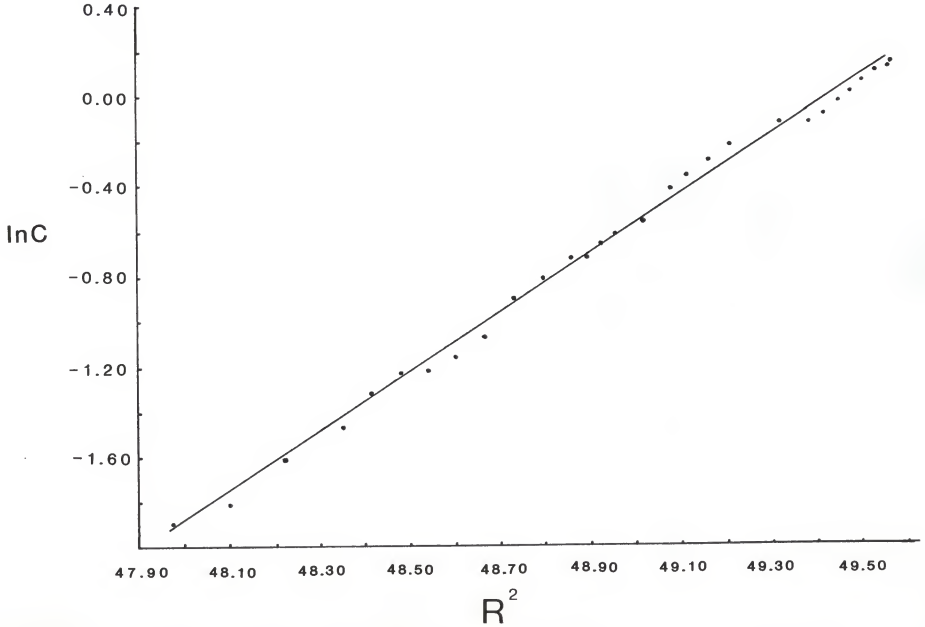

0.40
0.00
-0.40
-0.80
-1.20
-1.60
lnC
47.90
48.10
48.30
48.50
48.70
48.90
49.10
49.30
49.50
R2

Fig. 14. Far UV circular dichroism spectra of HA-prep (solid line) and pH 4.5-prep (broken line) α-globulin in 0.05 M Tris-Cl (pH 8.2)/0.5 M NaCl.

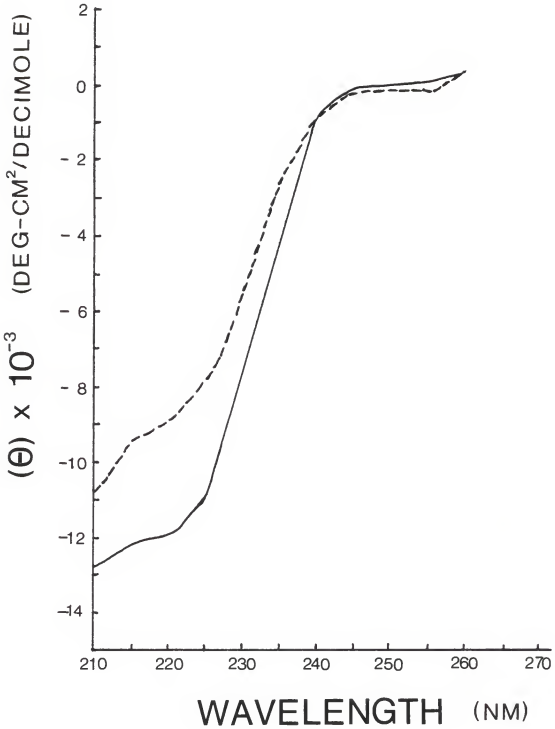
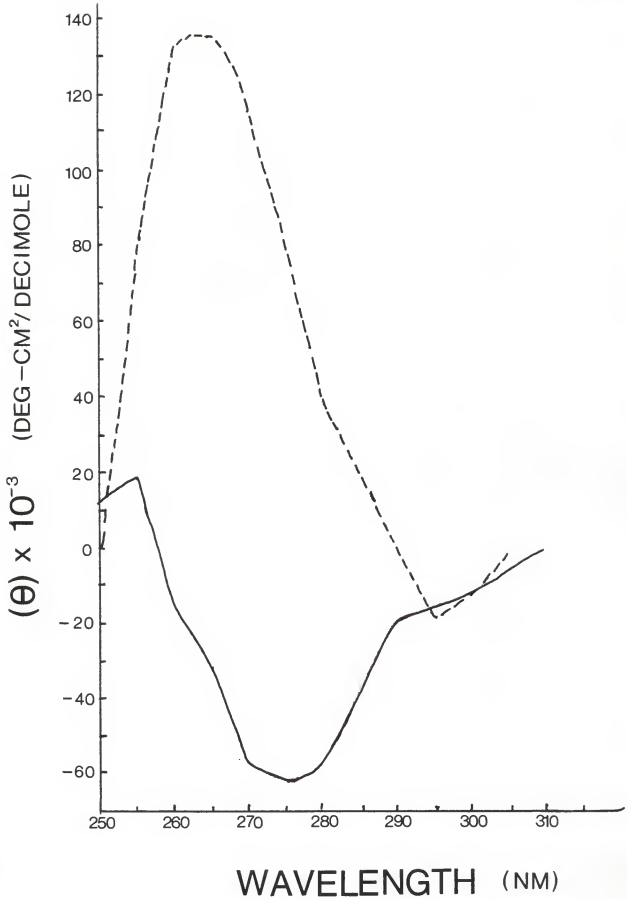

(Θ) x 10^{-3} (DEG-CM^2/DECIMOLE)
2
0
- 2
- 4
- 6
- 8
-10
-12
-14
210
220
230
240
250
260
270
WAVELENGTH (NM)

Fig. 15. Near UV circular dichroism spectra of HA-prep (solid line) and pH 4.5-prep (broken line) α-globulin in 0.05 M Tris-Cl (pH 8.2)/0.5 M NaCl.


140
120
100
80
60
40
20
0
-20
-40
-60
(θ) x 10^-3 (DEG-CM²/DECIMOLE)
250
260
270
280
290
300
310
WAVELENGTH (NM)

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ELECTROPHORETIC STUDIES OF RICE PROTEINS AND CHARATERIZATION OF RICE ENDOSPERM α-GLOBULIN

by

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B.A., Chung Hsing University, Taiwan, 1981

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF FOOD SCIENCE

Department of Biochemistry

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1984

Investigation of the grain proteins of several cereals has shown that valuable information about the genotype, pedigree and genetic origins can be decoded by protein analysis using techniques such as gel electrophoresis and isoelectric focusing. Several different electrophoresis procedures have been applied to rice proteins. These are all one-dimensional procedures. Because of the complexity, including various pI's, molecular weights and abundance, rice proteins can not be well separated by a method that involves one parameter only. My study is the first that combines two techniques, pH gradient electrophoresis (PHGE) and SDS polyacrylamide gel electrophoresis (SDS-PAGE), to provide a clear picture of rice endosperm proteins. This two-dimensional gel electrophoresis system separates proteins according to their charges in the first dimension (PHGE) and in the second dimension (SDS-PAGE), their molecular weights. I have figured out the conditions of the best resolution for each of the four Osborne protein fractions as well as the total rice endosperm proteins.

More detailed studies of major rice endosperm globulin, α-globulin, have been conducted in this study. Compared with the commonly used pH-4.5 precipitation method, Hydroxyapatite chromatography is a better method for α-globulin isolation, it gives higher yield and keeps the protein under nondenatured condition. The α-globulin so obtained has a molecular weight of 13,300 estimated by sedimentation equilibrium method. Amino acid analysis shows that Glx, Ser, Gly and Arg are the four most abundant amino acids in this protein.