Extraction and Characterization of Rice Proteins

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ABSTRACT

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Protein content of 60-mesh, defatted rice (*Oryza sativa* L.) meal was 10.6% on a dry weight basis. Fractionation of rice proteins yielded albumin, globulin, prolamin, and glutelin in the proportions of 8:9.5:12.5:70, respectively. The ultraviolet absorption spectrum, amino acid composition, isoelectric points, and subunit constitution of proteins were distinctly different for each fraction. Nonoverlapping of isoelectric points and molecular weights of protein subunits suggested the absence of cross-contamination between various fractions. The respective chemical scores of albumin, globulin, prolamin, and glutelin fractions were 47.5, 53.0, 23.0, and 46.7. Leucine, lysine, sulfur-containing amino acids, and threonine were the limiting amino acids of total proteins in rice with respective scores of 65.1, 66.3, 67.9, and 78.9. Estimates of biological values of protein fractions in human nutrition qualified albumins as superior and prolamins as inferior proteins. Two-dimensional slab gel electrophoresis, with phenol-acetic acid-mercaptoethanol-urea (PAMU) system in the first dimension and sodium dodecyl sulfate system in the second dimension indicated that the mobility of protein in PAMU may depend on its molecular size.

Rice has been an important staple cereal since ancient times. Through the decades, natural spoilage of rice has furnished a basis for its desirable fermented products such as sake, amarillo, and idli (van Veen 1972) by virtue of their appeal and nutritional qualities. As a part of our biochemical studies on the legume, black gram (*Phaseolus mungo* L.), we proposed to investigate the fermentation for idli. This necessitated the studies on the constituents of idli—black gram and rice.

The studies were undertaken to investigate the Texas long-grain rice, on which work has not been reported. Extraction and composition of other varieties of rice have been studied (McIntyre and Kymal 1956, Cagampang et al 1966, Tecson et al 1971). Because the composition of rice is influenced by the variety and environment (McCall et al 1953), the reported results could not be extrapolated to Texas long-grain rice. This article presents the results on the extraction and characterization of the proteins in Texas long-grain rice. We evaluated rice proteins for their relative amounts and amino acid compositions and attempted to predict their nutritional quality. To our knowledge, this is the first report of the isoelectric points of rice proteins.

MATERIALS AND METHODS

Material

Polished rice (Texas long grain) was pulverized in a Wiley mill with a 60-mesh screen and defatted in a Soxhlet extractor with *n*-hexane for 20 hr. The meal was stored at 5° C after removal of hexane.

Fractionation of Rice Proteins

The rice proteins were extracted according to the procedure detailed earlier (Padhye and Salunkhe 1977). All the freeze-dehydrated samples were stored at 5° C.

Polyacrylamide Gel Electrophoresis

The protein fractions were electrophoresed on flat-bed, polyacrylamide gel with two different systems in two directions. Dissociating medium, phenol-acetic acid-mercaptoethanol-urea (PAMU), was used in the first dimension and sodium dodecyl sulfate (SDS) system in the second. The molecular weights of the subunits were determined separately by the one-dimensional SDS method. The electrophoretic procedures and the protein standards for molecular weight determination were reported earlier (Padhye and Salunkhe 1977).

Isoelectric Focusing

Polyacrylamide gels (0.5 cm diameter, 11 cm length) containing 6*M* urea and LKB-ampholine (pH 3.5–10) were prepared according to Llewellyn and Flaherty (1976). Freeze-dehydrated protein samples were dissolved in deionized water containing 6*M* urea, 2% mercaptoethanol, and 5% sucrose. The focusing was performed for 5 hr during which the current flowing through each gel was reduced from 1 to 0.16 mA. Anode buffer was 0.02*M* NaOH containing 6*M* urea (pH 12.4) and cathode buffer was 0.06% orthophosphoric acid with 6*M* urea (pH adjusted to 1.7). Gels having protein samples were stained with bromphenol blue. The gels run without protein samples were cut 1 cm in length, dispensed in small vials, and immersed in 2 ml of deionized water. The pH was noted with a combined electrode and the pH gradient along the gel length was constructed.

Amino Acid Analysis

Protein samples (1–2 mg) were hydrolyzed for 24 hr at 110° C in 3 ml of 3× glass distilled 5.7*N* HCl in sealed and evacuated tubes. The resultant hydrolysate was lyophilized to dryness and dissolved in 0.2*M* citrate buffer, pH 2.2. Analyses were made on a Beckman model 120-B amino acid analyzer, using the Durrum single column, three buffer elution system. Tryptophan content was determined by the spectrophotometric method (Bencze and Schmid 1957).

The amino acid composition was used to estimate the nutritional value of the protein based on the provisional amino acid scoring pattern (WHO 1973). The evaluated nutritional parameters included the proportion of total essential amino acids with total amino acids in the protein (E/T%), limiting essential amino acids, chemical score, protein efficiency ratio (PER), and biological value (BV). PER values were estimated by the regression equations proposed earlier (Alsmeyer et al 1974). BVs of proteins in human nutrition were predicted using equations developed by Mørup and Olesen (1976).

Analytical Procedures

The semimicro Kjeldahl method was used to quantitate the nitrogen content of rice (AOAC 1960). The crude protein content was obtained by employing the conversion factor of 5.95. Protein contents in the extracts were assayed by Lowry's method (Lowry et al 1951). Protein fractions were scanned for absorbances in the ultraviolet region from 220 to 320 nm on a Beckman DB-G spectrophotometer equipped with a recorder.

RESULTS AND DISCUSSION

Fractionation of Rice Proteins

The crude protein content of Texas long-grain rice on moisture free basis was 10.6 ± 0.1%. Osborn's protein fractionation procedure, when performed as outlined earlier (Padhye and

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Salunkhe 1977), extracted 26.4% of the proteins. The extraction depends on the sequence in which the solvents are used, vigor of extraction, and the condition of starting material (Bietz and Wall 1975). A part of the isolated globulins were denatured during their separation from albumins. The irreversibly denatured proteins were centrifuged out and were not accounted for. Thus, the fractions isolated herein conformed to their expected dissolution properties, although percent protein extraction remained low.

Cagampang et al (1966) have reported the extraction of 97% of the endosperm proteins when 100-mesh rice was treated with a 20-fold amount of 0.1*N* NaOH for 6 hr. McIntyre and Kymal (1956) extracted 97–98% of the proteins when alkyl benzene-sulfonate (4%), sodium carbonate (2%), and sodium bisulfite (0.29%) were used in fivefold excess amounts for three extractions lasting 2, 4, and 9 hr. Earlier work on black gram (Padhye and Salunkhe 1977) has confirmed that protein solubilization is more effective under conditions such as pH extremes or the presence of chemicals like polyphosphates and detergents. Proteins so extracted, however, have contaminating residues or modified moieties.

The albumins, globulins, prolamins, and glutelins extracted from 60-mesh defatted rice flour were in the proportions of 8:9.5:12.5:70, respectively. This ratio of the solubilized fractions varied markedly with the milling fraction. Juliano (1972) has reported this ratio to be 30:14:5:51 for the bran and 5:9:3:83 for the milled rice. When compared with the reported values, the relative proportion of the prolamins (12.5%) seemed to be higher. In addition to the variety of rice employed, removal of a part of globulins and glutelins due to their irreversible denaturation in certain fractionation steps might have increased the prolamin content in this study.

TABLE I
Amino Acid Composition of Rice Proteins and Their Fractions (mol %)

Amino Acids	Protein Fraction				Rice Proteins
	Albumin	Globulin	Prolamin	Glutelin	
Aspartic acid	9.6	8.2	8.3	9.7	9.1
Threonine	5.1	2.7	1.3	3.0	3.5
Serine	4.7	7.1	5.1	5.4	4.8
Glutamic acid	12.0	16.4	19.9	16.9	16.9
Proline	5.9	4.2	5.5	6.0	5.4
Glycine	13.0	9.8	6.2	8.9	8.9
Alanine	11.1	8.1	9.5	7.9	8.6
Cysteine	2.2	0.9	Trace	1.7	0.9
Valine	6.8	7.1	7.0	6.8	6.7
Methionine	1.5	2.6	0.8	1.7	1.3
Leucine	3.3	4.0	4.4	4.1	4.6
Isoleucine	5.9	6.5	12.3	7.0	8.4
Tyrosine	2.8	2.4	6.4	3.7	3.7
Phenylalanine	2.6	3.7	4.4	4.1	4.0
Lysine	5.1	3.2	1.0	2.3	3.4
Histidine	2.2	2.2	1.7	2.1	2.2
Arginine	5.3	10.0	4.6	7.8	6.6
Tryptophan	1.0	0.9	1.6	1.0	1.1
Classified Distribution of Amino Acids					
1. Hydrophobic	38.1	37.1	45.5	38.6	40.1
2. Uncharged polar	27.8	22.9	19.0	22.7	21.8
3. Basic	12.6	15.4	7.3	12.2	12.2
4. Acidic	21.6	24.6	28.2	26.6	26.0

Amino Acid Composition

Amino acid analyses of rice proteins and their fractions are presented in Table I. Gross comparison between the protein fractions suggests that the albumins contained higher proportions of the uncharged polar amino acids (27.8 mol %) and lower amounts of acidic amino acids (21.6 mol %). Basic amino acid content (15.4 mol %) was the highest in globulins that registered low in hydrophobic amino acids (37.1 mol %). The classified distribution (based on acid-base properties) of amino acids in glutelin indicated that the values were intermediate for all classes of amino acids. The most striking was the composition of the prolamin fraction. Among four fractions, prolamin had the highest amounts of hydrophobic (45.5 mol %) and acidic (28.2 mol %) amino acids, whereas the contents of basic (7.3 mol %) and

TABLE II
Nutritional Evaluation of Rice Proteins and Their Fractions

Nutritional Parameter	Albumins	Globulins	Prolamins	Glutelins	Total Rice Proteins
E/T, %[a]	40.8	38.0	43.2	38.4	41.0
Chemical Score[a]	47.5	53.0	23.0	46.7	65.1
Limiting amino acids[a]					
First	Leu (47.5)	Leu (53.0)	Lys (23.0)	Lys (46.7)	Leu (65.1)
Second		Thr (55.6)	Met + Cys (26.8)	Leu (57.9)	Lys (66.3)
Third		Lys (72.0)	Thr (31.2)	Thr (66.1)	Met + Cys (67.9)
Estimates of PER[b]					
I	0.65	0.96	1.09	0.97	1.2
II	−0.66	0.78	0.56	0.85	1.1
III	−1.76	−2.24	−6.26	−2.29	−2.1
Estimate of Biological value[c]	74.5	54.9	3.1	31.2	42.0

[a] E/T% = Proportion of total essential amino acids and total amino acids. Limiting amino acids and chemical scores were evaluated with WHO-UN (1973) provisional amino acid pattern as the reference. The figures in parentheses correspond to the amino acid scores.

[b] Protein efficiency ratio (PER) values were estimated according to the regression equations proposed by Alsmyer et al (1974), as follows:

I. PER = −0.684 + 0.456 (Leu) − 0.047 (Pro)
II. PER = −0.468 + 0.454 (Leu) − 0.015 (Tyr)
III. PER = −1.816 + 0.435 (Met) + 0.780 (Leu) +0.211 (His) − 0.944 (Tyr)

[c] Biological values (BV) of proteins were predicted according to the regression equation computed by Mørup and Olesen (1976).

$$BV = 10^{2.15} \times q_{Lys}^{0.41} \times q_{Phe+Tyr}^{0.60} \times q_{Met+Cys}^{0.77} \times q_{Thr}^{2.4} \times q_{Trp}^{0.21}$$

where $q_i = \frac{a_i \text{ sample}}{a_i \text{ reference}}$, for a_i sample $\leq a_i$ reference and $q_i = \frac{a_i \text{ reference}}{a_i \text{ sample}}$, for a_i sample $\geq a_i$ reference

a_i represented milligrams of the amino acid per gram of total essential amino acids.

uncharged polar (19.0 mol %) amino acids were the lowest. The contrast between the compositions of prolamin and other fractions seemed more magnified when comparison was made on the basis of individual amino acids. Threonine, glycine, cysteine, methionine, lysine, histidine, and arginine contents were the lowest in prolamins that were constituted with the highest amounts of glutamic acid, leucine, isoleucine, tyrosine, phenylalanine, and tryptophan. These observations regarding the relative composition of prolamin with other fractions coincided with the data (Tecson et al 1971) presented by Juliano (1972) except for tryptophan content, which they reported to be low for prolamins.

Estimation of Nutritional Quality and Quantity

The nutritional quality of a protein is principally governed by its amino acid composition. Amino acid contents in Table I were further used to quantify certain nutritional parameters (Table II).

The World Health Organization of the United Nations (1973) has suggested the provisional amino acid scoring pattern for an ideal protein. The pattern recommends that for a good protein, the E/T ratio should be at least 36%. Rice proteins and protein fractions individually exceeded this criterion. Contrary to the expectation, the prolamins with E/T value of 43.2% ranked the highest. The known inferior quality of the prolamin fraction is, however, demonstrated by its essential amino acid scores. Computation of PER and BVs for rice proteins based on their amino acid compositions was rather unsatisfactory. PER values were estimated according to the regression equations (in footnote of Table II) formulated by Alsmeyer et al (1974). Biologically evaluated PER values for milled rice have been reported as 1.9 to 2.1 (Kennedy 1975) and 1.38 to 2.56 (Juliano 1972). It is clear from the calculated values in Table II that these equations, originally designed for meat products, were inapplicable to rice proteins. Predicted BVs (Table II) suggest that the prolamins had the lowest value (3.1), whereas albumins marked the highest (71.5) and the value for the total proteins was intermediate. Lozsa and Koller's work in 1954, as referred to by Juliano (1972), indicated that the BVs for milled rice, albumin + globulin fraction, prolamins, and glutelins when fed to animals were 81.4, 85.4, 35.0, and 85.4, respectively. The BVs estimated by Mørup and Olesen's (1976) equation were, thus, consistently lower than the reported values.

Ultraviolet Spectra

The ultraviolet absorption spectra were recorded to characterize the isolated protein fractions (Fig. 1). All protein fractions showed a trough between 250 and 262 nm and a peak between 272 and 285 nm. Protein fractions separated on the basis of solubility often contain nonproteinaceous contaminants such as carbohydrates, nucleic acids, and pigments. The ratios of absorbances at 280 and 260 nm (A_{280}/A_{260}) were computed to judge possible nucleic acid contamination. A_{280}/A_{260} for albumin, globulin, prolamin, and glutelin fractions was 1.5, 0.8, 1.1, and 1.4, respectively (Fig. 1). Globulins contained lower amounts of tryptophan, tyrosine, and phenylalanine. this partly explained the low absorption ratio for the globulins. The absorption ratio of 0.8 suggested, however, that the globulins may be contaminated with the nucleotides.

Isoelectric Focusing

The isoelectric points of rice proteins are defined in Fig. 2. Incorporation of 6*M* urea and 2% β-mercaptoethanol reduced protein aggregation and increased its solubility. The dissociated proteins remain soluble, even at their isoelectric points, in 6*M* urea and the use of dissociating medium restricts the microheterogeneity aspects mainly to differences in the primary structure (Catsimpoolas and Wang 1971). In spite of the dissociating conditions, a part of the glutelins remained unfocused. Although two-dimensional slab gel electrophoresis demonstrated multiplicity of subunits, the albumins showed a single band at pH 6.42. Isoelectric points of globulins varied between pH 5.85 and 7.27. The proportion of protein(s) having isoelectric pH of 5.85 seemed to be higher as the corresponding band was broad and pronounced. Five bands closely spaced in the pH span of 6 to 6.5 characterized the prolamins. Glutelins demonstrated two ranges of isoelectric points. They showed the presence of seven acidic proteins (pH range, 5.70–6.86) in addition to five basic proteins (pH range, 8.00–8.72).

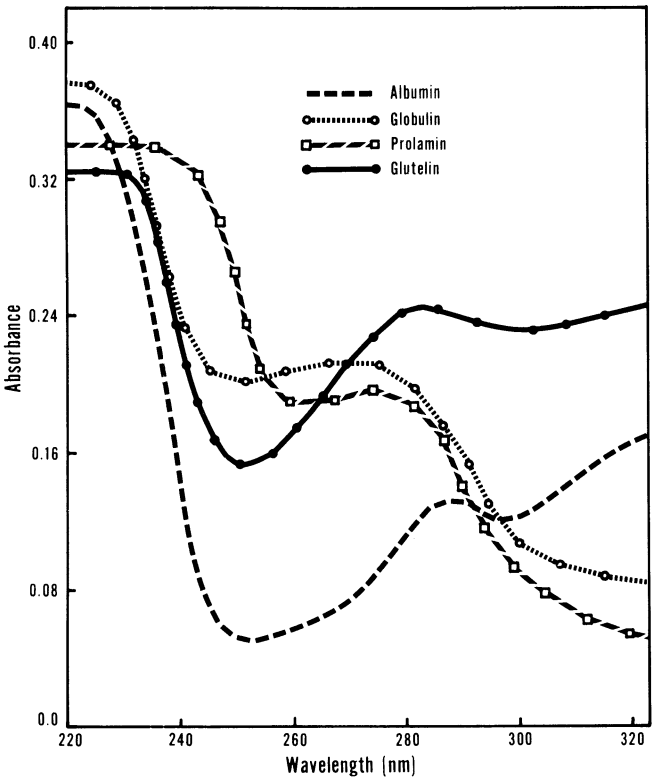

Fig. 1. Ultraviolet absorption spectra for the protein fractions isolated from Texas long-grain rice.

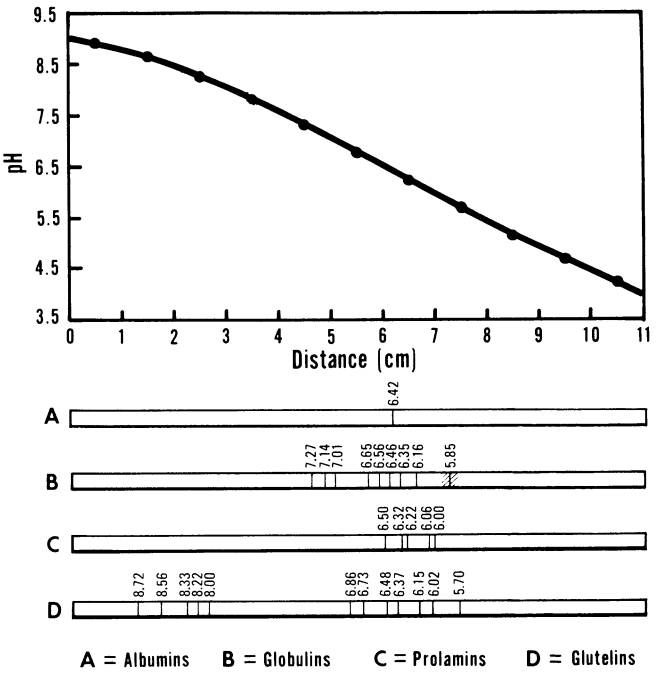

Fig. 2. Isoelectric focusing of protein fractions isolated from Texas long-grain rice. The graph demonstrates the pH profile established when ampholines (pH 3.5–10) were electrophoresed in polyacrylamide gels for 5 hr, during which current flowing through each gel was dropped to 0.16 mA from its initial value of 1 mA.

Two-Dimensional Gel Electrophoresis

Separation of rice protein fractions on two-dimensional slab gels is seen in Fig. 3A–D. Presence of six major proteins in rice albumins is evident from Fig. 3A. The molecular weights as computed by the mobilities in the SDS system were 135,000, 43,700, 31,600, 20,300, 15,300, and 7,400. The protein(s) of molecular weight 7,400 was the most prominent. It seems that the globulins were composed of several polypeptides. In addition to seven distinct proteins, there were 9 to 10 minor moieties (Fig. 3B). The molecular weights of the most pronounced globulin proteins were 60,300, 54,300, 32,600, and 13,200. Cagampang et al (1976) reported 20,000 and 12,000 as the molecular weights of two major polypeptides of rice globulins. Our results did not coincide with their observations. The findings differed from those of Juliano and Boulter (1976) regarding the molecular size of prolamin constitutents. Juliano and Boulter (1976) reported that rice prolamin has a single subunit of 23,000 daltons. Figure 3C indicates that the prolamin fraction was composed of two polypeptides of 12,600 and 7,200 daltons. Even though the separation obtained for the glutelins was far from ideal, presence of at least five major proteins could be concluded (Fig. 3D). Their molecular weights were 29,200, 15,300, 9,900, 9,200, and 7,700. Variations in the number of subunits and their molecular weights may be emerging from the systems employed for electrophoresis and the protein constitution of different cultivars of rice. Two-dimensional gel electrophoresis with urea system (first dimension) followed by SDS system (second dimension) has been reported to possess a better power of protein resolution than SDS electrophoresis alone (Johnson et al 1975). Our observations with rice proteins were similar. PAMU, however, failed to resolve glutelin proteins, particularly those of higher molecular weights such as of 29,200 and 15,300 daltons.

Johnson et al (1975) separated the envelope proteins of *Escherichia coli* across the entire surface of the slab gel when 8*M* urea system was followed by SDS system in the second dimension. They reported that the separation in urea system depended on the intrinsic charge on protein. Houston and Mohammad (1970) had proposed that urea (7.5*M*) could differentially denature two

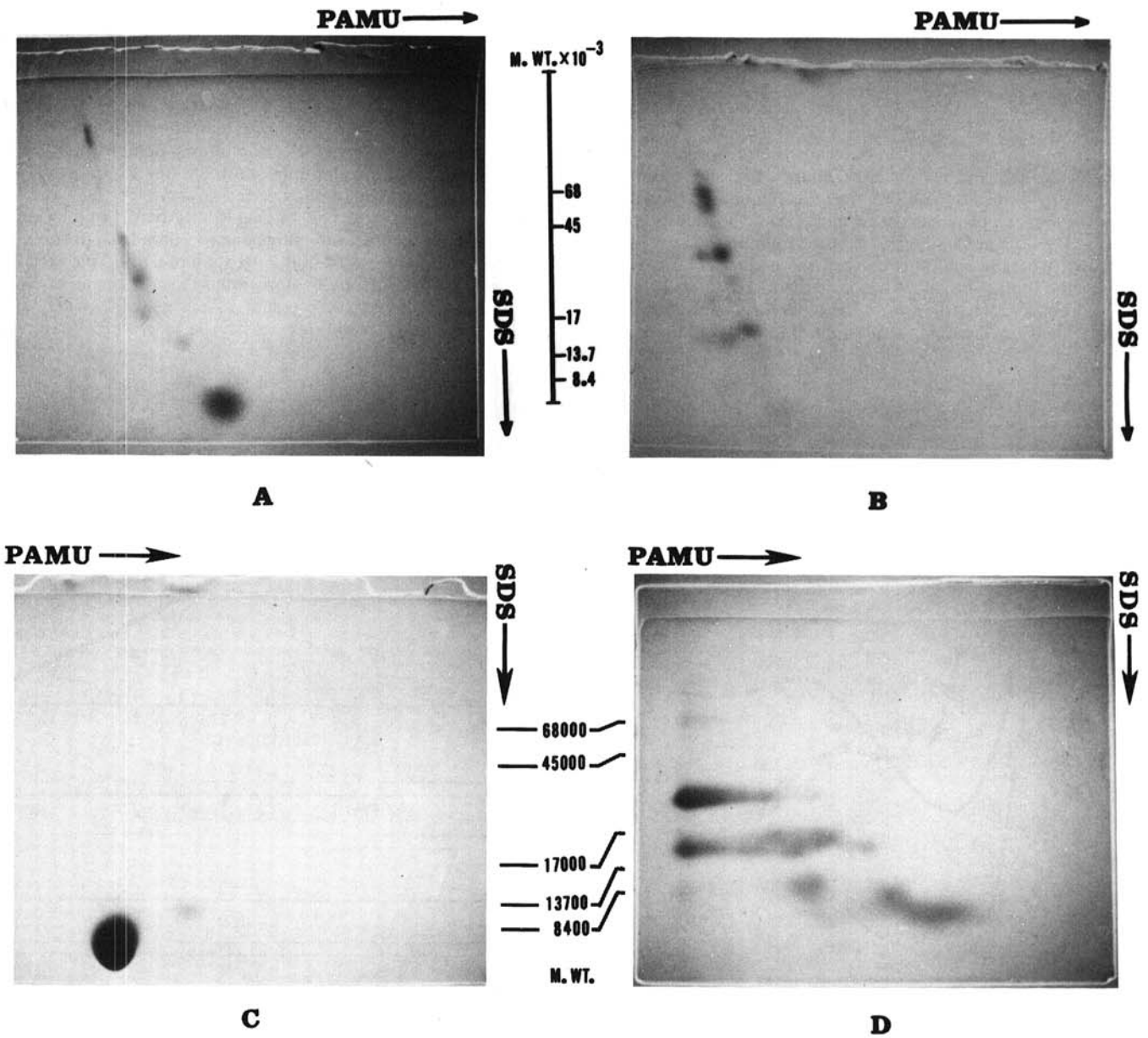

Fig. 3. Two-dimensional slab gel electrophoresis of protein fractions (Padhye and Salunkhe 1977) isolated from Texas long-grain rice. Phenol-acetic acid-mercaptoethanol-urea (PAMU) system was in the first dimension and sodium dodecyl sulfate (SDS) was in the second. **A** = Albumin fraction, **B** = globulin fraction, **C** = prolamin fraction, and **D** = glutelin fraction.

proteins having equal charges and molecular weights. The majority of the proteins in Fig. 3A,B were aligned along the diagonal, suggesting that the mobilities in PAMU and SDS systems were dictated by the related parameters. Similar were the observations of Hoffman and Ilan (1977) for ribosomal proteins and of Padhye and Salunkhe (1979) for black gram proteins. It is important to note that all proteins did not follow the "diagonal rule." The most interesting were the observations with rice globulins in Fig. 3B, which distinctly had three different types of proteins. Each type had a diagonal relationship for the two systems of electrophoresis but the mobility for each type was hindered to different extents in the urea system. Thus, three diagonals with common origin were seen on the slab gel (Fig. 3B).

Hydrodynamic properties of proteins in a urea system such as PAMU have not been studied. From the observed diagonal relationship, it seems that the mobility of a protein denatured by PAMU may depend on its molecular size. Concentration of urea and atypical composition of the protein seem to be critical factors and may alter the proposed behavior.

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