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SUBUNIT STRUCTURE AND IMMUNOLOGICAL PROPERTIES OF A BASIC 7S GLOBULIN FROM SOYBEAN SEEDS

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Key Word Index—*Glycine max*; Leguminosae; soybean protein; subunit structure; basic 7S globulin.

Abstract—Basic 7S globulin from defatted soybean (var. Miyagishirome) meal was isolated from the 0.5 M NaCl-soluble fraction by ion-exchange chromatography on CM-Sepharose CL-6B. The basic 7S globulin was distinguished immunologically from the other soybean proteins. SDS–urea gel electrophoresis of basic 7S globulin gave two kinds of subunits (M_r 16 000 and 26 000), designated as LMWS (low M_r subunit) and HMWS (high M_r subunit), respectively, in the presence of a 2-mercaptoethanol (2-ME)-containing system. They gave only one intermediary subunit (M_r 42 000) without 2-ME. Two kinds of HMWS (H-I and H-II) and two kinds of LMWS (L-I and L-II) were separated by polyacrylamide gel electrophoresis in a urea–acetic acid system. The basic 7S globulin was fractionated into two components (designated as basic 7S-I and basic 7S-II) on CM-Sepharose CL-6B. The subunits of basic 7S-I and basic 7S-II comprising H-I and L-I, and H-II and L-II, respectively.

INTRODUCTION

The soybean proteins are composed of multiple components, referred to as 2S, 7S, 11S and 15S globulins [1]. The 7S and 11S globulin are the major species and the 7S globulin fraction contains β-, γ-conglycinin and many other minor components. β-Conglycinin [2] and γ-conglycinin have already been isolated and identified in soybean globulin [3, 4]. We have previously described the isolation and partial characterization of a new globulin, namely, basic 7S globulin. This protein had higher isoelectric points at pHs 9.05 and 9.26 than did water-extractable globulins. SDS-gel electrophoresis of the basic 7S globulin gave two kinds of subunits (M_r 16 000 and 26 000) with the 2-ME-containing system and showed only one intermediate subunit (M_r 42 000) without 2-ME. The M_r was calculated as 168 000 determined by a cross-linking reagent followed by SDS-gel electrophoresis [5]. The present paper describes the subunit structure and immunological properties of the basic 7S globulin.

RESULTS AND DISCUSSION

A basic 7S globulin was purified by partially modified methods as described previously [5]. Figure 1B shows the polyacrylamide gel electrophoresis pattern of the purified basic 7S globulin. It consists of a high M_r subunit (HMWS) and low M_r subunit (LMWS), both subunits form intermediary subunit (IS) when subjected to electrophoresis a dissociating buffer without 2-ME (Fig. 1C). The result was identical with that of a previous report [5]. The basic 7S globulin showed a single band on disc gel electrophoresis according to Reisfeld *et al.* [6] (Fig. 1D).

Double gel immunodiffusion showed a single precipitation band between the purified basic 7S globulin and an antiserum to the basic 7S globulin. This precipitation band fused with the bands between the NaCl-soluble fraction and the antiserum of basic 7S globulin. There was no precipitation reaction between the major soybean globulins (glycinin and β-conglycinin) and the antiserum to the basic 7S globulin. A similar result was obtained using immunoelectrophoresis. Thus, immunochemical evidence suggests that the basic 7S globulin is different from the other soybean globulins. The basic 7S globulin can be found in both the water-soluble and NaCl-soluble fractions. The basic 7S globulin was fractionated into two components (designated as 7S-I and 7S-II) by chromatography on CM-Sepharose CL-6B (Fig. 2). Characterization of the two basic 7S globulins by SDS–urea polyacrylamide gel electrophoresis is shown in Fig. 3. The relative mobilities of HMWS and intermediary subunit were slightly different between the two components.

The fractionation profile of the reduced *S*-carboxyamidomethylated (RCAM)-basic 7S globulin subunits was illustrated in Fig. 4. It can be seen from the electrophoretic patterns of the fractions (Fig. 5) that both HMWS and LMWS consist of two subunits (designated as H-I and H-II, L-I and L-II, respectively), and that H-I and H-II elute in fraction-2 (F-2) and fraction-1 (F-1). However, L-I and L-II could not be separated with either CM-Sepharose CL-6B in urea (Fig. 5) or DEAE–Sepharose CL-6B in urea (data not shown). The fractionation profiles of the RCAM-basic 7S-I and RCAM-basic 7S-II are illustrated in Figs 6 and 7. Although both basic 7S-I and basic 7S-II were not completely pure, it can be seen from the electrophoretic patterns shown in Fig. 6 and 7 that basic 7S-I was made of H-I and L-I and basic 7S-II was H-II and L-II. Both H-I and L-I, H-II and L-II were selectively linked by disulphide bonds with a 1:1 molar ratio giving an intermediary subunit. These results and those previously reported [5] suggested that basic 7S-I molecule consisted of four identical intermediary subunits having H-I and L-I subunits, similarly basic 7S-II molecule consisted of four identical intermediary subunits having H-II and L-II subunits.

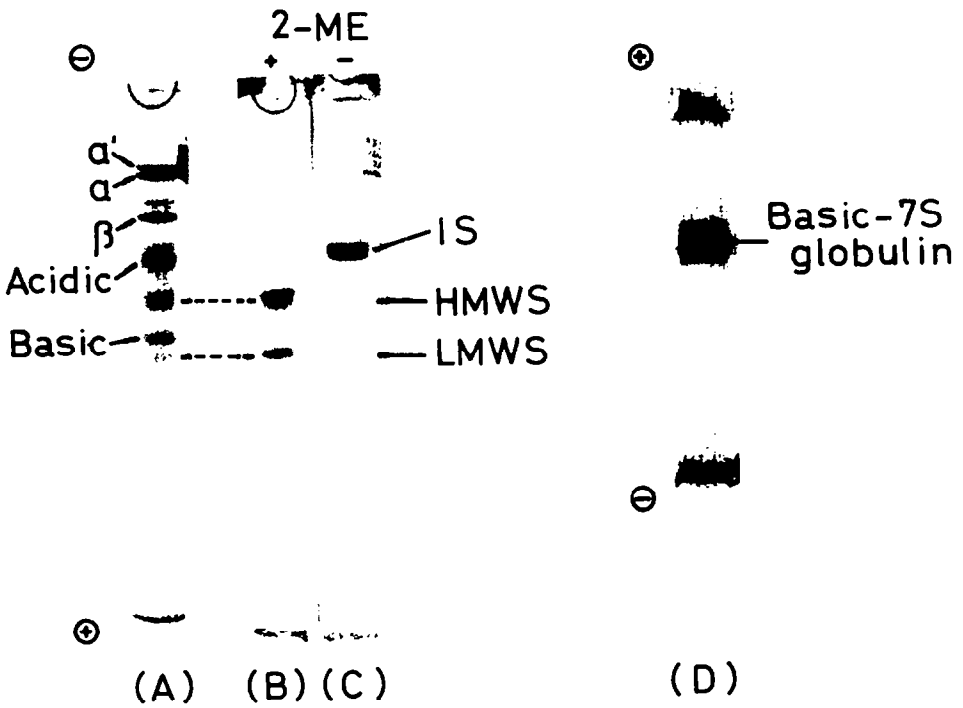

Fig. 1. Polyacrylamide gel electrophoretic patterns of basic 7S globulin. (A) SDS-urea gel electrophoresis of 0.5 M NaCl-soluble fraction toward the anode (+); (B) and (C) SDS-urea gel electrophoresis of the purified basic 7S globulin toward the anode (+); (D) electrophoresis of the purified basic 7S globulin toward the cathode (−) in pH 4.5 gel.

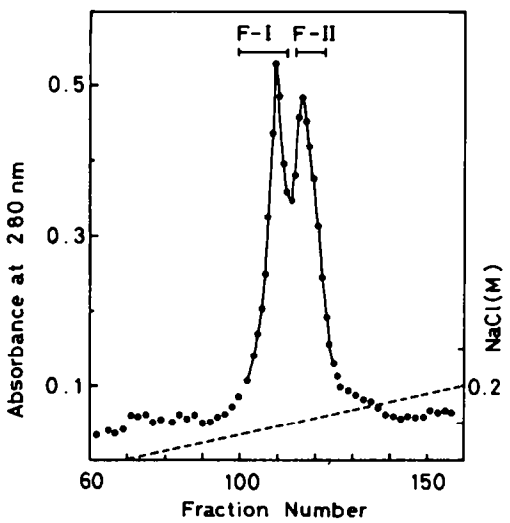

Fig. 2. Fractionation of basic 7S globulin on CM-Sepharose CL-6B. – Absorbance at 280 nm; - - - - - - - -, NaCl concentration.

On gel electrofocusing, basic 7S globulin showed multiple bands (Fig. 8). The isolated LMWS (L-I and L-II) and HMWS (H-I and H-II) were subjected to gel electrofocusing. The isoelectric point of HMWS was higher than that of LMWS, but there is little difference between H-I and H-II, L-I and L-II. The isoelectric points for HMWS and LMWS are from 7.7 to 7.9 and 6.5 to 7.0, respectively (Table 1). The lower isoelectric points of HMWS and LMWS than those of native protein may depend on a decrease of surface positive charge caused by the destruction of the steric protein structure with urea. From these results, the isoelectric points for HMWS seem to have a higher contribution from that for the basic 7S globulin. It is interesting to see a comparison with *Pisum sativum* legumin [7], where the LMWS have a higher p*I* than that for the HMWS and the latter contribute most significantly to the p*I* of the native molecule.

The *N*-terminal amino acid of the isolated subunits of H-I and H-II was valine and that of L-I and L-II was serine (Table 2). The amino acid compositions of each of the subunits are shown in Table 3. Although there are similarities in composition between H-I and H-II, and L-I and L-II, HMWS contained more histidine and proline and less methionine than LMWS. Both HMWS and LMWS contained more sulphur-containing amino acids than β-conglycinin and glycinin. This result was consistent with that of the previous report [5]. These data indicated that the basic 7S globulin has heterogeneous intermediate subunits like soybean glycinin [8, 9] and *Pisum sativum* legumin [7]. The present quantitative analysis by densitometry on gel electrophoresis showed that the basic 7S globulin contained 3.0% of the total extracted soybean protein [10].

EXPERIMENTAL

Materials. Soybean seeds (var. Miyagishirome, 1982, 1983 crops in Japan) were stored at 5°, ground with a coffee mill,

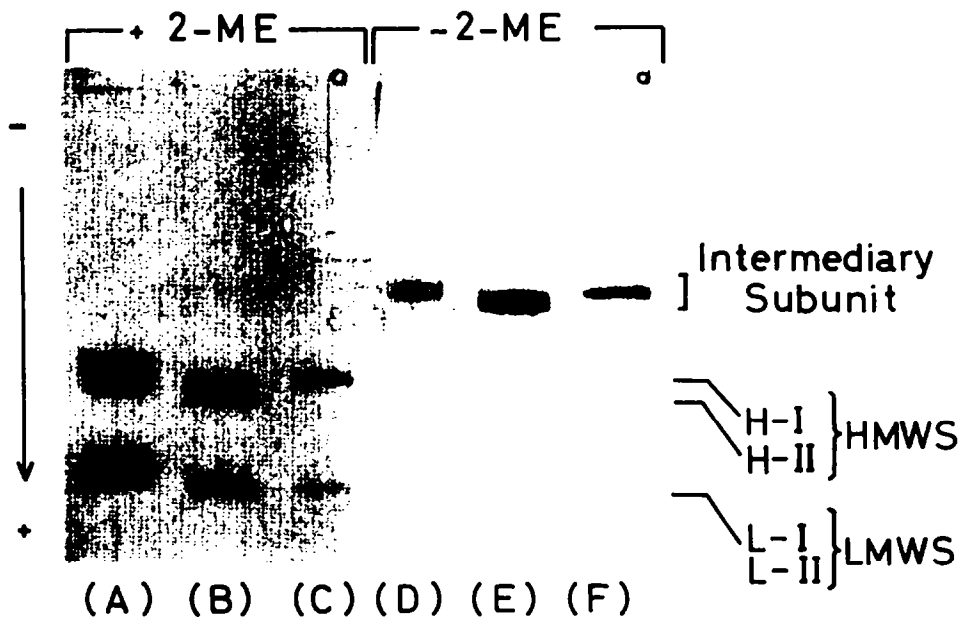

Fig. 3. SDS-urea polyacrylamide gel electrophoretic patterns of basic 7S globulin (A) (D), basic 7S-I (C) (F) and basic 7S-II (B) (E). (A), (B) and (C) contained 2-mercaptoethanol in the sample buffer.

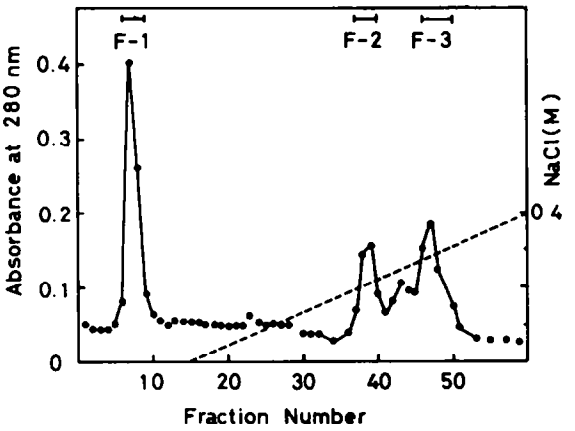

Fig. 4. Fractionation of the RCAM-basic 7S globulin on CM-Sepharose CL-6B in urea. —·—·—, Absorbance at 280 nm; – – – – – – –, NaCl concentration.

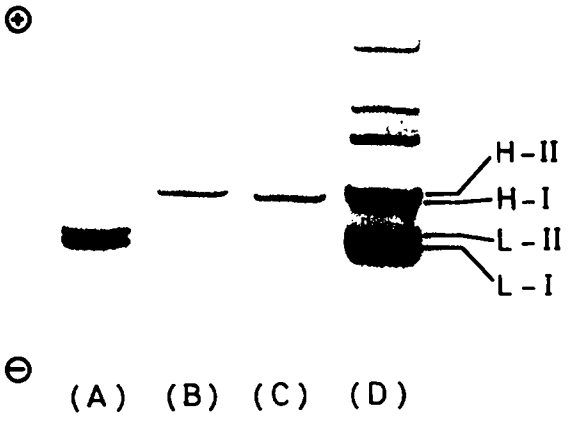

Fig. 5. Polyacrylamide gel electrophoretic patterns of RCAM-basic 7S globulin in the urea–acetic acid solvent system and the fractions in Fig. 4. (A) F-1; (B) F-2; (C) F-3; (D) RCAM-basic 7S globulin.

screened through a 60 mesh sieve, and defatted with hexane. Water- and NaCl-soluble fractions were prepared from defatted soybean seeds as described previously [5]. The β-conglycinin (7S globulin) and glycinin (11S globulin) were isolated by the method of ref. [2]. All the reagents were of the highest grade available. CM-Sepharose CL-6B was obtained from Pharmacia.

Purification of the basic 7S globulin. A basic 7S globulin was purified by partially modified methods as described previously [5]. Defatted soybean meals were extracted with 0.03 M Tris–HCl buffer (pH 6.5) containing 0.5 M NaCl and 0.01 M 2-ME (meal–buffer, 1:20) for 1 hr and centrifuged (10000 rpm for 20 min at 15°). The ppt. was extracted the same way once more. The mixed NaCl-soluble fractions were made 60% saturated $(NH_4)_2SO_4$. The soln was stirred for 30 min at room temp. and the ppt. was obtained by centrifugation at 10000 rpm for 15 min. The ppt. of the crude basic 7S globulin was dissolved in a buffer (2.6 mM KH_2PO_4, 32.5 mM K_2HPO_4, 0.4 M NaCl, 0.01 M 2-ME, 0.05% NaN_3, pH 7.6, ionic strength = 0.5: standard buffer). The crude basic 7S globulin was dialysed against the phosphate buffer without NaCl (ionic strength = 0.1, pH 7.6) overnight and then applied on a 2 × 28 cm column of CM-Sepharose CL-6B equilibrated with the same buffer. After elution of the non-adsorbed fraction with the starting buffer at a rate of 20 ml/hr, elution of the adsorbed fraction with NaCl in gradient concn of 0–0.4 M was carried out using a mixed chamber (containing 300 ml of the starting buffer) and a reservoir chamber (containing an equal vol. of the phosphate buffer made 0.4 M in NaCl, pH 7.6). Column effluents were collected in 5 ml fractions and monitored at 280 nm. The adsorbed fraction containing the basic 7S globulin was dialysed against H_2O and lyophilized.

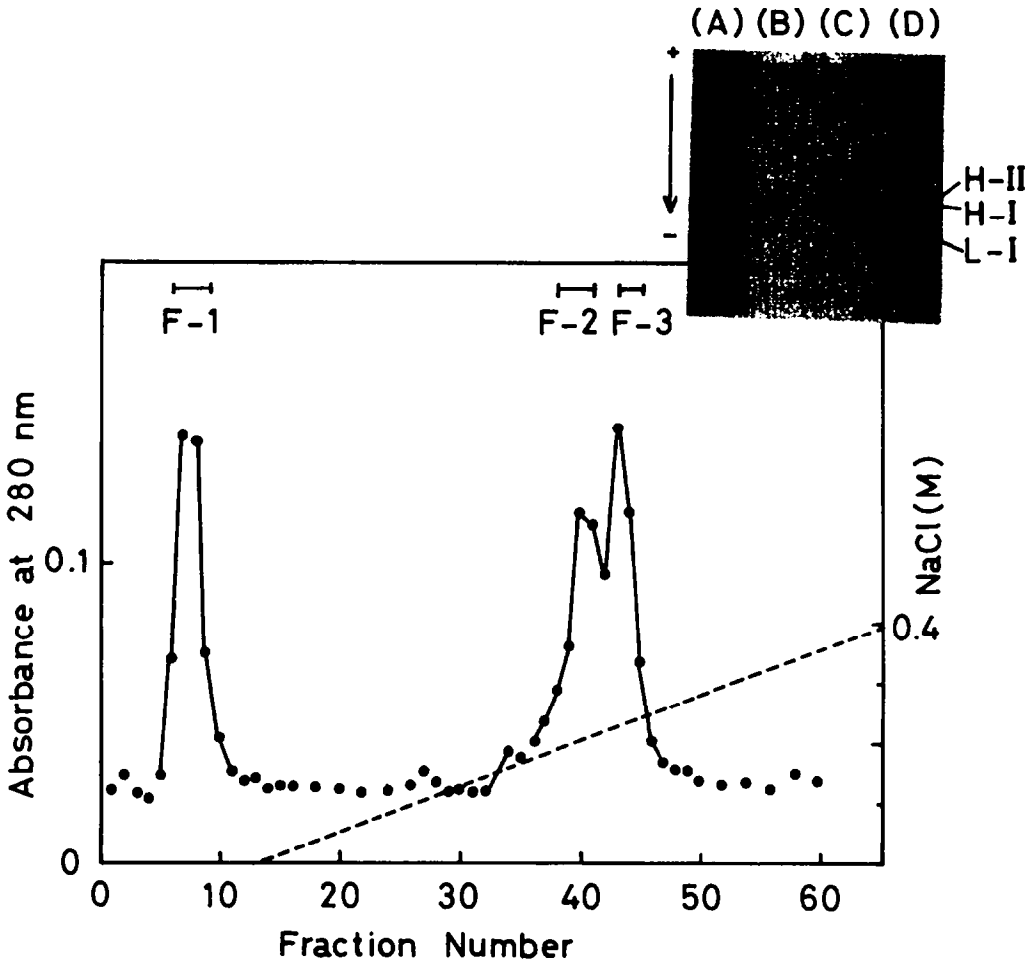

Fig. 6. Fractionation of the RCAM-basic 7S-I on CM-Sepharose CL-6B in urea. Chromatographic conditions are the same as in Fig. 4. Inset shows gel electrophoretic patterns of RCAM-basic 7S-I and the fractions in the urea–acetic acid solvent system. (A) F-1; (B) F-2; (C) F-3; (D) RCAM-basic 7S-I.

Table 1. Isoelectric point of the basic 7S globulin subunits

	Isoelectric point
Basic 7S globulin	pH 9.1–9.3*
HMWS	pH 7.7–7.9†
LMWS	pH 6.5–7.0†

*Without urea in the buffer.
†With urea in the buffer.

Table 2. M_r and *N*-terminal amino acid of the basic 7S globulin subunits

Subunit	M_r*	*N*-Terminal amino acid
H-I	26000	Val
H-II	26000	Val
L-I	16000	Ser
L-II	16000	Ser

*Estimated by SDS-polyacrylamide gel electrophoresis.

Immunochemical methods. The antiserum to basic 7S globulin was prepared by the procedure of ref. [11]. Double gel immunodiffusion was carried out according to the method of ref. [12]. The gel medium consisted of 1.2% (w/v) agar in the standard buffer. Protein samples were dissolved in the same buffer. The reactants were allowed to diffuse at room temp. for 24 hr. Immunoelectrophoresis in agar gel was carried out according to ref. [13]. The gel medium consists of 1.2% agar in pH 8.6 buffer, Tris–HCl, ionic strength 0.025. Electrophoresis was carried out for 1 hr with a current of 7.5 mA per microscope slide.

Fractionation of basic 7S globulin on CM-Sepharose CL-6B. Fractionation of basic 7S globulin was carried out on a 2 × 28 cm column of CM-Sepharose CL-6B equilibrated with a phosphate buffer (pH 7.6, ionic strength = 0.1). The adsorbed fraction was obtained by elution with NaCl in a gradient from 0 to 0.2 M (chambers contained 150 + 150 ml of the buffer) at a rate of 20 ml/hr. Fractions of 5 ml were collected and monitored of 280 nm.

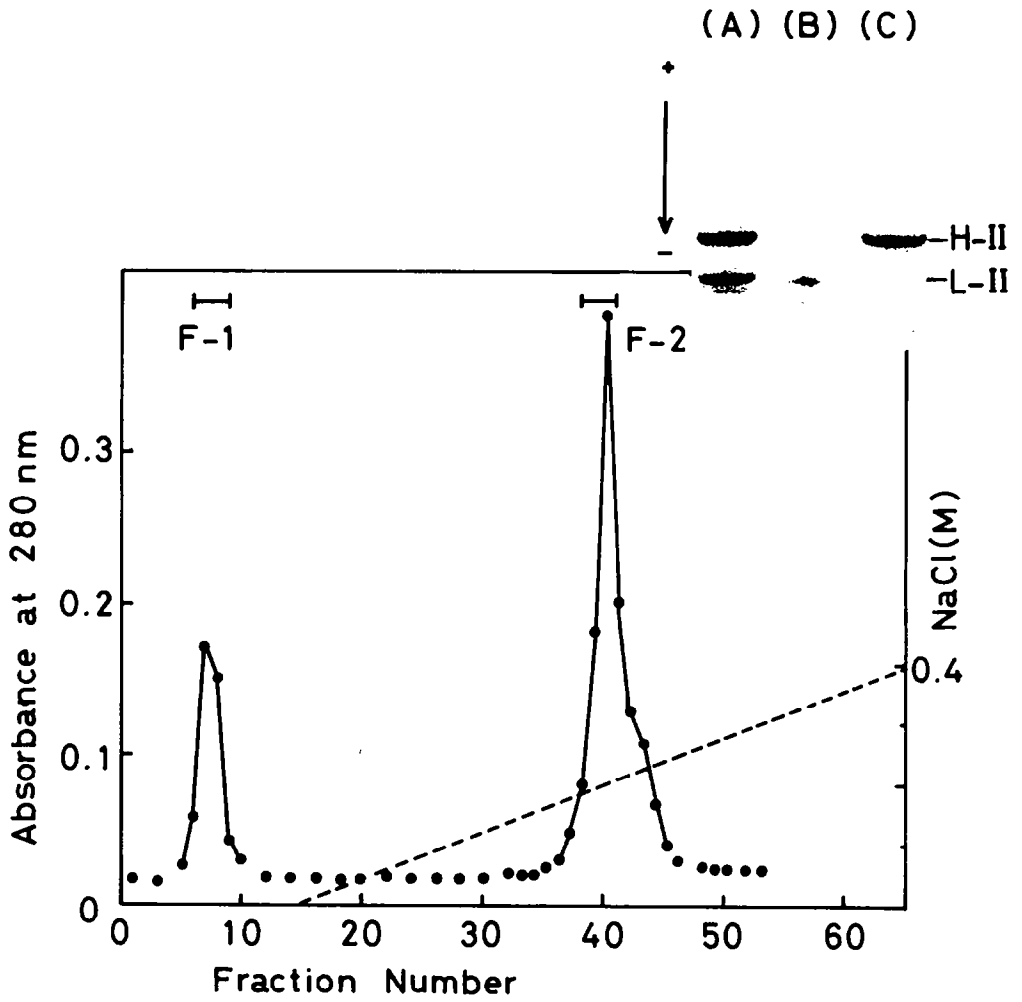

Fig. 7. Fractionation of the RCAM-basic 7S-II on CM-Sepharose CL-6B in urea. Chromatographic conditions are the same as in Fig. 4. Inset shows gel electrophoretic patterns of RCAM-basic 7S-II and the fractions in the urea–acetic acid solvent system. (A) RCAM-basic 7S-II; (B) F-1; (C) F-2.

Table 3. Amino acid composition of the subunits and basic 7S globulin

Amino acid	A.A. res.%/T.A.A. res.				
	H-I	H-II	L-I	L-II	Basic 7S globulin
Asx	9.32	9.46	8.48	8.96	9.45
Thr	7.66	8.16	5.94	5.47	7.50
Ser	7.24	6.49	9.43	8.12	8.27
Glx	12.42	12.19	12.36	11.06	12.47
Gly	7.76	7.58	9.57	9.62	8.42
Ala	6.55	5.98	7.20	8.60	6.81
Cys	3.07*	3.57*	2.03*	2.27*	1.38
Val	5.95	6.69	9.38	8.91	7.87
Met	1.72	1.56	4.62	4.77	2.29
Ile	3.83	3.93	2.97	3.13	3.80
Leu	10.33	10.95	8.55	8.52	10.43
Tyr	1.64	1.69	1.29	1.62	1.50
Phe	3.39	3.83	4.11	4.51	4.43
Lys	3.35	2.58	4.06	3.45	2.96
His	5.15	4.16	1.93	2.09	3.60
Arg	2.93	3.75	3.47	3.63	3.28
Pro	7.77	7.50	4.66	5.29	7.59

*Determined as carboxymethyl cysteine.

Preparation of RCAM-basic 7S globulin. Carboxyamidomethylation of basic 7S globulin was carried out as essentially described in ref. [14] with iodoacetamide and 0.15 M 2-ME and 8 M urea. The modified protein (RCAM-basic 7S) was dialysed against deionized water in the dark and then lyophilized.

Ion exchange chromatography of subunit. About 40 mg of the RCAM-protein in 5 ml of a phosphate buffer (41.8 mM NaH_2PO_4, 9.4 mM Na_2HPO_4, 1 mM EDTA·2Na, 0.02% NaN_3, pH 6.0) containing 6 M urea was fractionated on a column 2 × 22 cm) of CM-Sepharose CL-6B equilibrated with the same buffer. Ionic strength linear elution was achieved by adding 0.4 M NaCl soln to a mixing chamber (chambers contained 150 + 150 ml of the buffer) at a rate of 12 ml/hr. Fractions (10 ml of No. 1–15 and 5 ml of No. 16–60) were monitored at 280 nm.

Gel electrophoresis. Disc gel electrophoresis was performed in a 10% polyacrylamide gel according to ref. [8]. SDS-urea polyacrylamide gel electrophoresis was carried out in a 8% gel according to ref. [15]. Gel electrophoresis in urea–HOAc solvent was performed in a 8% polyacrylamide gel according to ref. [2]. All gels were stained with Coomassie Brilliant Blue G-250 and destained by diffusion in 7.5% HOAc–5% MeOH–H_2O.

Gel electrofocusing. Electrofocusing gels were prepared with a 5.0% acrylamide, 0.17% *N,N'*-methylene bisacrylamide, 2.5% ampholytes (pH 3.5–10) and 6 M urea, according to ref. [2]. Electrode solns were 0.01 M H_3PO_4 (anode) and 0.02 M NaOH (cathode). Gel electrofocusing was performed with 5 × 130 mm glass tubes. After electrophoresis had been completed, another

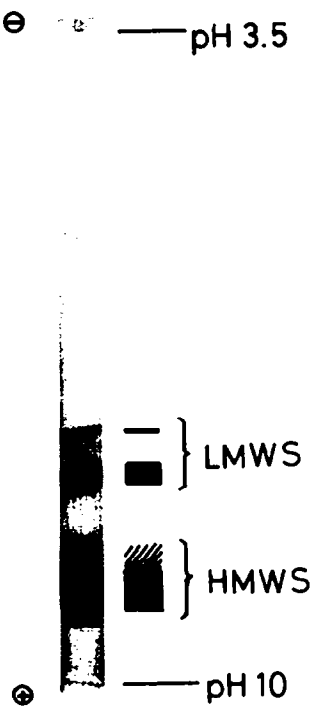

Fig. 8. Gel electrofocusing of the purified basic 7S globulin. Gel contained 5.0% polyacrylamide, 6 M urea and 2.5% ampholytes (pH 3.5–10).

gel was used for the pH-gradient measurement in gel cylinders by suspending 5 mm gel slices in 1 ml of distilled water overnight.

N-Terminal analysis. The *N*-terminal amino acid residue was determined by a gas-phase protein sequencer [16, 17].

Amino acid analysis. The protein samples were hydrolysed with 6 M HCl in evacuated and sealed tubes at 110° for 24 hr. The hydrolysate was subjected to analysis with an amino acid analyser (Hitachi, model KLA-3B).

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