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SUBUNIT STRUCTURE OF γ-CONGLYCININ IN SOYBEAN SEEDS

F. YAMAUCHI, W. SATO and Y. KAMATA

Department of Food Chemistry, Faculty of Agriculture, Tohoku University, Sendai 980, Japan

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Key Word Index—*Glycine max*; Leguminosae; soybean protein; subunit structure; electrophoresis; γ-conglycinin.

Abstract—Dissociated subunits of purified γ-conglycinin were isolated on a DEAE–Sephadex A-50 column. A single band was seen on two kinds of gel electrophoresis and isoleucine was shown as the only *N*-terminal amino acid. The isolated subunit reacted with antisera to the native γ-conglycinin. The M_r of the subunit was 51 000–51 500 estimated by urea–acetic acid and SDS–urea gel electrophoresis. A value of 50 000 was obtained by gel filtration with guanidine–hydrochloric acid on Sepharose CL-6B. The γ-conglycinin molecule was found to be made up of three subunits. This was determined by cross-linking the subunits and then submitting them to gel electrophoresis. Differences and similarities of subunit structure among γ-conglycinin, β-conglycinin and glycinin are discussed.

INTRODUCTION

Catsimpoolas and Ekenstam [1] have isolated four major antigenically different components from the soybean reserve proteins. One of them was given the name γ-conglycinin. Some of the properties of this protein have been described by Koshiyama and Fukushima [2]. Since γ-conglycinin is a minor protein among soybean proteins, complicated procedures for its purification are needed to remove the major proteins [2]. In a recent paper [3], we reported a simple procedure for the isolation of γ-conglycinin.

It has been reported that γ-conglycinin gave nine subunit bands [4] on gel electrophoresis in a system containing urea, however, our recent study showed only one subunit band of the γ-conglycinin molecule on SDS gel electrophoresis. Conversely, Koshiyama and Fukushima [2] have reported that γ-conglycinin has a M_r of 104 000 when determined by equilibrium centrifugation. However, we found a higher M_r [3]. This paper describes the subunit structure of γ-conglycinin and the M_r calculated from its subunit composition.

RESULTS AND DISCUSSION

Purified γ-conglycinin was prepared using the procedure we reported in ref. [3]. The protein was dissociated by 6 M urea and isolated by ion-exchange chromatography on a DEAE–Sephadex A-50 column. A main peak, which showed a single band on SDS–urea and acetic acid–urea gel electrophoresis, and a minor peak were obtained. The isolated γ-conglycinin subunit reacted with the antisera against native γ-conglycinin, which suggests that either the isolated subunit regenerates the three dimensional structure corresponding to the native one to a high degree in an environment without urea, or the antigenic determinants are primarily dependent on the amino acid sequence for this protein. The *N*-terminal amino acid of γ-conglycinin was dansylated and analysed on an polyacrylamide sheet and a spot of isoleucine was observed. This is in accord with the single subunit band observed on gel electrophoresis.

An apparent M_r of 51 000 was obtained using urea–acetic acid gel electrophoresis. M_rs of 51 000 and 51 500 were obtained on electrophoresis in 5% and 10% gels of the SDS–urea system, respectively. The M_r determined by gel filtration on Sepharose CL-6B with 6 M guanidine–hydrochloric acid was 50 000. Thus, the values obtained using gel electrophoresis agreed with those obtained using gel filtration and are near those of the α- (57 000), α'- (58 000) and β-subunits (42 000) of β-conglycinin [5], but distinct from those of the acidic subunits (28 000) and basic subunits (18 000) of glycinin [6].

Isoelectric focusing of γ-conglycinin is shown in Fig. 1. In the region of the sharp bands, several appeared in the pH range 5.80–6.10, indicating microheterogeneity of the subunit. These values nearly equal the p*I* of the native protein (5.80) reported by Koshiyama and Fukushima [2]. The microheterogeneity of the β-subunit of β-conglycinin using gel electrofocusing has also been reported [7]. The p*I*s of the γ-conglycinin subunit are similar to those of the β-subunit of β-conglycinin which is located between 5.66 and 6.00 [7].

A minor subunit of β-conglycinin, the γ-subunit, has been reported [5] using urea–acetic acid gel electrophoresis. Its mobility was similar to that of the γ-conglycinin subunit. However, the γ-subunit was inseparable from the β-subunit of β-conglycinin using SDS–urea gel electrophoresis. However, the γ-conglycinin subunit definitely was separable from the β-subunit of β-conglycinin. Moreover, the *N*-terminal isoleucine of the γ-conglycinin subunit was different from that (glutamic acid [5]) of the γ-subunit of β-conglycinin.

The SDS–urea gel electrophoretic pattern of cross-linked proteins is shown in Fig. 2. The reference protein of modified rabbit muscle aldolase (c) gave four bands [8]: monomer (40 000), dimer (80 000), trimer (120 000) and tetramer (160 000). Modified γ-conglycinin (b) gave three bands with the one corresponding to the trimer as the most intense. From their mobilities on the gel, the M_rs of the oligomers were found to be 54 500 (monomer), 109 000 (dimer) and 154 000 (trimer). These results show

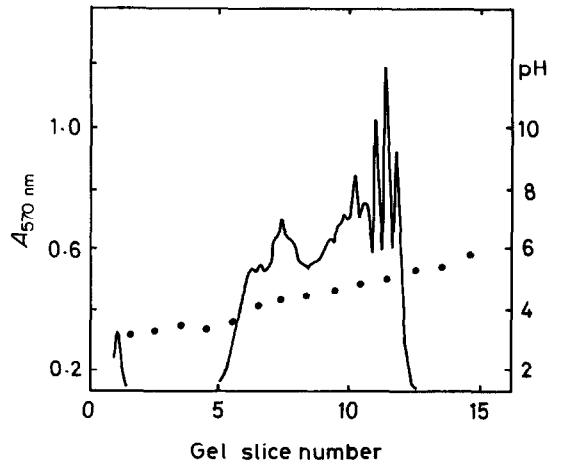

Fig. 1. Isoelectric focusing of the γ-conglycinin subunit. (——) $A_{570\,nm}$; (· · ·) pH.

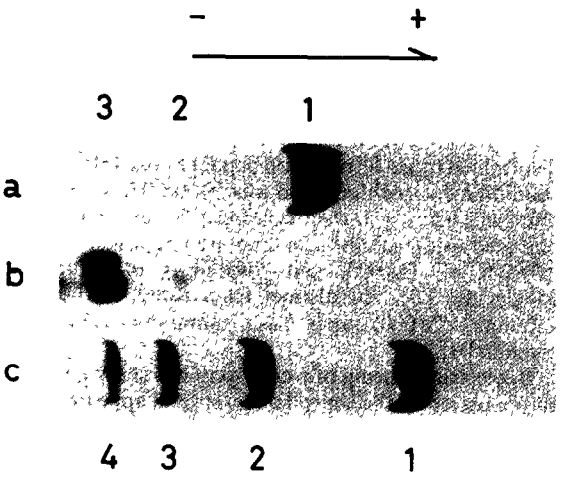

Fig 2. Polyacrylamide gel electrophoresis of the cross-linked subunits of γ-conglycinin and rabbit muscle aldolase. γ-Conglycinin (a), cross-linked γ-conglycinin (b) and rabbit muscle aldolase (c). The subunit numbers of cross-linked oligomers are shown.

that one γ-conglycinin molecule is made up of three subunits. These subunits are essentially identical, as shown by gel electrophoresis and *N*-terminal analysis. Similarly, it is suggested that β-conglycinin is made up of three subunits; however, this protein has several heterogeneities formed by different combinations of α-, α′- and β-subunits.

The M_r of γ-conglycinin was found to be 154000 using cross-linking reagent and SDS–urea gel electrophoresis. This is in agreement with the values determined by physico-chemical methods (163000–177000) [3] and is also three times the M_r of the subunit (50000–51500) estimated by gel electrophoresis and gel filtration. There is a difference, however, in the above values for γ-conglycinin's M_r and the value (104000) reported by Koshiyama and Fukushima [2].

EXPERIMENTAL

Proteins. Purified γ-conglycinin was prepared using fractionation at pH 6.4–5.7, pptn at 60% satn with $(NH_4)_2SO_4$, DEAE-Sepharose CL-6B chromatography and, finally, Sepharose CL-6B chromatography [3] Glycinin and β-conglycinin, from soybean were prepared by the method of ref. [9].

DEAE–Sephadex A-50 chromatography. Purified γ-conglycinin was dissociated into its subunits with a KPi–urea buffer (3.1 mM KH_2PO_4, 15.8 mM K_2HPO_4, 6 M urea, 1 mM EDTA, 0.02% NaN_3, pH 7.6). The sample soln containing the dissociated subunits was added to a column of DEAE–Sephadex A-50. The subunit was eluted by NaCl gradient elution from 0 to 0.5 M.

Polyacrylamide gel electrophoresis Urea–HOAc gel electrophoresis [6] was performed using a 6% acrylamide gel. SDS–urea gel electrophoresis was performed with 5% and 10% acrylamide gels according to the methods of ref. [5]. M_rs were determined by comparisons with the following standards: bovine serum albumin (68000), bovine liver catalase (58000), bovine γ-globulin (50000), porcine gastric pepsin (35000), bovine γ-globulin light chain (23500) and egg white lysozyme (14300). Isoelectric focusing was performed with a 5% acrylamide gel, 2% Ampholyte (pH 3.5–10) and 6 M urea [7]. The pH gradient in the gel cylinder was measured at 20° by suspending 5 mm slices of the gel in 1 ml H_2O for 24 hr.

Immunochemical methods Antisera of native γ-conglycinin was prepared using the procedure of ref. [1]. Double gel immunodiffusion in agar was carried out according to refs [3, 10].

Determination of M_r on a Sepharose CL-6B. This was performed by the method of ref [5]. Purified γ-conglycinin was dissociated into its subunits with 6.5 M guanidine–HCl in 1 M Tris–HCl (pH 8.5) containing 0.05 M dithiothreitol for 2 hr at room temp The soln containing the dissociated subunits was added to a column of Sepharose CL-6B.

N-Terminal analysis. The *N*-terminal amino acid residue was determined by the dansyl chloride method [11].

Cross-linking of subunits [8]. Native γ-conglycinin (4 mg/ml) was dissolved in 0.2 M triethanolamine–HCl (pH 8.5). Cross-linking reagent (dimethyl suberimidate) (4 mg) was added to the soln (0.5 ml), which was incubated for 3 hr at room temp A reference protein, rabbit muscle aldolase, was also similarly treated The cross-linked subunits were analysed by SDS–urea gel electrophoresis with 5% acrylamide gel in a slab gel apparatus.

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