High-Resolution Carbon-13 Nuclear Magnetic Resonance Study of the Soybean 7S Storage Protein Fraction in Solution

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The molecular structure and dynamics of soybean 7S storage proteins were investigated by high-resolution ^{13}C NMR at high fields (7.05 and 11.75 T). The spectrum of the alkali-denatured proteins (pH 12.0) is well-resolved, and tentative assignments of 53 peaks are given. At pH 12.0 and 12 mM ionic strength the proteins dissociate into partially unfolded subunits that exhibit fast local motion (10^{-9}–10^{-10} s), while they maintain a relatively immobile hydrophobic core. The spectra of "native", multisubunit proteins consist of broad peaks as a result of the slower protein tumbling in solution and the expected chemical shift nonequivalence. There is evidence that Phe residues are in the hydrophobic subunit core, whereas Tyr residues are found at the subunits' interface. No major spectral differences were observed between pH 7.6 and 10.3 (at 0.5 M ionic strength, where proteins exist as trimers) or between 13 mM ionic strength and 0.5 M NaCl (at pH 10.2, where a trimer–hexamer interconversion occurs). Potential applications of these results, and of the ^{13}C NMR techniques, in general, include quality control of soy protein isolates in food engineering and the analysis of complex foods that include soy protein ingredients.

INTRODUCTION

Soybean seeds contain the highest amount of protein in Leguminosae (30–50%), comprising an important protein source, hence the considerable amount of related research (Derbyshire et al., 1976; Koshiyama, 1983; Nielsen, 1985; Prakash and Narasinga Rao, 1986; Kinsella et al., 1985).

β-Conglycinin, the major 7S soybean globulin, is a heterogeneous class of glycoproteins, composed of at least seven distinct trimeric combinations of four major and two minor subunits that are structurally similar (Thanh and Shibasaki, 1976b, 1978; Sykes and Gayler, 1981; Coates et al., 1985; Hirano et al., 1987). The β-conglycinin protomer consisting of three monomers (subunits) appears to be a disk of 85-Å diameter, 35-Å thickness, and 180 000 molecular weight (Koshiyama, 1968b; Thanh and Shibasaki, 1978; Tulloch and Blagrove, 1985) and undergoes mostly reversible association–dissociation reactions with changes in pH and ionic strength (Roberts and Briggs, 1965; Koshiyama, 1968a; Iibuchi and Imahori, 1978b; Thanh and Shibasaki, 1979).

Nuclear magnetic resonance spectroscopy (NMR) has become a principal experimental technique in the investigation of the molecular structure and dynamics of biopolymers (Dwek, 1973; Wüthrich, 1976, 1986). Despite its relatively low sensitivity, ^{13}C NMR is a powerful method for the characterization of proteins in solution (Howarth and Lilley, 1978; Allerhand, 1979) and has been recently applied in the study of plant storage proteins such as wheat gliadins, barley hordein, and corn zein (Baianu et al., 1982; Tatham et al., 1985; Augustine and Baianu, 1986). A previous paper has shown the usefulness of ^{13}C NMR in the study of soy protein fractions, such as 11S (Kakalis and Baianu, 1989).

The present study was undertaken to identify by high-field, high-resolution ^{13}C NMR spectroscopy the amino acid residues of the 7S soybean protein fraction in solution and to examine the usefulness of ^{13}C NMR in monitoring the conformational changes and association–dissociation reactions induced by pH and ionic strength.

Several series of experiments were conducted at alkaline pH because of the increased solubility of soy proteins (Shen, 1976) and the relatively well studied behavior of 7S globulins in this pH range (Ishino and Okamoto, 1975; Thanh and Shibasaki, 1979; Ishino and Kudo, 1980).

MATERIALS AND METHODS

Materials. All reagents used were of analytical grade or "ACS certified" from Sigma, Aldrich, Merck, Fisher, Baker, and Mallinckrodt. The D_2O used (99.8 atom % D) was obtained from Sigma Chemical Co. (St. Louis, MO), whereas the NaOD (minimum 99.0 atom % D) was purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI).

Soybean Protein Samples. The soybean 7S fraction used in this study was prepared from defatted, minimally heat processed soy flour (Nutrisoy 7B from ADM, Decatur, IL) according to the method of Thanh and Shibasaki (1976a). The finally purified 7S globulin solution was exhaustively dialyzed against water at 4 °C, lyophilized, and stored at −20 °C.

Gel Electrophoresis. Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS–PAGE) was carried out in a continuous buffer system using a $T = 10\%$, $C = 2.6\%$ horizontal slab gel (250 × 115 × 2 mm). An LKB 2117 Multiphor system (Bromma, Sweden) operating with an LKB 219 regulated voltage/current/power supply was used for electrophoresis (Fehrstrom and Moberg, 1977). A mixture of protein standards (MW-SDS-70L, Lot 55F6048 from Sigma) was run in parallel with soy protein samples.

After pre-electrophoresis (150 mA for 15 min), 50 μg of soy protein and 150 μg of SDS in 5 μL of sample buffer (0.045 M Tris·HCl, pH 8.9, 0.1% SDS, 0.05% Bromophenol Blue), placed in boiling water for 2 min and allowed to cool, were loaded into the gel sample slots. Reduced samples contained 50 μg of dithiothreitol. Electrophoresis was performed in a 0.375 M Tris·HCl, pH 8.9, buffer system at a constant current of 30 mA for 15 min and then at 180 mA for 3 h. Gels were fixed in a 12% trichloroacetic acid–3.5% sulfosalicylic acid solution in 30% aqueous CH_3OH for 2 h, stained in a 0.25% solution of Coomassie Brilliant Blue R250 in H_2O–CH_3OH–CH_3COOH (6:6:1) for 4 h, diffusion-destained with several changes of a 30% C_2H_5OH–10%, CH_3COOH aqueous solution over 24 h, and finally soaked

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in a preserving 30% C_2H_5OH–10% CH_3COOH–10% glycerol aqueous solution for 2 h.

Amino Acid Analysis. Acid hydrolysis of the 7S protein fraction was carried out at 105 °C for 24 h (Spitz, 1973) after 8 mL of 6 N HCl (that had been flushed with N_2 was added) to 30 mg of protein. Ion-exchange chromatograms of two separate hydrolysates were obtained with an amino acid analyzer (Model 119CL, Beckman Instruments, Palo Alto, CA; application note 118/119 CL-AN-002, April 1977) using a 22 cm long sulfonated polystyrene column (W3P from Beckman).

^{13}C NMR Measurements. Natural abundance carbon-13 NMR experiments were carried out with a GN-300 NB (75.45 MHz) as well as a GN-500 (125.76 MHz) multinuclear spectrometer (General Electric Co., NMR Instruments, Freemont, CA) equipped with 7.05- and 11.75-T superconducting magnets, respectively (Oxford Instruments, Inc., U.K.), and Nicolet 1280 dedicated computers. Three hundred milligrams of protein was dispersed in 4 mL of solvent (7.5% w/v) that contained 0.02% NaN_3 together with 0.5 mg/mL of sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as an internal chemical shift standard. The pH (pD) was adjusted with NaOH (NaOD) by using a microsyringe. For samples in D_2O, the conversion to pD values was made according to the relation pD = pH + 0.45 (Covington et al., 1968), where pH is the pH-meter reading for a D_2O solution with the electrode calibrated in standard H_2O buffers.

Portions of about 4 mL of 7.5% w/v protein samples in 10-mm high-resolution NMR tubes (Wilmad, Buena, NJ) were used for ^{13}C NMR measurements. Fully proton decoupled ^{13}C NMR spectra were accumulated over a period of 24 h by using the MLEV-64 pulse sequence (Levitt et al., 1982); the decoupler was centered 4.8 ppm downfield from the ^{1}H resonance of tetramethylsilane (TMS), and a ^{1}H-decoupling field ($B_2/2\pi$) of 3.02 (GN-300) or 4.9 kHz (GN-500) was used. Data were stored in a 32K point array. Fourier transforms/data processing were carried out on line with the NIC-1280 computer. Chemical shifts and integrated intensities were routinely listed by the computer. Line widths were measured digitally at half-height.

The effect of field inhomogeneity was estimated in each case by comparing the measured line widths $\Delta\nu_{1/2} = 1/(\pi T_2)$ of a 1 M sucrose solution in D_2O with $1/(\pi T_1)$. The spin–lattice relaxation times T_1 were measured by inversion recovery (Vold et al., 1968); values were in good agreement with those reported by McCain and Markley (1986).

Despite the low radio frequency power decoupling method used, some radio frequency heating of the sample was observed, which, as expected, was most pronounced for the high ionic strength samples (0.5 M NaCl) at the higher magnetic field (500-MHz ^{1}H NMR frequency), where the temperature rose by 11 °C, from 21 to 32 °C. No heat-induced protein conformational changes are expected to take place under the experimental conditions used (Bikbov et al., 1983; Varfolomeyeva et al., 1986).

RESULTS AND DISCUSSION

Isolated 7S Globulin. The method of Thanh and Shibasaki (1976) for the simultaneous isolation of the two major soybean storage proteins, glycinin (11S) and β-conglycinin (7S), is suitable for obtaining the relatively large amount of protein required for ^{13}C NMR spectroscopy. The purity of a 7S fraction prepared in this manner has been found to be about 86% by ultracentrifugation (Thanh and Shibasaki, 1976) or 70% by densitometry of SDS–PAGE gels (Damodaran and Kinsella, 1986), although single radial immunodiffusion yielded a β-conglycinin content as low as 52% (Iwabuchi and Yamauchi, 1987). It would seem, however, that the two previous estimates were more reliable, (e.g., 70–86% 7S content) in view of the additional analyses presented here.

SDS–PAGE under reducing conditions yielded a pattern that consisted of a very intense band at 64 kDa and two other major ones at 60 and 49 kDa, which were identified as the α′, α, and β subunits of β-conglycinin, respectively (Thanh and Shibasaki, 1977). The measured molecular weights generally agree with those reported in the literature (Thanh and Shibasaki, 1977; Iibuchi and Imahori, 1978a; Sykes and Gayler, 1981), although they may represent an overestimate, since β-conglycinin subunits are glycoproteins (Segrest and Jackson, 1972; Thanh and Shibasaki, 1977). The reason for the variability of the reported molecular weight of β-conglycinin subunits, as estimated from SDS–PAGE, is not clear at present (Nielsen, 1985).

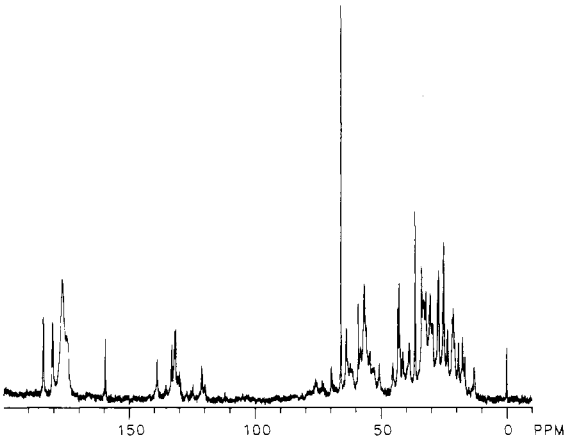

Figure 1. Natural abundance ^{13}C–{^{1}H} NMR spectrum (75.45 MHz) of a 7.5% 7S soy protein fraction in D_2O at pD 12.0, 12 mM ionic strength, and 21 °C: 15-μs pulse width (45° flip angle), 0.82-s acquisition time, 2.82-s recycle time, 20-kHz spectral width, 30 380 scans, and 5-Hz line broadening.

The contaminants were glycinin (38 and 19 kDa) and 2.8S protein fraction (33 kDa; Vaintraub and Shutov, 1969; Thanh and Shibasaki, 1976a). A minor band beyond the upper limit of the log MW–distance of migration linear range may be due to lipoxygenase (Pacheco Batista Fontes et al., 1984; Hirano et al., 1987) or urease (Beachy et al., 1981; Bond and Bowles, 1983).

Assignment of Resonances. Chemical shift assignments are easier to make for the ^{13}C NMR spectra of the denatured proteins where peaks are sharper as a result of increased segmental mobility; also contributing to the narrowing of the NMR peaks is the decreased variation in the chemical shifts, which for identical chemical groups could be caused by the differences in their chemical environments (Wüthrich, 1976; Howarth and Lilley, 1978).

Tentative assignments of most of the peaks in the ^{13}C NMR spectrum of the alkali-denatured 7S protein (Figure 1) were made by comparing the observed chemical shifts with those of other diamagnetic proteins, peptides, and free amino acids after the effect of amino acid incorporation into peptides on the chemical shift was corrected for (Freedman et al., 1971, 1973; Christl and Roberts, 1972; Keim et al., 1973a,b, 1974; Bradbury and Norton, 1973; Quirt et al., 1974; Deslauriers et al., 1975; Van Binst et al., 1975; Oldfield et al., 1975b; Wüthrich and Baumann, 1976; Wüthrich, 1976; Richarz and Wüthrich, 1978; Howarth and Lilley, 1978; Baianu et al., 1982; Tatham et al., 1985; Kakalis and Baianu, 1989), as well as by taking into account the amino acid analysis data.

Reported chemical shift values usually refer to neutral solutions. In the assignment of peaks of ionizable groups the values for the ionic form(s) expected to be present at pH 12 were used. The ^{13}C chemical shifts of nonionizable groups are relatively insensitive to changes in the ionic state of nearby residues, unless they are actually H-bonded to them (Howarth and Lilley, 1978). The chemical shift values and the proposed assignments for soy 7S proteins are given in Table I.

The multiplets between 71 and 78 ppm are most probably due to the carbohydrate moiety of β-conglyci-

Table I. C-13 Chemical Shifts of 7S Soy Proteins in D_2O, pD 12.0

peak no.	chemical shift (ppm from DSS)	proposed assignments
1	13.0	Ile δCH_3
2	16.6	Ile γ_2CH_3
3	17.5–17.6	Ala βCH_3
4	19.1–19.3	Val γ_2CH_3
5	20.7	Val γ_1CH_3, Thr γCH_3
6	21.1	Leu δ_2CH_3
7a	21.5	Leu δ_1CH_3
7b	21.6	Lys γCH_2
8	23.5	Arg γCH_2
9	25.0	Gln βCH_2
10	27.0–27.2	Glu βCH_2
11	29.4	Arg βCH_2
12	30.3	Pro βCH_2, Val βCH, His βCH_2
13	32.0	Lys βCH_2
14	32.8–33.0	Lys δCH_2
15	33.2–33.4	-
16	33.7	Gln γCH_2
17	36.3	Glu γCH_2
18	38.4–3..6	Asn βCH_2, Phe βCH_2
19	41.2–41.4	Asp βCH_2, Leu βCH_2
20	42.8	Lys ϵCH_2
21	43.2	Arg δCH_2
22	44.9–45.5	Gly αCH
23	50.6–50.9	Pro δCH_2
24	52.4–53.9	Ala αCH, Asx αCH, His αCH
25	54.2–54.5	Arg αCH, Leu αCH
26	55.2–56.7	Glx αCH, Lys αCH, Tyr αCH
27	57.7–58.5	Phe αCH, Ser αCH
28	60.7–62.2	Thr αCH, Ser βCH_2, Val αCH, Ile αCH
29	63.0–63.7	Pro αCH, Ser βCH_2
30	6..6–69.8	Thr βCH
31	119.8–120.1	His δCH
32	121.0–121.1	Tyr ϵCH
33	124.6–125.0	Tyr γC
34	126.7–127.1	Trp δ_2C
35	129.8	Phe ζCH
36	130.8–130.9	Phe CH
37	131.4	Phe ϵCH
38	131.8	Phe δCH
39	133.0	Tyr δCH
40	133.8–133.9	His ϵCH
41	138.8	Phe γC, His γC
42	159.4	Arg ζC
43	173.7–174.7	Ser δCO, Gly δCO, Asn δCO, Thr δCO, His δCO, Ile δCO, Val δCO, Asp δCO
44	175.0–176.7	Glu δCO, Asn γCO, Gln δCO, Lys δCO, Arg δCO, Leu δCO, Pro δCO, Phe δCO, Ala δCO
45	180.2–180.3	Asp γCO, Gln δCO
46	183.9	Glu δCO

nin (Neeser et al., 1985). The tallest peak (65.8 ppm) is present in all spectra. Chemical shift/amino acid profile considerations suggest that it may originate from a nonprotein component of the sample. It is known that the 7S protein fraction binds strongly nonprotein material (Anderson, 1974; Yamauchi et al., 1980). The sharp peak at 59.0 ppm is present in all spectra except that at pH 7.6 (Figure 3A). It is most likely due to alkali-induced chemical modification of proteins such as the peptide bond hydrolysis (Whitaker, 1980) or the $H_2C{=}C<$ group. Our peak assignment for the Lys ϵCH_2 (Table I) is supported by the observed upfield shift of this peak, from 42.8 to 41.9 ppm, at pD 10.1 (Figure 2). This is in agreement with the effect of Lys ionization on the chemical shift of Lys ϵCH_2 (Howarth and Lilley, 1978).

The absence of the Tyr ζC peak from the spectrum in Figure 1 may be due to the chemical shift nonequivalence at pH 12.0, resulting in weak and probably broad peaks that are not readily observable. The expected

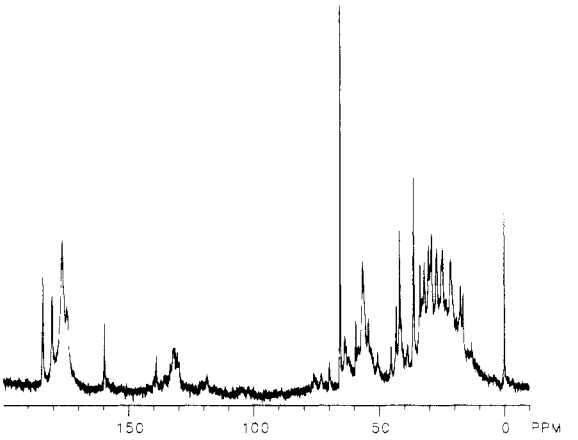

Figure 2. Natural abundance ^{13}C-{^1H} NMR spectrum (75.45 MHz) of a 7.5% 7S soy protein fraction in D_2O at pD 10.1, 13 mM ionic strength, and 21 °C: 15-μs pulse width (45° flip angle), 0.82-s acquisition time, 2.62-s recycle time, 20-kHz spectral width, 35 924 scans, and 5-Hz line broadening.

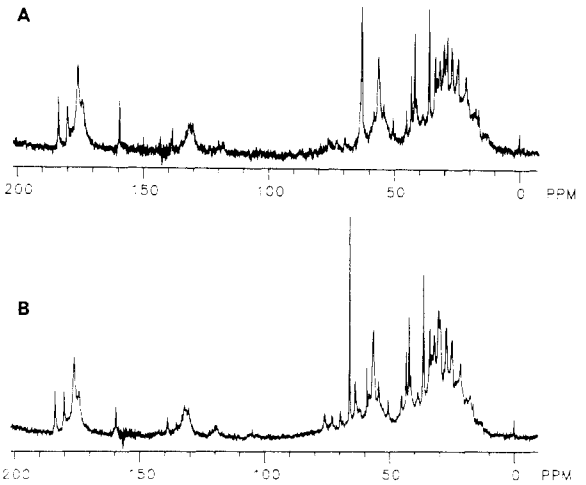

Figure 3. Natural abundance ^{13}C-{^1H} NMR spectrum (125.76 MHz) of a 7.5% 7S soy protein fraction in 35 mM sodium phosphate buffer in 20% D_2O, pH 7.6, that contained 0.4 M NaCl (0.5 M ionic strength) and 0.02% NaN_3 at 32 °C (A) or in 0.5 M NaCl in D_2O at pD 10.3 and 32 °C (B): 20-μs pulse width (45° flip angle), 0.6- (A) or 0.7-s (B) acquisition time, 3.04- (A) or 1.54-s (B) recycle time, 30-kHz spectral width, 17 539 (A) or 45 304 (B) scans, and 10-Hz line broadening.

chemical shift for the Tyr ζC at pH 12.0 is 165 ppm, and a 10 ppm upfield shift is observed when Tyr is protonated (Howarth and Lilley, 1978). A peak at 150 ppm which, is observed at pH 7.6 (Figure 3A), is most likely due to the Tyr ζC. At intermediate pH values, the observed chemical shift is the weighted average of two states, the protonated and nonprotonated forms, if there is fast exchange on the NMR time scale; this is true for most pH titrations (Allerhand, 1979). At pD 10.3, there is a low-amplitude peak downfield from that of Arg ζC and partly overlapping with it (Figures 3B and 5B) which can be attributed to Tyr ζC. At pD 10.1, this peak seems to be buried under that of Arg ζC (Figures 2 and 4B). Similar results have been reported for the Tyr residues of myoglobins (Wilbur and Allerhand, 1976).

Although the recycle time (2.82 s for 45° flip angle) may not be long enough to allow a quantitative determination of the different chemical groups, a comparison between the peak heights at 183.9 (Glu δCO) and 180.3 ppm (Gln δCO) can be meaningful: they both are due to Glx δ carbonyls, and their line widths are similar. One may conclude from such measurements that the Glx residues of 7S

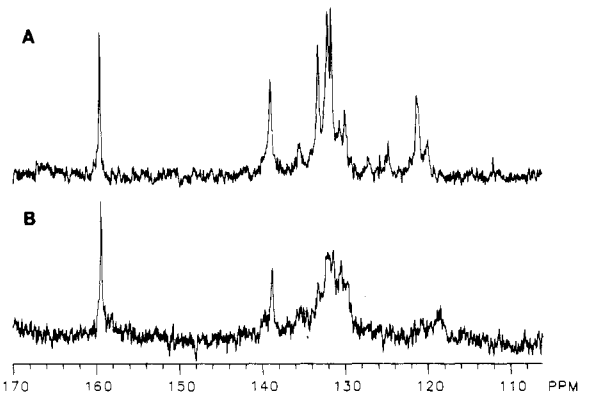

Figure 4. Comparison of the aromatic regions of soybean 7S proteins' ^{13}C NMR spectra in D_2O at pD 12.0 (A) and pD 10.1 (B). Conditions as in Figures 1 and 2.

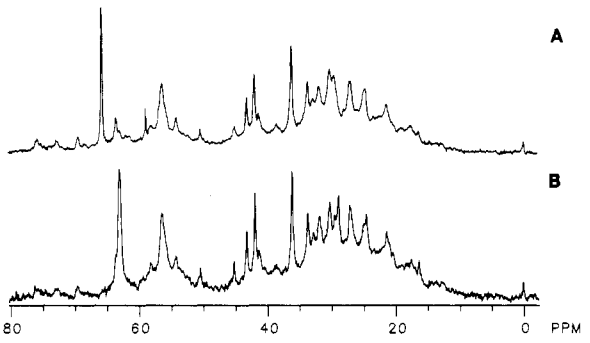

Figure 5. Comparison of the aliphatic regions of soybean 7S proteins' ^{13}C NMR spectra at 0.5 M ionic strength and pD 10.3 (A) or pH 7.6 (B). Conditions as in Figure 3.

proteins are approximately 50% amidated. Since a 45% amidation has been reported for the total acidic residues of 7S globulins (Fukushima, 1968), about half of the Asx residues should be in the amide form.

Structure of the Alkali-Denatured Soybean 7S Proteins. The observed chemical shift nonequivalence of Ile δCH_3 (13.0 ppm) and that of several peaks in the aromatic spectral region (110–165 ppm), combined with the absence of well-resolved, sharp peaks in the αCH spectral region (45–65 ppm), suggest that even at pH 12.0, the 7S proteins maintain a relatively "immobile" hydrophobic core which is disrupted above pH 12.9 (Ishino and Okamoto, 1975; Thanh and Shibasaki, 1979). Optical rotatory dispersion studies have shown that 7S globulins in aqueous solutions at pH 12.5 are not completely denatured (Fukushima, 1965).

The protein concentration that we used for the ^{13}C NMR measurements (75 mg/mL of solvent) is around the minimum required for gelation (Ishino and Kudo, 1979, 1980), and the type of protein–protein interactions is important (Kinsella et al., 1985). In addition to the electrostatic repulsive forces at pH 12.0 and the hydrophobic interactions mentioned above, hydrogen bonding is present as evidenced by the ~1 ppm downfield shift of the carbonyl peaks (Howarth and Lilley, 1978). This shift is significantly larger than that of the simple isotope effect of the proton substitution by deuterium (Oldfield et al., 1975b).

Comparison of ^{13}C NMR Spectra. A comparison of the alkali-denatured 7S protein spectrum (Figure 1) with those obtained at lower pH (Figures 2 and 3) shows several differences: in the latter, the resolution in the region containing the aliphatic side chain resonances (10–45 ppm) is obviously lower (the Ile δCH_3 peak is barely discernible), and there is significant coalescence of the peaks in the aromatic region (Figure 4). This is partly the result of restricted mobility of the corresponding side chains, as expected for the hydrophobic acid residues present in the interior of globular proteins; the chemical shift nonequivalence also contributes to peak broadening (Howarth and Lilley, 1978; Allerhand, 1979).

At pH 10.1 and 13 mM ionic strength, β-conglycinin protomers (trimers) associate to form dimers of such protomers (hexamers) (Iibuchi and Imahori, 1978b; Thanh and Shibasaki, 1979). Comparison of the aromatic spectral region with that of the alkali-denatured protein (Figure 4) shows that the Tyr δCH and Tyr γC peaks in the former are significantly reduced in intensity, whereas that of Phe γC is not affected to the same degree. A plausible explanation is that the hydrophobic core maintained at pH 12.0 contains the Phe residues that are also in the interior of the β-conglycinin subunits, whereas Tyr residues are at the intersubunit contact area. Then the observed sharpness of the Tyr peaks at pH 12.0 is the result of their exposure to the solvent and the possibility of fast local motion of the Tyr residues. On the basis of their ionization behavior, it has been suggested that Tyr residues are located at the interface of the β-conglycinin subunits (Koshiyama, 1971; Thanh and Shibasaki, 1979).

Ultracentrifugation studies have shown that at pH 7.6 or 10.3 and 0.5 M ionic strength the β-conglycinin exists as a trimer (Koshiyama, 1971; Thanh and Shibasaki, 1979); the absence of disulfide-bond reducing agents from our samples had no effect on the structure of β-conglycinin (Koshiyama, 1968a; Fukushima, 1968). A comparison between the corresponding ^{13}C NMR spectra (Figure 3) indicates no obvious difference in the aromatic regions. However, in the aliphatic region, the most intense peak shifts from 65.8 ppm at pD 10.3 to 63.2 ppm at pH 7.6, whereas the peak assigned to Arg βCH_2 shifts 0.6 ppm upfield (Figure 5). There is generally an increase in peak line widths with increasing pH (Figure 5) that may be due to the increased solution viscosity (Ishino and Kudo, 1979; Diep et al., 1982) and the consequent slower tumbling of proteins in solution.

No major spectral differences were observed when the ionic strength was increased from 13 mM to 0.5 M (Figures 2 and 3B) and β-conglycinin undergoes a reversible hexamer-to-trimer interconversion (Iibuchi and Imahori, 1978b; Thanh and Shibasaki, 1979). The apparent better resolution for certain aliphatic side chain peaks in Figure 2 is most likely due to the smaller line broadening applied (5 Hz in Figure 2 vs 10 Hz in Figure 3B) and also to the possible presence of α subunits that have dissociated from the hexamer at 13 mM ionic strength (Thanh and Shibasaki, 1979).

Molecular Dynamics of Soybean 7S Proteins. The dynamic state of specific side chains in a protein (time scale of 10^{-7}–10^{-12} s) can be determined from ^{13}C NMR relaxation measurements with some reasonable assumptions; generally, the rotational correlation times for the backbone carbon atoms are essentially identical with the correlation time for the overall tumbling of the entire protein molecule, whereas substantially higher mobilities are derived for the side chain carbon atoms that are further away from the protein backbone (Glushko et al., 1972; Howarth and Lilley, 1978).

By measuring the line width at half-height, $\Delta\nu$, of well-resolved and individually assigned absorption peaks that have Lorentzian line shapes (after correcting for the digital line broadening as well as the estimated inhomogeneity line broadening), one can calculate the effective correlation

Table II. Natural Line Widths at Half-Height and Rotational Correlation Times for Certain Resonances in the ^{13}C NMR Spectra of 7S Soy Proteins

	7S soy protein sample			
	pD 12.0[b]		pD 10.1[c]	
assignment[a]	$\Delta\nu$, Hz	τ_R, ns	$\Delta\nu$, Hz	τ_R, ns
Ile γ_2CH_3	1.6	0.08	–	–
Gln βCH_2	7.5	1.7	–	–
Glu γCH_2	1.2	0.1	15.3	5.2
Lys ϵCH_2	–	–	15.3	5.2
Tyr δCH	3.6	1.6		
Arg ζC	0.5	1.3	–	–
Glu δCO	3.4	13.1	24.5	–

[a] From Table I. [b] Figure 1. [c] Figure 2.

time τ_R that characterizes the "isotropic" or "effectively isotropic" rotation of the corresponding chemical groups (Oldfield et al., 1975a; Norton et al., 1977). At the magnetic fields that we employed (7.05 and 11.75 T) the relaxation rates of protonated carbons are dominated by the ^{13}C–^{1}H dipolar interactions (D), whereas the chemical shift anisotropy (CSA) is the major source of relaxation for the nonprotonated carbons (Norton et al., 1977). In each case, τ_R can be calculated from the equations

$$\Delta\nu_D = \frac{n\hbar^2\gamma_C^2\gamma_H^2}{20\pi r_{CH}^6}\left(4\tau_R + \frac{\tau_R}{1+(\omega_C-\omega_H)^2\tau_R^2} + \frac{3\tau_R}{1+\omega_C^2\tau_R^2} + \frac{3\tau_R}{1+\omega_C^2\tau_R^2} + \frac{6\tau_R}{1+\omega_H^2\tau_R^2} + \frac{6\tau_R}{1+(\omega_C+\omega_H)^2\tau_R^2}\right)$$

$$\Delta\nu_{CSA} = \frac{\gamma_C^2H_0^2(\Delta\sigma)^2}{45\pi}\left(4\tau_R + \frac{3\tau_R}{1+\gamma_C^2H_0^2\tau_R^2}\right)$$

where $\Delta\nu = (\pi T_2)^{-1}$ is the "natural" (corrected) line width at half-height; n is the number of protons attached to the carbons; the gyromagnetic ratios γ_C and γ_H are 6728 and 26 753 rad s^{-1} G^{-1}, respectively; the C–H bond length r_{CH} is 1.1×10^{-8} cm (Dill and Allerhand, 1979); $\hbar = 1.055 \times 10^{-27}$ erg s; and the chemical shift anisotropy $\Delta\sigma$ is 2×10^{-4} (Norton et al., 1977). The ^{13}C NMR frequency is $\omega_C/2\pi$, with $\omega_C = 4.74 \times 10^8$ rad s^{-1} (GN-300) or 7.90×10^8 rad s^{-1} (GN-500); the ^{1}H NMR frequency is $\omega_H/2\pi$ with $\omega_H = 1.88 \times 10^9$ rad s^{-1} (GN-300) or 3.14×10^9 rad s^{-1} (GN-500). The magnetic field strength H_0 is 75 000 or 117 500 G for the GN-300 or the GN-500 spectrometer, respectively.

The estimated natural line widths at half-height and the corresponding calculated rotational correlation times τ_R are given in Table II. In the case of the alkali-denatured proteins at pD 12.0, the τ_R values we obtained are typical for fast segmental motion in protein molecules (Glushko et al., 1972; Oldfield et al., 1975a; Howarth and Lilley, 1978). The τ_R of δCO Glu (13.1 ns) is probably an overestimate since the τ_R of Glu γCH_2 is only 100 ps; the reason is most likely a slight chemical shift nonequivalence in the Glu δCO peak. At pH 10.1, the τ_R of Glu γCH_2 and Lys ϵCH_2 (5.2 ns) is above typical values for fast local motion but well below those for "immobile" residues; a small chemical shift nonequivalence could be the reason for the large apparent τ_R. Side-chain motion of the Lys residues in several native proteins (Howarth and Lilley, 1978) and in Glu residues of a soy protein isolate (Kakalis and Baianu, 1989) was previously reported.

Concluding Remarks. The high molecular weight of soybean storage proteins affects adversely both the resolution and the signal-to-noise ratio in proton-decoupled ^{13}C NMR spectra (Norton et al., 1977; Howarth and Lilley, 1978). However, ^{13}C NMR spectroscopy does provide new and detailed information about the molecular structure of soybean 7S proteins and can offer important insights into processes such as protein gelation (Clark and Lee-Tuffnell, 1986), protein modification (Feeney and Whitaker, 1985), and texturization (Lillford, 1986).

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