CARBOHYDRATE CONTENT OF SOYBEAN PROTEINS[1]

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ABSTRACT

Several fractions of soybean proteins were analyzed with phenol-sulfuric acid to determine the range of carbohydrate content of soybean proteins. Protein fractions were prepared by a variety of purification techniques, including isoelectric precipitation, ammonium sulfate precipitation, alcohol washing, and chromatography on hydroxylapatite. A final purification common to all samples was exhaustive dialysis against distilled water, followed by freeze-drying. All protein preparations gave a positive test for carbohydrates with phenol-sulfuric acid. Carbohydrate analyses expressed as percent glucose included: water-extractable proteins, 1.6–2.0; isoelectrically precipitated globulins, 1.5–2.3; whey proteins, 2.8–4.2; glycinin, 1.5–1.9; cold-insoluble fraction, 0.3–0.5; and purified 11S component, 0.2.

Effluents of soybean proteins chromatographed on hydroxylapatite columns were analyzed with phenol-sulfuric acid. Although carbohydrates were eluted with all the proteins present in the water-extractable mixture, definite fractions were indicated. The first carbohydrate fraction was non-dialyzable, was eluted as a sharp peak slightly ahead of the first protein fraction, and occurred largely in whey upon isoelectric precipitation of the globulins. Four or more carbohydrate fractions were eluted with the other water-extractable protein components.

The presence of carbohydrates in soybean proteins was first reported in 1929 by Tillmans and Philippi (1). Using an orcinol procedure, they obtained a carbohydrate content of 5.1% for the globulin fraction purified by repeated solution in alkali and precipitation with acid. In 1957 Gladyshev (2) reported hexosamines in an acid hydrolysate of glycinin but did not identify the amino sugars. Wada *et al.* (3) subsequently found glucosamine in soybean hemagglutinin. Lis and coworkers (4) confirmed the presence of glucosamine in hemagglutinin but also found mannose; furthermore, the glycoprotein nature of hemagglutinin was established by isolation of a glycopeptide containing the two sugars. Using an anthrone procedure, Vaintraub (5) found no carbohydrates in the globulin fraction (glycinin) or the cold-insoluble protein fraction. Subsequently, Hullar and Smith (6) hydrolyzed isoelectrically precipitated soybean globulins and isolated arabinose, galactose, glucose, mannose, ribose, and xylose. Later, Roberts and Briggs (7) reported carbohydrate contents of 7S com-

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ponent and the cold-soluble fraction (~ 80% 11S), the major proteins of the globulin fraction, and found the 7S component to contain four times as much carbohydrate as the partially purified 11S protein.

Sialic acid, possibly as the N-glycolyl form, has recently been reported in defatted soybeans by Mayer *et al.* (8), but it is not yet known whether sialic acid is combined with protein or other meal constituents.

The major proteins of soybeans have no known biological activity that can be used for analytical purposes in conjunction with present physical methods of analysis. We have therefore analyzed a number of available protein fractions of soybeans to determine whether carbohydrates could be used as markers for the isolation of specific proteins. Here we report the results of our survey and also the elution behavior of the carbohydrate constituents when soybean proteins were chromatographed on hydroxylapatite.

Materials and Methods

Soybean Meal. Soybeans were cracked, dehulled, and flaked, then defatted with hexane at room temperature.

Protein Samples. The protein samples analyzed in this study are listed in Table I. Water-extractable proteins (samples 1–7) were obtained by stirring defatted meal with water at 25°C. for 1 hr. and centrifuging (12,000 × *g*) for 10 min. For samples 1–4 a re-extraction was made for 15 min., and the extracts from each meal were combined. The extracts were dialyzed for 2 days against cold potassium phosphate-sodium chloride buffer, pH 7.6, ionic strength 0.5, 0.01*M* mercaptoethanol; this buffer is subsequently referred to as standard buffer (7,10). The extracts were then dialyzed against cold distilled water for 10 days and freeze-dried.

Isoelectrically precipitated globulins (samples 9–12) were prepared by acidifying a part of the water extracts used for samples 1–4 to pH 4.4 with hydrochloric acid and centrifuging. The precipitates were dispersed and dialyzed in the cold against standard buffer for 2 days and against distilled water for 10 days, and freeze-dried. Sample 13 was prepared in the same manner except that the acid-precipitated curd was suspended in 10% sodium chloride and adjusted to pH 6.6 with alkali. The protein was then dialyzed in the cold against salt solution for a week and against distilled water for 10 days, and then adjusted to pH 4.5 with acetic acid. The precipitate was washed twice with water and freeze-dried.

A portion of sample 13 was stirred with 25 volumes of 86% (v./v.) ethanol at 38°C. for 17 hr. The alcohol was then filtered off on a Büchner funnel and the protein was washed several times with 86%

TABLE I

CARBOHYDRATE CONTENT OF SOYBEAN PROTEINS

Sample No.	Protein Preparation	Soybean Variety	Crop Year	Nitrogen Content of Protein	λ max[a]	Carbohydrate Content[b]		
						Mean	95% Confidence Limits	
							Lower	Upper
				%	mμ	%	%	%
	Water-extractable proteins							
1	(1:10; 1:5)[c]	Clark	1957	16.51	...	1.74	1.63	1.86
2	(1:10; 1:5)	Harosoy	1963	16.59	483	2.02	1.89	2.16
3	(1:10; 1:5)	Hawkeye	1963	16.53	484	1.60	1.50	1.71
4	(1:10; 1:5)	Adams	1963	16.24	...	1.76	1.65	1.88
5	(1:10)	Clark	1957	16.50	486	1.62	1.51	1.73
6	(1:5)	Clark	1957	16.38	486	1.89	1.77	2.02
7	(1:5)	Clark	1957	16.82	...	1.87	1.75	2.00
	Sodium chloride-extractable							
8	proteins	Clark	1957	16.42	486	1.55	1.45	1.65
	Isoelectrically precipitated							
9	globulins	Clark	1957	16.26	...	1.68	1.58	1.80
10		Harosoy	1963	16.16	485	1.78	1.67	1.91
11		Hawkeye	1963	16.44	484	1.47	1.38	1.57
12		Adams	1963	15.92	...	1.73	1.62	1.85
13		Adams	1960	16.24	484	2.27	2.12	2.43
	Isoelectrically precipitated globulins, washed with							
14	alcohol at 38°C.	Adams	1960	17.04	484	2.14	2.09	2.19
	Washed with							
15	boiling alcohol	Adams	1960	16.96	486	1.50	1.40	1.60
16	Glycinin	Clark	1960	16.52	486	1.85	1.73	1.98
17		Clark	1957	16.83	487	1.51	1.41	1.61
18	Whey proteins	Clark	1957	15.26	486	3.34	3.13	3.57
19		Harosoy	1957	15.02	484	3.64	3.40	3.88
20		Hawkeye	1957	14.94	485	4.20	3.93	4.48
21		Adams	1957	15.66	486	2.81	2.63	3.01
	Cold-insoluble							
22	fraction	Clark	1957	17.26	485	0.47	0.44	0.51
23		Harosoy	1957	17.29	...	0.52	0.49	0.56
24		Adams	1957	17.50	483	0.25	0.24	0.27
	Cold-insoluble fraction,							
25	NEMI-treated	Clark	1957	17.58	483	0.26	0.24	0.28
26		Clark	1957	17.62	484	0.24	0.22	0.25
	Cold-soluble							
27	fraction	Clark	1957	15.99	...	2.60	2.43	2.77
28		Clark	1957	16.51	485	2.03	1.90	2.17
29		Clark	1957	15.86	482	2.39	2.24	2.56

TABLE I — *continued*

Sample No.	Protein Preparation	Soybean Variety	Crop Year	Nitrogen Content of Protein	λ max[a]	Carbohydrate Content[b]		
						Mean	95% Confidence Limits	
							Lower	Upper
				%	mμ	%	%	%
30	Purified 11S	Clark	1957	17.56	484	0.19	0.18	0.20
31		Clark	1957	17.66	483	0.24	0.22	0.25
32		Clark	1957	17.64	...	0.17	0.16	0.18
33	Purified 11S, alcohol-washed	Clark	1957	17.51	483	0.17	0.16	0.18
	Hydroxylapatite column							
34	Fraction A	Clark	1957	15.25	485	4.27	3.99	4.56
35	Fraction A	Clark	1957	14.08	484	5.59	5.23	5.97
36	Fraction B	Clark	1957	15.80	...	1.71	1.60	1.83
37	Fraction B	Clark	1957	15.67	484	1.76	1.65	1.88
38	Fraction C	Clark	1957	16.94	485	0.77	0.72	0.82
39	Fraction C	Clark	1957	16.75	486	1.05	0.99	1.13
40	Fraction D	Clark	1957	16.89	489	2.37	2.22	2.53
41	Fraction D	Clark	1957	16.50	488	2.42	2.27	2.59
42	7S Protein	.....	....	16.82	488	3.53	3.30	3.77
	Hemagglutinin							
43	Laboratory	Clark	1957	15.40	488	3.74	3.50	3.99
44	Pilot plant	Clark	1957	15.16	488	5.70	5.34	6.09
	Ovalbumin	.....	....	15.76[d]	489	2.31[e]	2.16	2.47

[a] Wave length of maximum absorption of phenol-sulfuric acid reaction mixture.
[b] Expressed as glucose.
[c] Numbers in parentheses indicate meal:water ratio for extraction. Second ratio was used in reextraction.
[d] Value reported by Tristram (9).
[e] Expressed as mannose.

ethanol, rinsed with diethyl ether, and air-dried. The air-dried protein was moistened with water, dried *in vacuo* to remove residual ether, and designated as sample 14. A portion of sample 14 was stirred with 10 volumes of boiling 80% (v./v.) ethanol for 2 hr. The alcohol was decanted and extraction was repeated a total of seven times. Residual alcohol in the protein sample was removed in a rotary evaporator; the sample was moistened with water and redried *in vacuo* to yield sample 15.

Glycinin sample 16 was prepared by extracting 200 g. of defatted soybean flakes with 2 liters of 10% sodium chloride adjusted to pH 7.8 with dilute alkali for 1 hr., and centrifuging. The extract was then saturated with ammonium sulfate and allowed to stand at room temperature overnight. The precipitate was separated by filtration, dispersed in water, and dialyzed against water in the cold for 12 days. The pH was then adjusted from 6.1 to 4.6 with 2*N* acetic acid; the

resulting precipitate was separated by centrifuging, washed three times with distilled water, and freeze-dried.

Glycinin sample 17 was prepared according to Vaintraub (5). Defatted meal (10 g.) was suspended in 100 ml. of 1*N* sodium chloride and sufficient Na_2HPO_4 to adjust the pH to 7.0. After being extracted overnight at 4°C., the residue was removed by centrifuging and was re-extracted with 50 ml. of 1*N* sodium chloride for 30 min. The salt extracts were combined; a portion was removed, dialyzed against standard buffer for 3 days, dialyzed against distilled water for 10 days, and freeze-dried to yield sample 8. The remainder of the combined sodium chloride extracts was adjusted to 70% saturation with ammonium sulfate. The resulting precipitate was separated by centrifuging; then it was redissolved in 0.5*M* sodium chloride, and the solution was dialyzed in the cold against distilled water for 5 days. The pH was adjusted from 6.0 to 5.6 by adding acetic acid. The precipitated protein was removed by centrifuging; after it was redispersed in 0.5*M* sodium chloride, the pH was adjusted to 7.0 with dilute alkali. A turbid suspension containing amber-colored, gellike particles resulted. The gel particles were separated by decantation, dissolved in standard buffer, and recombined with the supernatant. The resulting turbid solution was dialyzed against distilled water for 3 days, adjusted to pH 5.6 with acetic acid, and centrifuged. The precipitate was dissolved in standard buffer, dialyzed against several changes of buffer, and finally dialyzed salt-free and freeze-dried to yield sample 17.

The wheys obtained in preparing samples 9–12 were dialyzed against standard buffer for 2 days and against distilled water for 2 days, and then freeze-dried to yield samples 18–21.

Cold-insoluble fraction (samples 22–24) was prepared as described elsewhere (11). Cold-insoluble fraction treated with N-ethylmaleimide (NEMI-treated, samples 25 and 26) was similar to material prepared earlier (12).

Samples 27–29, designated cold-soluble fractions, were the proteins remaining soluble when a concentrated water extract of defatted meal was cooled. The starting material for each sample was the supernatant remaining after removal of cold-insoluble fractions (11). Sample 27 was prepared by dialyzing supernatant exhaustively against distilled water and freeze-drying. Sample 28 was prepared by dialyzing supernatant against several changes of standard buffer, followed by exhaustive dialysis against distilled water and freeze-drying. Sample 29 was prepared by adjusting supernatant to pH 4.5 with acetic acid, removing the precipitate by centrifuging, and redispersing it in standard buffer. The dispersion was then dialyzed against distilled water for 6

days and adjusted to pH 4.5 with acetic acid; the resulting precipitate was washed three times with water and freeze-dried.

Purified 11S protein was prepared by ammonium sulfate precipitation of the 11S component from the cold-insoluble fraction (13). Sample 30 was a preparation of fraction VIII;[3] samples 31 and 32 were fraction X. All were dialyzed salt-free and freeze-dried. Sample 33 was a freeze-dried preparation of fraction X washed five times with 5 volumes of 86% (v./v.) ethanol and dried *in vacuo*.

Samples 34–41 were prepared by hydroxylapatite chromatography of water-extractable soybean proteins (13,14). The 2.5 × 50-cm. columns used were packed with hydroxylapatite (Hypatite C, Clarkson Chemical Co., Williamsport, Pa.) and were equilibrated with starting buffer, 0.03*M* potassium phosphate, pH 7.6. Protein samples of 1 g. in 45–50 ml. of starting buffer were applied to the columns and were eluted stepwise with the following series of potassium phosphate buffers at pH 7.6:

Molarity		*Fraction Eluted*
	ml.	
0.03	25	A
0.15	150	B
0.25	150	C
1.0	385	D

The column effluents were collected with a fraction collector and were monitored by absorbancy measurements at 280 mμ. After tubes of each fraction were pooled, the samples were dialyzed exhaustively against distilled water and freeze-dried.

Sample 42 was a sample of 7S component obtained from R. C. Roberts. It was purified to the "final precipitate 6" stage as described by Roberts and Briggs (7). To ensure complete removal of ammonium sulfate, the sample was dissolved in a small volume of water by adding solid sodium chloride. The solution was then dialyzed against distilled water for 1 week and freeze-dried.

Soybean hemagglutinin (samples 43 and 44) was prepared by the method of Pallansch and Liener (15). Sample 43 was prepared from a 1-kg. batch of defatted meal, and sample 44 came from 40 lb. of defatted meal prepared in pilot-plant equipment through the ammonium sulfate precipitation steps. Ovalbumin was a five-times-crystallized preparation obtained from Mann Research Laboratories, New York, N.Y., Lot C 2982.

Carbohydrate Analysis. Carbohydrate contents of the protein sam-

[3] Fraction designation refers to preparation obtained by ammonium sulfate precipitation as described elsewhere (13).

ples were determined by the phenol-sulfuric acid method (16) with glucose as the standard, with one exception: mannose was used for analysis of ovalbumin. Absorption spectra of the phenol-sulfuric acid-protein reaction mixtures were determined in a Cary 14 recording spectrophotometer (Applied Physics Corporation). Protein concentrations were determined by micro-Kjeldahl analysis.

The experimental design used for investigating the samples was a form of balanced incomplete block design. This design and the appropriate analysis have been described (17). Sets of several samples were selected, and replicates of each set were run at two different times. Triplicate determinations were made on each sample within a replicate. Sample 14 was common to all sets.

Since variability in results increased as the carbohydrate content increased, the logarithm of carbohydrate content was used in computing the analysis. The appropriate antilogarithms were taken to obtain the means and confidence limits in Table I. The probability is 0.95 that the 95% confidence limits include the true mean carbohydrate content for any sample. The sample mean is the best estimate of the "true" carbohydrate content. The relative precision associated with an experimental unit was ±4.5% where the experimental unit is represented by the mean of triplicate determinations. Any two samples in Table I are statistically significantly different in carbohydrate content at approximately the 0.95 probability level if the ratio of the larger to smaller mean exceeds 1.15.

Gradient Elution Chromatography on Hydroxylapatite. Chromatography of soybean proteins on hydroxylapatite by gradient elution analysis was carried out as described earlier (14). Water-extractable proteins, globulins, whey proteins, cold-insoluble fraction, and cold-soluble fraction were prepared as previously described (14).

Results

Carbohydrate Analysis. Carbohydrate contents of 44 soybean protein samples are listed in Table I. The water-extractable proteins (samples 1–7), which constitute the bulk of the proteins present in soybeans, contained 1.6–2.0% carbohydrate. Changing the extraction ratio of meal to water caused some variation in carbohydrate content, but the variations were within the range obtained for proteins isolated from four different meals when a constant extraction ratio was used (samples 1–4). Previous studies (10) showed no significant difference between the proteins extracted from defatted meal with either water or a sodium chloride solution. The carbohydrate content of sodium

chloride-extractable proteins (sample 8) also was in the range obtained for water-extractable proteins.

Isoelectrically precipitated globulins (samples 9–13), which comprise nearly 90% of the water-extractable proteins, contained about the same amount of carbohydrate as the water-extractable proteins. Recent work indicates that isoelectrically precipitated soybean globulins contain noncovalently bound carbohydrate constituents which are extractable by aqueous alcohols (18,19). Washing sample 13 with alcohol at 38°C. removed 3.7% of a light-brown semisolid (19) containing 6.9% carbohydrate as determined by the phenol-sulfuric acid method. Alcohol washing thus removed about 12% of the total carbohydrate from the protein (sample 14); on exhaustive washing of sample 14 with boiling 80% alcohol, the carbohydrate content was reduced to 1.5% (sample 15).

When the globulins were prepared by salt extraction, ammonium sulfate precipitation, and dialysis as in the preparation of glycinin (samples 16 and 17), the carbohydrate contents were in the range obtained for isoelectrically precipitated globulins. Whey proteins, obtained on isoelectric precipitation of the globulins, had appreciably higher sugar contents than either the water-extractable proteins or the globulins. In contrast, the cold-insoluble fraction contained only 0.25–0.52% carbohydrate, and the cold-soluble fraction (samples 27–29) was higher in sugars than the water-extractable proteins. When the 11S protein, the major constituent of the cold-insoluble fraction, was purified further, the sugar content was reduced to 0.17–0.24%; alcohol washing did not affect the carbohydrate content. Analysis of the four protein fractions obtained by hydroxylapatite chromatography of water-extractable proteins (13,14) showed a range of 0.8–5.6% carbohydrate. Hydroxylapatite chromatography fraction C (samples 38–39) contained the 11S component as a major component and, like the cold-insoluble fraction, contained less carbohydrate than the other three fractions. Fractions A and D were highest in sugar contents. Ultracentrifugal analysis of fraction D indicated 53–56% 7S component, which dimerizes at 0.1 ionic strength (14) and which has recently been reported to contain carbohydrate (7). Sample 42, a partially purified 7S preparation obtained by ammonium sulfate precipitation, contained 3.5% carbohydrate. Two partially purified hemagglutinin preparations varied considerably in sugar content. The mannose content for a commercial preparation of ovalbumin was 2.31% compared with a value of 2.13% reported by Lee and Montgomery (20) when the same method was used.

Wave lengths at which maximum absorption occurs in the phenol-

sulfuric acid reaction for the sugars reported to be present in soybean proteins (6) are tabulated below. In agreement with results of Dubois

Sugar	λ *max* mμ
Glucose	488
Galactose	489
Mannose	489
Arabinose	480
Ribose	479
Xylose	480

et al. (16), hexoses gave absorption maxima near 490 mμ; the pentoses absorbed maximally near 480 mμ. Since the absorption spectra for the phenol-sulfuric acid reaction with the various soybean proteins were similar to those for the pure sugars, there appears to be no interference by the proteins or the nonprotein materials associated with the proteins (19). The absorption maxima obtained (see Table I) indicate that most of the protein fractions contained mixtures of pentoses and hexoses. Exceptions were hydroxylapatite column fraction D, 7S protein, and hemagglutinin which gave spectra having maxima at 488–489 mμ. Ovalbumin contains only mannose and glucosamine (20) and gave a maximum at 489 mμ. Samples 40–44 therefore appear to contain hexose, with little or no pentose.

Hydroxylapatite Chromatography. Additional information regarding the distribution of carbohydrates among the various soybean proteins was obtained by hydroxylapatite chromatography of several protein fractions. Figure 1 shows the elution diagrams obtained for water-extractable proteins, isoelectrically precipitated globulins, and whey proteins. This chromatographic procedure separates the water-extractable proteins into four fairly distinct fractions designated A–D (13,14). Fraction A consists primarily of 2S ultracentrifugal component(s); fraction B, of 2S and 7S components; fraction C, primarily of 11S component plus small amounts of 2S, 7S, and 15S; and fraction D contains mainly 7S component plus faster-sedimenting proteins. Analysis of the column effluent from the water-extractable proteins with phenol-sulfuric acid showed the presence of a large carbohydrate peak that was eluted just ahead of protein fraction A. A definite carbohydrate peak coincided with protein fraction B; three or more carbohydrate constituents were eluted in the region of protein fraction C. Carbohydrate continued to be eluted through the region of fraction D. Chromatography of globulins isoelectrically precipitated from a water extract showed a marked reduction in the first and second large carbohydrate peaks of the water-extractable proteins. The carbohydrate

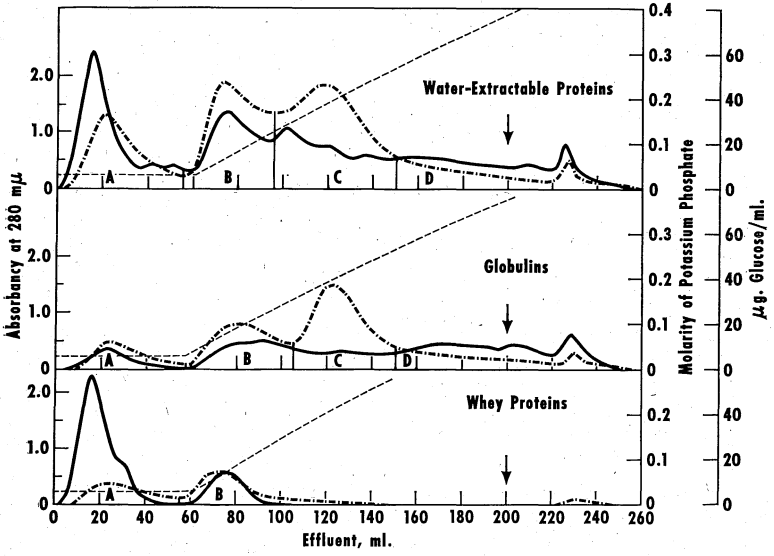

Fig. 1. Hydroxylapatite chromatography elution diagrams for water-extractable soybean proteins, globulins, and whey proteins. Column load for water-extractable proteins was 200 mg. Curves correspond to: dot and dash, absorbancy; solid line, glucose content; and dashes, elution gradient of potassium phosphate. Letters A to D designate protein fractions as described elsewhere (13,14). Vertical arrow indicates point of addition of 1*M* potassium phosphate to complete elution of the proteins.

constituents lost from the globulins go mainly into the whey protein fraction; some carbohydrate may also be associated with the portion of the globulins irreversibly insolubilized by isoelectric precipitation (21).

Figure 2 shows elution patterns for water-extractable proteins, cold-insoluble fraction, and cold-soluble fraction. The protein elution curve for the water-extractable proteins in Fig. 2 indicates a higher content of fraction C than in the water-extractable proteins used to obtain Fig. 1. This difference apparently is the result of using dissimilar extraction conditions for preparing the water-extractable proteins (14). In agreement with the results in Table I, the elution diagram for the cold-insoluble fraction indicates only traces of carbohydrate. About one-half of the carbohydrate in the cold-insoluble fraction is eluted with the starting buffer; the remainder is eluted with fraction C, which consists of 11S and 15S components (13,14). The cold-soluble fraction gave an elution pattern for carbohydrates similar to the elution pattern for the water-extractable proteins, as expected, since the cold-insoluble fraction was low in carbohydrate content. Figure 3 shows hydroxylapatite elution patterns for the first

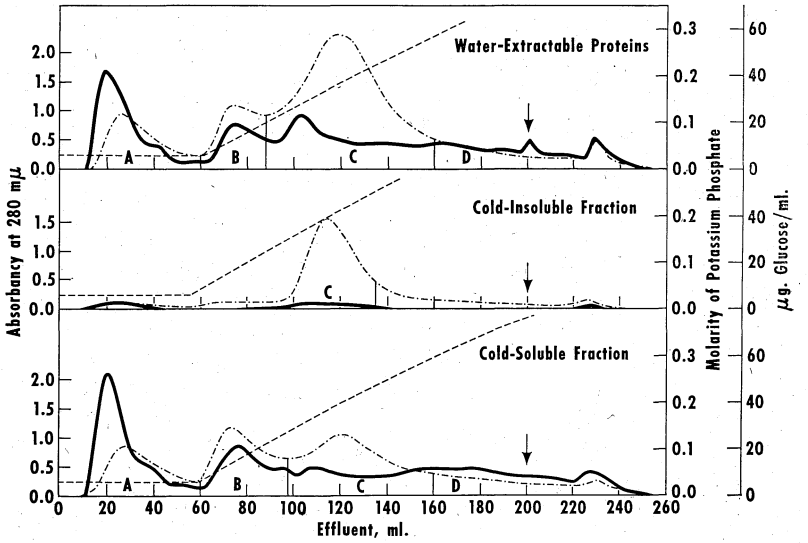

Fig. 2. Hydroxylapatite chromatography elution diagrams for water-extractable proteins, cold-insoluble fraction, and cold-soluble fraction. Column load for water-extractable proteins was 201 mg. Curves are designated as in Fig. 1.

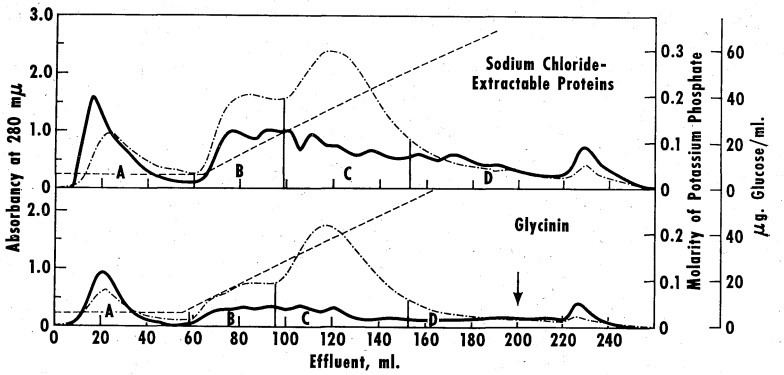

Fig. 3. Hydroxylapatite chromatography elution diagrams for sodium chloride-extractable proteins and glycinin. Respective column loads were 198 and 144 mg. of protein. Curves are designated as in Fig. 1.

and the final steps in the preparation of glycinin according to Vaintraub as described under "Methods" (5). The sodium chloride-extractable proteins gave an elution pattern similar to that of the water-extractable proteins in Figs. 1 and 2. When proteins in the sodium chloride extract were precipitated with 70% saturated ammonium sulfate, the elution pattern did not change significantly; consequently, carbohydrate constituents were not dissociated from

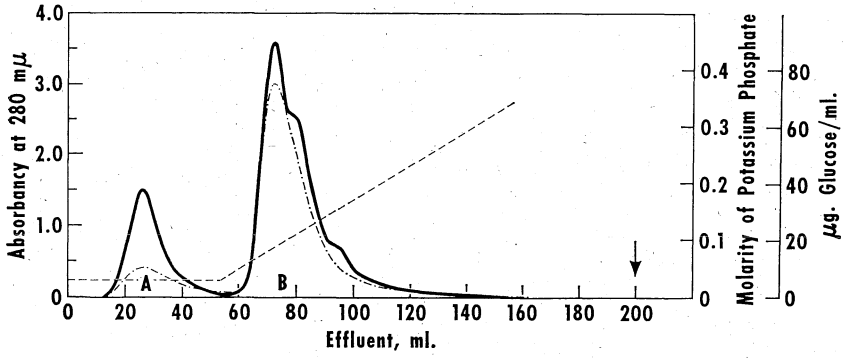

Fig. 4. Hydroxylapatite chromatography elution diagram for 46 mg. of soybean hemagglutinin. Curves are designated as in Fig. 1.

proteins in the presence of high salt concentrations. After dialysis against water and precipitation at pH 5.6 twice, the final glycinin preparation gave an elution pattern (Fig. 3) which did not differ greatly from that for the globulins prepared by isoelectric precipitation (Fig. 1). The large carbohydrate peak which was eluted ahead of protein fraction A in the salt-extractable proteins was greatly reduced, and the remaining carbohydrate of this fraction peaked with the protein fraction A of glycinin.

Figure 4 shows the hydroxylapatite elution pattern for partially purified hemagglutinin (sample 44, Table I). Two protein fractions designated A and B were obtained, and carbohydrates were associated with both fractions. Fraction B contains hemagglutinin on the basis of ultracentrifugal analysis (14).

Discussion

Our results in Table I confirm and extend earlier reports of carbohydrates in soybean proteins (1,4–7). All samples of the various fractions analyzed contained carbohydrate, but there was a wide range in values. Highest values were obtained for hemagglutinin, whey proteins, and hydroxylapatite column fraction A. Purified hemagglutinin contains 4.5% neutral sugar which has been isolated as a mannose-containing glycopeptide (4) to establish the glycoprotein nature of this protein. Hemagglutinin occurs largely in soybean whey when water-extractable proteins are fractionated by isoelectric precipitation; therefore, the carbohydrate associated with hydroxylapatite column fraction B of the whey proteins (Fig. 1) may be largely due to hemagglutinin. The high carbohydrate content of whey proteins and of hydroxylapatite column fraction A is due to the presence of the carbo-

hydrate which is eluted with the starting buffer in the chromatography experiments (Fig. 1). This carbohydrate fraction in the water-extractable proteins consists of at least two components. On isoelectric precipitation of the globulins, the carbohydrate remaining with the globulins peaked with protein fraction A, whereas most of the carbohydrate in the whey was eluted ahead of protein fraction A, with indication of resolution into a second peak.

Also high in carbohydrate is the 7S preparation (sample 42). Ultracentrifugal analysis of this sample indicated 85% 7S with 4% 2S and 11% of material sedimenting slightly faster than the 7S component. Hydroxylapatite fraction D, which appeared to contain the same 7S component as sample 42 on the basis of its ability to form dimers at 0.1 ionic strength, also contained relatively high concentrations of sugars. Sample 40 contained 56% 7S, and sample 41 contained 53% 7S component; the remainder of the protein in both samples sedimented in the 11–15S range. Since samples 40–42 gave absorption maxima at 488 $m\mu$ with phenol-sulfuric acid, evidently the carbohydrate in them is mostly hexose.

Our results, in contrast to those of Vaintraub (5), indicated the presence of carbohydrates in glycinin and in the cold-insoluble fraction. The content of sugars in glycinin agreed with values obtained for the globulins prepared by isoelectric precipitation, and elution diagrams were similar for the two globulin preparations (Figs. 1 and 3). We are unable to explain the discrepancy between our results and those of Vaintraub using an anthrone procedure, but tryptophan released from proteins during the reaction of anthrone with sugars is known to interfere with carbohydrate determination (22), and pentoses give a low color yield with anthrone (23).

The cold-insoluble fraction, which is a crude 11S protein preparation, is among the lowest in carbohydrate content of the 44 samples in Table I. On further purification of the 11S component, the sugar content is reduced to 0.2%. Since the purified 11S component still contains 5–10% 15S protein (13), the possibility exists that the 11S component is free of sugars and that the residual carbohydrate is associated with the 15S contaminant. The 7S fraction (samples 40–42) contains 12–18 times as much carbohydrate as the 11S component (samples 30–33); this difference supports previous conclusions regarding the nonidentity of subunits of the 7S and 11S components (7,13).

Since soybean meal is high in carbohydrates, the question arises whether those in the isolated proteins are contaminants or integral parts of the protein molecules. A definitive answer cannot be given as yet, except for hemagglutinin which has already been demonstrated to

be a glycoprotein (4). A variety of purification procedures, including salt extraction, ammonium sulfate precipitation, hydroxylapatite chromatography, and alcohol washing, failed to remove the carbohydrates completely. Washing with alcohol (samples 14 and 15) removed some carbohydrates not removed by dialysis; evidently this carbohydrate fraction must be tightly complexed with the protein but is not covalently bound to it. Glycoproteins other than hemagglutinin may be present, but further purification of the various proteins appears desirable before isolation of the carbohydrates in the form of glycopeptides is attempted.

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