Albumins and Globulins in Extracts of Corn Grain Parts[1]

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ABSTRACT

Experiments were run to determine the nature of differences between water-soluble and saline-soluble proteins in parts of the corn kernel. Of several extractants tested, neutral 0.5*M* NaCl yielded the most protein from corn meal, and the protein gave the best starch-gel electrophoretic patterns. Extracts with this solvent were prepared from separate germ and endosperm portions of corn. Dialysis against distilled water separated these extracts of germ and endosperm into water-soluble albumins and globulin precipitates. Amino acid analysis of albumins and globulins from both germ and endosperm showed only small differences. Starch-gel electrophoresis demonstrated differences between albumin and globulin fractions both in endosperm and germ; however, patterns of endosperm and germ fractions were similar.

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Albumins of endosperm were chromatographed in different buffers on columns of cross-linked dextran. The chromatographic peaks contained components differing in mobilities on gel electrophoresis. Chromatography with an alkaline buffer resulted in larger amounts of materials that eluted early than when acid buffers were used. This effect of alkali was not reduced by addition of 6*M* urea to this buffer or by reduction and alkylation of disulfides of the protein. The isolated germ protein could only be completely resolubilized in alkaline buffers in which it exhibited more of a high-molecular-weight component than does endosperm albumin.

In their early studies Chittenden and Osborne (1) classified proteins in corn grain according to solubility in various solutions. Albumins and globulins were soluble in salt solutions, but only albumins could be dissolved in distilled water. Later Osborne (2) noted that corn albumins gradually became insoluble in water and questioned this classification, as did subsequent workers (3). The proteins of salt-solution extracts of corn grain are heterogeneous as to charge, as shown by moving-boundary electrophoretic studies by Foster *et al.* (4) and Mertz and co-workers (5). McGuire and associates (6) partially fractionated some of the salt-soluble proteins of corn by ion-exchange chromatography on columns of carboxymethyl cellulose. The salt-soluble proteins of corn grain were resolved into a large number of constituents by starch-gel electrophoresis by Boundy and co-workers (7). In our studies starch-gel electrophoresis facilitates analysis of albumins and globulins from extracts of separate germ and endosperm parts of the grain.

Jiménez (8) reported variations in the amino acid content of hydrolysates of water extracts and sodium chloride extracts of corn endosperm. The germ contributes a major portion of the albumins and globulins of whole corn grain according to early studies of Osborne and Mendel (9). A comparison was therefore made of the analyses of the fractions of salt-soluble proteins in germ and endosperm.

There is considerable evidence that the salt-soluble proteins of corn exist in different molecular sizes or states of aggregation. Danielsson (10) found at least two components in a corn globulin preparation that differ in sedimentation rates during ultracentrifugal analysis. Albumins from corn were separated by Moureaux and co-workers (11) into five components differing in rate of elution from Sephadex G-100 gel-filtration columns. In the present study effects of pH, such dissociating agents as urea, and sulfhydryl group oxidation on the molecular size of albumins from whole corn, germ, and endosperm were explored by means of gel-filtration chromatography.

MATERIALS AND METHODS

Proteins were extracted from field corn, Funk's G-83, 1966 harvest. Corn was milled by a method described by Brekke *et al.* (12). The endosperm and germ sections (12% moisture) were separated by hand-selection from the largest screened fraction (designated $-3\frac{1}{2}+4$). Partially defatted whole corn and endosperm meals were prepared by the method of Boundy *et al.* (7). The germ meal was ground in an intermediate-size Wiley mill through a No. 40 U.S. mesh screen. It was then covered with a layer

of petroleum ether at 5°C. for 2 hr. with occasional agitation of the slurry, for partial defatting.

Extraction of Proteins

Separate portions of defatted whole corn meal were extracted with water, 0.5*M* NaCl, 0.1*M* $NaHCO_3$-0.02*M* Na_2CO_3, and 0.1*M* acetic acid-0.5*M* NaCl. For all extractions, except with 0.1*M* acetic acid-0.5*M* NaCl, the defatted meal at 5°C. was stirred with the solvent, first in a 5:1 (v./w.) solvent-to-meal ratio and then in a 5:2 ratio for 1 hr. Each resulting suspension was centrifuged at 2,300 × g at 5°C. for 15 min. The supernatants from both extractions with a single solvent were combined and dialyzed at 4°C. against three 16-liter changes of distilled water for a total of 36 hr. The 0.1*M* acetic acid-0.5*M* NaCl extraction differed from those with the other solvents in that it was carried out once in a 5:1 (v./w.) solvent-to-meal ratio at room temperature for 3 hr., and the supernatants were dialyzed first against 0.1*N* acetic acid for several hours and then against water. The contents of the dialysis bags were centrifuged as before. The precipitates were washed twice with distilled water and then redissolved in their original extractant, after which the dialysis procedure was repeated. The water extract contained little precipitate after dialysis and was not fractionated further. All supernatants containing the albumins, as well as the precipitated globulins from each extraction, were separately combined and freeze-dried.

The albumins and globulins from the ground defatted endosperm and germ sections were extracted with 0.5*M* NaCl by the procedure used for whole corn, except that the germ extract was recentrifuged at 20,000 × g for 30 min. The lipid in the germ extract, which appeared as an upper phase after the second centrifugation, was discarded.

Analytical Methods

Aliquots of the extracts or portions of weighed dried material were assayed for nitrogen by a semimicro-Kjeldahl method.

Reducing sugars were determined with a Technicon Autoanalyzer by the colorimetric method of Hoffman (13) and calculated as glucose.

Amino acid analyses were carried out on 10-mg. protein samples hydrolyzed with 12 ml. constant-boiling HCl in sealed glass tubes at 105°C. for 24 hr. Amino acid compositions of aliquots of the hydrolysates were quantitatively determined with a Phoenix automatic amino acid analyzer, Model K-8000, by the Benson and Patterson (14) accelerated procedure.

Portions of the extracted proteins were reduced in a 0.1*M* phosphate pH 8 buffer containing 8*M* urea by reaction with a 30-fold molar excess of mercaptoethanol over sulfur for 3 hr. at room temperature. A tenfold excess of acrylonitrile over total mercaptan was then added for alkylation and reacted for 1 hr. at room temperature. The solutions were adjusted to pH 3 and then dialyzed.

Electrophoresis was carried out in starch gels according to a modified procedure of Beckwith et al. (15). The gels were prepared from a solution made by heating 48 g. of Connaught partially hydrolyzed starch in 300 ml.

aluminum lactate buffer (0.008*M* aluminum lactate and 0.5*M* lactic acid), pH 3.1, containing 8*M* urea. The proteins were applied as 5% solutions in electrophoresis buffer to filter paper strips inserted into the gels. Electrophoresis was carried out at a potential of 50 and 100 v. for large and small gels, respectively, at 60 to 80 ma. for 4 hr. Although the buffer containing 8*M* urea was heated during formation of the starch gel, any cyanate formation did not significantly alter the electrophoretic patterns. Lack of cyanate effect (16) was established by comparisons with patterns in polyacrylamide gels, developed with the same buffers.

Gel Filtration

Gel-filtration chromatography was performed with three different buffers: sodium acetate, pH 4.4, as described by Moureaux *et al.* (11); 0.05*M* H_3BO_3-0.05*M* KCl, pH 8.6; and 0.05*M* H_3BO_3-0.05*M* KCl, pH 8.6, containing 6*M* urea. Separate batches of Sephadex G-100 were equilibrated in several changes of each buffer and then poured in sections into 2.5 by 60-cm. glass columns. The columns were then washed for 16 to 20 hr. with 500 ml. of buffer. The exclusion volume of each column was determined by establishing the volume of elution of Blue Dextran-2000. A 6% (w./v.) solution (containing 13 to 22 mg. N) of either whole corn, endosperm, or germ albumins was dissolved in the column buffer and eluted with the same buffer at room temperature at a flow rate of about 26 ml. per hr. The effluent protein concentration was monitored by measuring the absorbancy at 280 mμ with a Vanguard Model 1030 recording flow spectrophotometer. Fractions of 5 to 6 ml. were collected in a refrigerated fraction collector. Total carbohydrate was determined by the phenol-sulfuric acid method (17) on fractions of effluent with glucose as a standard. The contents of the tubes containing separate absorption peaks were combined, dialyzed against cold water, and freeze-dried.

RESULTS

Composition of the Meals

The defatted whole corn, endosperm, and germ meals contained 1.5, 1.3, and 3.6% nitrogen, respectively. On the basis of a conversion factor of 6.25, these meals contained 9.3, 8.3, and 22.5% protein. The germ fraction consisted of about 10% of the grain. The bran was 7% of the whole meal, but its proteins were not studied.

Extraction with Various Solvents

The amounts of nitrogenous materials extracted with various solvents from the whole corn meal are shown in Table I. Extraction with 0.5*M* NaCl removed the most nitrogen, 17%; distilled water yields the least, 7%. Yields of soluble albumins and insoluble globulins obtained by dialysis of the extracts against water are also given in Table I. Differences between the total nitrogen of isolated albumins and globulins and the total nitrogen extracted with each solvent represent losses on dialysis. These losses constitute nonprotein nitrogen-containing substances of low molecular weight (MW) and, in part, may be due to digestion of protein by proteases in the extracts.

TABLE I. EFFECT OF SOLVENTS ON EXTRACTION OF ALBUMINS AND GLOBULINS FROM WHOLE CORN MEAL

Extractant	pH	Extracted Percent Nitrogen	Albumins		Globulins	
			Percent of Extract Nitrogen	Percent Nitrogen in Solid	Percent of Extract Nitrogen	Percent Nitrogen in Solid
Water	..	7	62	8	..	..
0.5M NaCl	6	17	50	9	22	15
0.1M $NaHCO_3$-0.02M Na_2CO_3	9	15	35	11	25	13
0.1M Acetic acid-0.5M NaCl	3	13	31	7	6	14

The losses on dialysis were highest for the pH 2.8 extract. More nitrogen, 72%, was recovered from the 0.5*M* NaCl extract after dialysis than from any of the others. In each extract the amount of protein soluble in distilled water (albumins) exceeded that which was insoluble in water (globulins). No appreciable precipitate of globulins was obtained from the water extract. The globulin preparations were mostly protein, since they contained 13 to 15% nitrogen. The lyophilized solids from dialyzed supernatants (albumins) had lower nitrogen contents, 7 to 11%. Most of the nonprotein material in these solids was established to be carbohydrate by phenol-sulfuric acid assay.

Differences between the starch-gel electrophoretic patterns of the albumin and globulin preparations from the various extracts are shown in Fig. 1. The patterns of albumins and globulins of corn meal differ. The globulins from the saline and carbonate buffer extracts migrate in sharply defined bands, which have slightly different mobilities than those of the corresponding al-

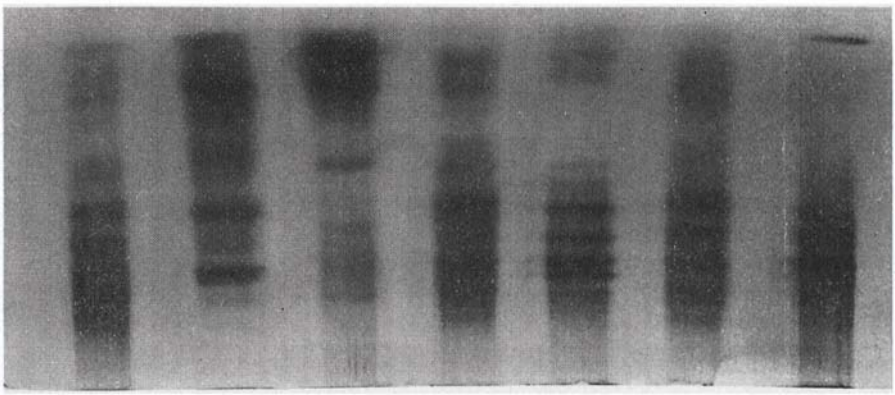

Fig. 1. Starch-gel electrophoretic patterns of albumin and globulin protein fractions from different extracts of whole corn meal. Acetate-saline consist of 0.1M acetic acid-0.5M NaCl buffer; saline, 0.5M sodium chloride; and carbonate 0.1M $NaHCO_3$-0.02M Na_2CO_3. Origin is at bottom of gel. Direction of migration is upward to negative electrode.

bumins. The patterns of albumin fractions from the water, saline, and carbonate extracts appear similar. The saline-extracted globulin electrophoretic pattern shows slightly more and better-defined bands than that of the carbonate extract. The acetate-saline extract albumins and globulins were quite different in gel patterns from those of the other extracts. Protein of high electrophoretic mobility on starch gel predominated in the acidic extracts. The acetic acid solution may preferentially extract the more basic proteins.

Since the 0.5*M* NaCl extract gave the best yields and electrophoretic patterns, it was used for studying endosperm and germ fractions.

Saline Extracts of Endosperm and Germ

Only 6% of the total nitrogen of the endosperm meal was extracted by 0.5*M* NaCl (Table II). Half of this nitrogen was recovered after dialysis, of which 60% was souble in distilled water (Table II). The solids recovered from the water-soluble fraction contained only 6% N. The nonprotein material in this fraction was predominantly carbohydrate, and about 8% of the carbohydrate analyzed for reducing sugar. The globulin precipitates are purer proteins, as evidenced by their higher content of nitrogen, 13%.

Extraction of germ with 0.5*M* NaCl dispersed 57% of its nitrogen into an initial milky extract. It was necessary to recentrifuge this supernatant to remove dispersed solids and lipid material. The resulting supernatant retained 65% of the extracted nitrogen. Values reported in Table II for yields of the albumins and globulins of germ are for fractions obtained after dialysis of the recentrifuged extracts. Considerably more nitrogen is extracted from germ than from endosperm. The germ extract contains more albumins than globulins, and these extracts of albumins have a high level of nitrogen, 13%. There is about twice as much dialyzable nitrogen in the germ extract as in the endosperm extract.

TABLE II. ALBUMIN AND GLOBULIN YIELDS OF 0.5M SODIUM CHLORIDE EXTRACTS OF CORN GERM AND CORN ENDOSPERM

		Albumins		Globulins	
Section	Extract Percent Nitrogen	Percent of Extract Nitrogen	Percent Nitrogen in Lyophilized Solid	Percent of Extract Nitrogen	Percent Nitrogen in Lyophilized Solid
Endosperm	6	31	6	20	13
Germ[a]	57	16	13	8	15

[a] After ultracentrifugation, 35% of the N in the germ extract was present in the sediment.

Figure 2 shows starch-gel electrophoretic patterns of albumins and globulins extracted with 0.5*M* NaCl from whole corn, germ, and endosperm. Marked differences exist between the globulins and albumins from the same seed parts. Two dark bands in albumins and four close distinct bands plus a band of high mobility in globulins are observed in all extracts. Some additional fast-moving bands in the germ albumins are the only major differences between the salt-soluble proteins of germ and endosperm.

Amino Acid Analyses

The amino acid compositions of the endosperm and germ albumins and

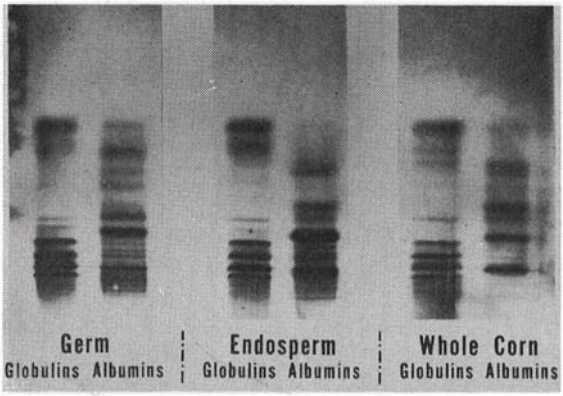

Fig. 2. Starch-gel electrophoretic patterns of albumin and globulin proteins separated from 0.5M NaCl extracts of corn grain, endosperm, and germ. Origin is at bottom of gel. Direction of migration is upward to negative electrode.

TABLE III. AMINO ACID ANALYSIS OF ENDOSPERM AND GERM ALBUMINS AND GLOBULINS

Amino Acid	Endosperm		Germ	
	Albumins	Globulins	Albumins	Globulins
	mM/100 g. protein (16 g. N)			
Lysine	44	41	51	37
Histidine	16	25	18	21
Ammonia	84	80	95	76
Arginine	43	72	44	63
Aspartic acid	73	58	72	53
Threonine	45	28	47	27
Serine	48	53	49	49
Glutamic acid	86	114	98	96
Proline	45	33	27	38
Glycine	93	73	82	70
Alanine	85	60	85	57
Valine	50	49	50	44
Methionine	10	7	8	5
Isoleucine	28	23	24	22
Leucine	49	45	45	44
Tyrosine	19	17	17	15
Phenylalanine	21	28	28	27

globulins are compared in Table III. Values are given as millimoles amino acid per 100 g. protein. Tryptophan and cystine were not determined. Slight differences exist between amounts of arginine, aspartic acid, alanine, threonine, proline, and glycine in the globulins and albumins from the same fraction of corn grain. These differences in the amino acid composition may account for differences noted in starch-gel electrophoretic patterns of albumins and globulins. Except for proline, only small differences in amino acids occur between similar proteins from germ and endosperm.

Gel Filtration

In Fig. 3, a and b compare gel-filtration chromatographic separations of whole corn albumins with sodium acetate-acetic acid, pH 4.4 buffer, and 0.05*M* borate-0.05*M* potassium chloride, pH 8.6 buffer. From 89 to 97%

of the total nitrogen of the albumin sample dissolved in each buffer, and recovery of 280-mμ absorbance after fractionation was 90 to 100%. All the globulin preparations were poorly soluble in these buffers at concentrations needed for chromatography.

Gel filtration of whole corn albumins at pH 4.4 (Fig. 3, a) results in five peaks and is similar to the fractionation pattern obtained by Moureaux *et al.* (11) under the same conditions. The first peak was eluted in the exclusion volume of the column. On starch-gel electrophoresis of the fractions, the initially eluted components have lower mobilities, which may result from their higher molecular weight. The last peak, although having a considerable absorption, contained little protein. When whole corn albumins were chromatographed with a more alkaline buffer (borate, pH 8.6), the number of peaks decreased from five to three and the amount of initially eluted material (peak A) increased (Fig. 3, b). Starch-gel electrophoretic patterns of the proteins in peak A indicate that the increased amount of this component was accompanied by more streaked material near the origin. However, it showed

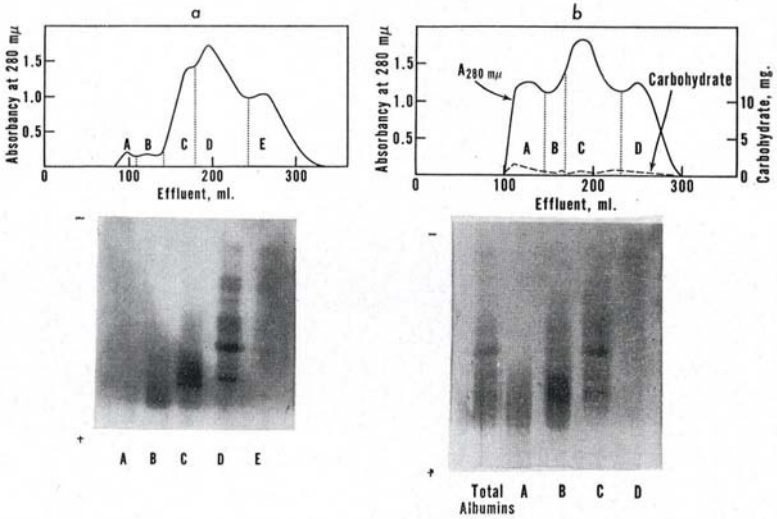

Fig. 3. Comparison of the influence of pH on chromatography of whole corn albumin extracts on Sephadex G-100. Gel electrophoretic patterns are of fractions in peaks obtained by chromatography. (A) Sodium acetate-acetic acid buffer, pH 4.4, μ = 0.025 and (B) 0.05M boric acid-0.05M potassium chloride adjusted to pH 8.6.

the same number of mobile bands as did the initial peak of the pH 4.4 fractionation of whole corn albumins.

When chromatographed with the same buffers, endosperm albumins gave patterns similar to those from whole corn albumins. The only difference was that with endosperm albumins the relative increase in peak A was slightly less upon chromatography in pH 8.6 buffer (Fig. 4, a). Starch-gel electrophoresis indicated no difference in the patterns of the endosperm albumin fractions compared to fractions of whole corn albumins.

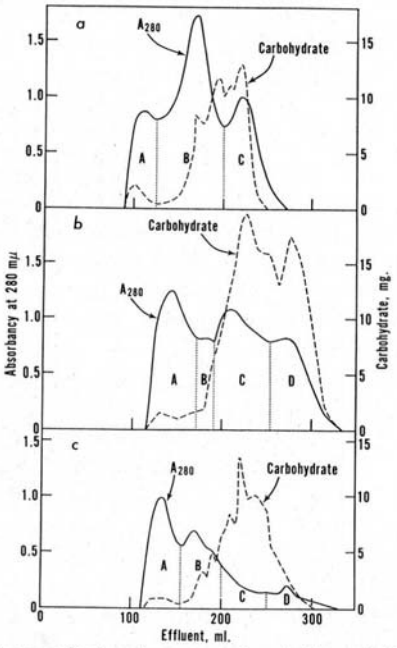

Fig. 4. Comparison of the effects of urea and reduction-alkylation on chromatography of endosperm albumins on Sephadex G-100 in pH 8.6 borate buffer. a, Endosperm albumins; b, endosperm albumins in presence of 6M urea in buffer; c, endosperm albumins after reduction of disulfides and cyanoethylation; chromatography buffer contains 6M urea.

Endosperm albumins were also fractionated with the buffer having pH 8.6 but containing 6*M* urea, a dissociating agent, to determine whether the increase in high-MW material observed at pH 8.6 compared to pH 4.4 was due to an increase in association of protein. The presence of 6*M* urea further increased the relative amount of initially eluted material (peak A) compared with the same buffer without urea (Fig. 4, b). Endosperm albumins, treated with mercaptoethanol to reduce disulfide bonds and alkylated with acrylonitrile to prevent reoxidation of the resulting sulfhydryls, were chromatographed in the same urea-containing buffer and showed a further relative increase in peak A (Fig. 4, c). Peak D is also decreased considerably. Since reduced and alkylated albumins also show a relative increase in initially eluted material, the more rapid elution of albumins in alkaline solution may not be attributed to disulfide cross-linking.

A large amount of carbohydrate elutes slightly after peak C in all the endosperm fractionations. An increase in reducing sugar appears in the latter part of the fractionations (150 to 300 ml.). An active amylase may be present in the endosperm extracts.

Because corn germ albumins were only slightly soluble in the acetate pH 4.4 buffer, they could not be chromatographed in this solvent. They were soluble in the borate pH 8.6 buffer, which was used for their Sephadex chromatography. The elution pattern for germ albumins was different from that

for whole corn and endosperm albumins at this pH value. Proportionally, more excluded material and less of the middle peak were formed as compared to whole corn and endosperm albumins. Peak A appeared streaked at the origin upon starch-gel electrophoresis and is probably high-MW material.

DISCUSSION

The albumins and globulins of corn constitute a spectrum of proteins differing in isoelectric points. Extraction at different pH values results in some preferential selection of certain of these. Small but significant differences between albumin and globulin fractions were observed in starch-gel electrophoretic patterns and amino acid analyses. The small differences in composition and properties between corn albumins and globulins may explain why separations of these two are not distinct and why yields of the two classes vary considerably with the method of separation.

A major problem that has confronted workers investigating albumin and globulins from corn is the difficulty of redissolving them in saline after they are dried. A possible cause of insolubilization is oxidation of sulfhydryls to form intermolecular disulfide bonds. Albumins from endosperm had more protein fractions eluting earlier when chromatographed on gel-filtration columns, in alkaline buffers, which accelerate disulfide interchange, than in acid buffers. However, reduction of disulfide bonds and stabilization of the sulfhydryls by alkylation did not change the elution positions of the protein in 6*M* urea solution. Urea alone increases the amount of material eluted in early fractions from the Sephadex column. Electrostatic interaction due to charged amino acids is not responsible for the aggregation at pH 8.6, since increasing the ionic strength of the buffer did not alter the chromatographic separation obtained by gel filtration. Possibly unfolding or other conformational changes of the protein in the pH 8.6 buffer and urea may be responsible for its change in properties.

Further studies are in progress in which corn albumin and globulin proteins are being purified by ammonium sulfate fractionation and ion-exchange chromatography. Investigations of factors influencing molecular properties will be continued on these purified proteins.

Acknowledgments

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