Much work remains to be done before the exact structure can be identified, but the final identification promises to be an important step toward the establishment of the chromium requirement and the assessment of the chromium nutrition in man.

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Heat-Induced Complex Formation between Myosin and Soybean 7S Globulins

Neville L. King

Myosin and β-conglycinin (a soybean 7S globulin fraction) were found to associate after exposure to temperatures in the range 75–100 °C. The complexes formed sedimented more slowly, and their subunit compositions were different from those of aggregates derived from β-conglycinin heated alone. The sedimentation coefficient of the β-conglycinin aggregate increased with incubation temperature, and its subunit composition became progressively richer in the component of lowest molecular weight. At lower temperatures (2 and 25 °C) no evidence for any complex formation was obtained when mixtures of myosin and β-conglycinin in phosphate buffer (pH 7.6, ionic strength 0.5) were examined by viscometry, gel chromatography, and density gradient centrifugation.

Changes in texture induced by the incorporation of soy proteins in manufactured meat products have been reported to be large in relation to the amount of soy protein added (Hermansson, 1973). It is possible that interactions between the proteins of muscle and soybeans might contribute to these changes. In view of the difficulties in examining whole meat products for the presence of interactions, solutions and suspensions of protein fractions from muscle and soybeans have been employed as model systems. Yamamoto et al. (1973) examined the 11S globulin and acid-precipitated proteins from soybeans in admixture with myosin and actomyosin. They detected no evidence for interactions at low temperatures, but found, by turbidimetric measurements, that myosin and soy protein aggregated to a lesser extent when heated in combination, than when heated separately.

In order to obtain further information on the above model systems, mixtures of the major protein components of muscle and soybeans are being investigated by alternative techniques. The present article deals mainly with the sedimentation properties and subunit composition of products formed from myosin and β-conglycinin after heating. β-Conglycinin is one of the two immunochemically distinguishable forms of soybean 7S globulins (Catsimpoolas and Ekenstam, 1969).

Changes in quaternary structure of proteins give rise to difficulties in the nomenclature used to describe the products formed (e.g., see Joly, 1965). For convenience, the term "aggregate" will be used here to describe a high molecular weight product derived from components of the same protein fraction, the term "complex" will be employed for products incorporating components of different protein fractions, while "interaction" will be used more widely to include effects such as hydrodynamic interactions which result in changes in physical properties but not necessarily in quaternary structure.

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MATERIALS AND METHODS

Phosphate buffer consisted of: 0.035 M potassium hydrogen phosphate, 0.4 M sodium chloride, and 0.02% sodium azide, pH 7.6.

Myosin. Myosin was prepared from longissimus dorsi muscles of rabbits by the extraction and ammonium sulfate fractionation procedures of Offer et al. (1973). The content of contaminating proteins (see, e.g., Offer et al., 1973; Pinset-Harstrom and Ehrlich, 1973) was reduced by using the additional purification steps recommended by Wikman-Coffelt et al. (1973). Finally, myosin was dialyzed against phosphate buffer and centrifuged at 30 000 rpm for 0.5 h and the supernatant (~10 mg/ml) was stored at 2 °C.

β-Conglycinin. Soybean 7S globulins were prepared by the following procedure, based on that of Roberts and Briggs (1965). Defatted soy flour was extracted with water (5 ml of water/g of flour) by stirring for 1 h at room temperature. After centrifuging at 20 000*g* for 15 min at 15 °C, the supernatant was passed through glass wool and allowed to stand overnight at 2 °C. The precipitated material (mainly 11S globulin) was removed by centrifuging at 0 °C. The supernatant was subjected to ammonium sulfate fractionation; the material precipitating between 0.80 and 0.95 saturation was dissolved in phosphate buffer (1 ml/g of soy flour) and the ammonium sulfate fractionation was repeated. The 0.80–0.95 fraction, after dissolving in phosphate buffer as above, was adjusted to pH 4.5 with acetic acid and dialyzed against water to precipitate the 7S globulins. After centrifuging, the precipitate was dissolved in phosphate buffer to give a

concentration of approximately 10 mg/ml and stored at 2 °C.

Catsimpoolas and Ekenstam (1969) reported that the method of Roberts and Briggs (1965) gave mainly β-conglycinin, but also some γ-conglycinin and glycinin (11S globulin). However, the modified procedure described above gave a product, which, on electrophoresis in the phenol–acetic acid–mercaptoethanol–urea (PAMU) system of Catsimpoolas et al. (1968), exhibited the four bands of β-conglycinin, but no detectable γ-conglycinin or glycinin. These three proteins are readily distinguishable in this system of electrophoresis (Catsimpoolas, 1970).

The above preparation gave sedimentation coefficients at 20 °C of 7.1 S at 6 mg/ml in phosphate buffer and 10.8 S at 4 mg/ml in phosphate buffer devoid of sodium chloride. These sedimentation coefficients are in good agreement with those obtained by Roberts and Briggs (1965) under the same conditions.

Heat Treatments. Solutions of myosin and β-conglycinin were placed in tubes in a heated water bath controlled to ±0.5 °C. After incubation, the tubes were allowed to cool by standing in air at room temperature. Samples were then stored at 2 °C.

Density Gradient Centrifugation. Samples (4 ml) were layered on sucrose gradients (linear, 60–20% in phosphate buffer) in a swinging bucket rotor (SW 25.1) and centrifuged at 25000 rpm for 16 h at 5 °C in a Beckman Spinco ultracentrifuge (Model L). The contents of each tube were pumped at 0.7 ml/min through an LKB Uvicord II in order to record the absorbance in the wavelength region of 280 nm. Fractions were collected, dialyzed against water, and freeze-dried for subsequent electrophoresis.

Sedimentation coefficients of β-conglycinin, as well as its aggregates, were measured at 20 °C in phosphate buffer in an analytical ultracentrifuge (Beckman Model E). The distances sedimented by aggregates of β-conglycinin in sucrose density gradients were also used to calculate their sedimentation coefficients in phosphate buffer at 20 °C by the numerical integration procedure of Martin and Ames (1961). Reasonable agreement was obtained by the two procedures. With regard to the rapidly sedimenting material formed in mixtures of β-conglycinin and myosin after heating, sedimentation coefficients were calculated by numerical integration and were estimated also from a calibration curve of sedimentation coefficient of β-conglycinin aggregates (in phosphate buffer at 20 °C) plotted against distance sedimented in the sucrose density gradient.

Gel Electrophoresis. (a) Gel electrophoresis, in the presence of sodium dodecyl sulfate, was carried out by the method of Weber and Osborn (1969). Samples were dissolved in a solution containing 1% sodium dodecyl sulfate, 8 M urea, and 2% mercaptoethanol, incubated for 30 min at 75 °C (except where otherwise indicated), and dialyzed into 0.01 M phosphate–0.1% sodium dodecyl sulfate–0.1% mercaptoethanol at pH 7. Gels were formed from a solution containing 5% acrylamide and 0.13% methylenebisacrylamide in glass tubes 120 mm in length and 5 mm in diameter. Staining of gels in 0.2% Coomassie Brilliant Blue R250 in water–methanol–acetic acid (4.5:4.5:10) for 20 min at room temperature was followed by destaining in several changes of water–methanol–acetic acid (87.5:5:7.5) at 37 °C over a period of 2 days. Gels were scanned in a Kipp and Zonen Densitometer.

(b) Gel electrophoresis, in the presence of 5 M urea in phenol, acetic acid, and mercaptoethanol (PAMU), was carried out by the method of Catsimpoolas et al. (1968).

Viscometry. Flow times were measured in a glass capillary viscometer in a water bath at 25 °C. Kinetic energy and surface tension corrections were applied as described by McKie and Brandts (1972).

Gel Chromatography. Samples (1–5 mg) were chromatographed on a column (40 cm × 2.5 cm) of Sepharose 4B by elution with phosphate buffer (without azide) at a flow rate of 10 ml/h at 2 °C. Fractions were collected and the absorbance of each was measured at 220 nm.

Protein determinations were carried out by the method of Lowry et al. (1951) using bovine serum albumin as a standard, or by absorbance measurements.

Criteria for Interactions. With gel chromatography, if the elution profile for the mixture of myosin and soy protein (at low column loadings) deviated from the sum of the elution profiles for the separate protein components, this was taken as evidence for the presence of interactions. Similarly, in sedimentation studies, if the distribution of protein after centrifuging for a certain time was different for the mixture than for the sum of the separate components, this was taken as evidence for the presence of interactions.

However, the problem of predicting the specific viscosity of a mixture of macromolecules, even when no changes in shape or quaternary structure are involved, has not yet been resolved. Equations 1–4 listed below have been

$$\eta_{sp(m)} = \eta_{sp(a)} + \eta_{sp(b)} \quad (1)$$

$$\eta_{sp(m)} = \eta_{sp(a)} + \eta_{sp(b)} + (K_a'[\eta]_a^2 + K_b'[\eta]_b^2)C_aC_b \quad (2)$$

$$\eta_{sp(m)} = \eta_{sp(a)} + \eta_{sp(b)} + 2(K_a'K_b')^{1/2}[\eta]_a[\eta]_bC_aC_b \quad (3)$$

$$\eta_{sp(m)} = \eta_{sp(a)} + \eta_{sp(b)} + (\eta_{sp(a)}\eta_{sp(b)}) \quad (4)$$

proposed, and they differ in the term intended to account for hydrodynamic interactions (eq 1 lacks such a term) (eq 1, Harrington and Burke, 1972; eq 2, Spencer and Boyer, 1945; eq 3, Krigbaum and Wall, 1950; eq 4, Eisenberg and Moos, 1967). Subscripts a and b refer to two separate protein components, while subscript m refers to the mixture; η_{sp} is specific viscosity; $[\eta]$ is intrinsic viscosity; K' is the slope constant in the Huggins equation $\eta_{sp} = [\eta]C + K[\eta]^2C^2$; and C is the concentration of protein.

Spencer and Boyer (1945) derived eq 2 by assuming that:

$$\frac{\eta_{sp(m)}}{\Sigma C_i} - [\eta]_m = \sum_i \left[\frac{\eta_{sp(i)}}{C_i} - [\eta]_i\right] = \sum_i K_i'[\eta]_i^2C_i$$

Alternatively, if $K_i[\eta]_iC_i$ is assumed additive, i.e.:

$$\frac{\eta_{sp(m)}}{[\eta]_m\Sigma C_i} - 1 = \sum_i \left[\frac{\eta_{sp(i)}}{[\eta]_iC_i} - 1\right] = \sum_i K_i'[\eta]_iC_i$$

the additional eq 5 arises, viz.:

$$\eta_{sp(m)} = \eta_{sp(a)} + \eta_{sp(b)} + (K_a' + K_b')[\eta]_a[\eta]_bC_aC_b \quad (5)$$

Since there is no general agreement on which of these equations correctly predicts the specific viscosity of a mixture which is noninteracting (apart from hydrodynamic interactions) viscosity data were taken to indicate evidence for complex formation only if the value for the mixture was outside the entire range covered by eq 1–5 above.

RESULTS

Composition of β-Conglycinin. β-Conglycinin has a subunit structure (see, e.g., Roberts and Briggs, 1965;

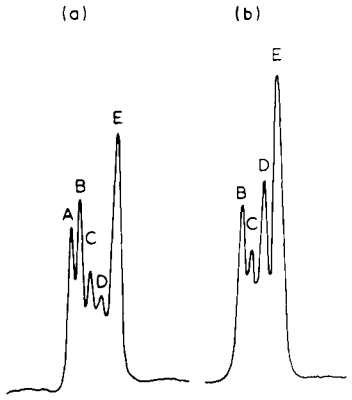

Figure 1. Densitometer scans of sodium dodecyl sulfate-polyacrylamide gels. β-Conglycinin was incubated in 0.01 M phosphate containing 1% sodium dodecyl sulfate, 8 M urea, and 2% mercaptoethanol for 0.5 h at 75 °C (a) or 15 min at 100 °C (b). Migration is from left to right toward anode.

Catsimpoolas, 1970) demonstrated in Figure 1 by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. The number of bands obtained by this technique depended on the temperature at which the sample was treated prior to loading on the gel. When β-conglycinin was digested in the sodium dodecyl sulfate–urea reagent at temperatures up to 75 °C, five bands (A to E) were obtained (Figure 1a). However, at 100 °C, the slowest moving band (A) was absent, while D and E were more intensely stained (Figure 1b). Breakdown of band A to D and E was also achieved by boiling β-conglycinin in phosphate buffer for 5 min, or by allowing the aqueous extract of soy flour to stand in a refrigerator for several weeks. It is possible that dissociation of subunits in the sodium dodecyl sulfate–urea reagent was incomplete after digestion at 75 °C. However, Shemer and Perkins (1975) reported partial degradation of soy protein polypeptide chains in boiling water. It is therefore likely that the disappearance of band A as a result of digestion at 100 °C was due to polypeptide breakdown, rather than further dissociation of subunits.

Despite homogeneity in the ultracentrifuge, Catsimpoolas and Ekenstam (1969) showed that β-conglycinin was heterogeneous since it yielded three bands on gel electrophoresis in a nondissociating solvent. The heterogeneity is further demonstrated in Figure 2a which shows the elution of β-conglycinin from DEAE Sephadex A-50. Several fractions from this column were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis, densitometer scans of which are shown in Figure 2b. The first peak to be eluted contained mostly band E with some D, but the other bands were absent. In the material eluted after the first peak, there was a progressive decrease in the proportion of E, accompanied by an increase in bands A and B.

Elution of β-conglycinin from Sepharose 4B with phosphate buffer gave only one peak, but the leading edge was richer in bands A and B, while the trailing edge was richer in band E. Heterogeneity of soybean 7S globulins, as revealed by gel chromatography and gel electrophoresis, has been reported previously by Thanh et al. (1975a,b).

No attempt was made in this work to fractionate β-conglycinin on a preparative scale; instead, investigations were made on mixtures of the heterogeneous preparation with myosin.

Heat-Induced Aggregation of β-Conglycinin. No change in the sedimentation behavior of β-conglycinin in phosphate buffer on sucrose density gradients was observed after exposure to temperatures up to 70 °C.

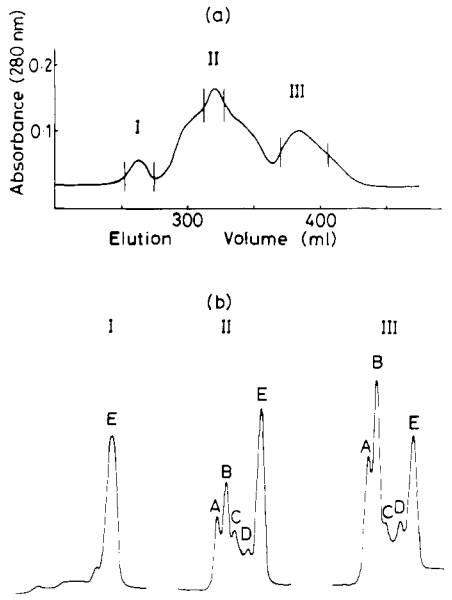

Figure 2. (a) Elution of 50 mg of β-conglycinin from DEAE Sephadex A-50 column (40 cm × 2.5 cm) with phosphate buffer (pH 7.6) containing gradient of sodium chloride from 0.1 to 1.0 M. (b) Densitometer scans of fractions I, II, and III from (a), run on sodium dodecyl sulfate-polyacrylamide gels.

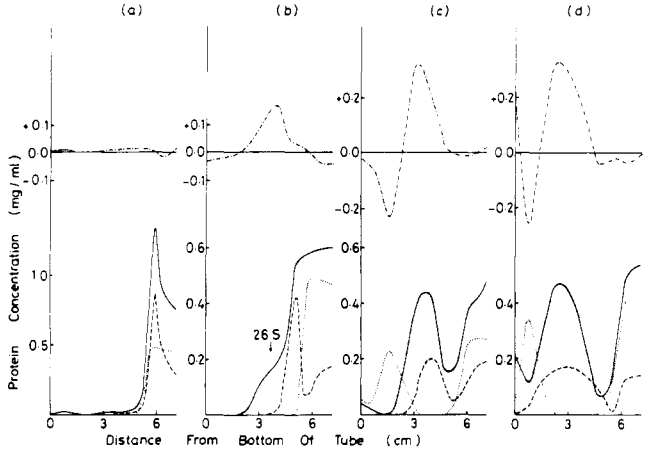

Figure 3. Distribution of protein in sucrose density gradient after centrifuging for 16 h at 25 000 rpm. Samples were incubated at 2 °C (a), 50 °C (b), 85 °C (c), and 95 °C (d) for 0.5 h, cooled at room temperature, and stored overnight at 2 °C prior to centrifugation. In each of the four parts of this figure, the curves shown were derived from three tubes centrifuged simultaneously. Any protein centrifuged to the bottom of a tube was not pumped through the Uvicord and consequently is not represented in this figure: (- - -) myosin (m); (· · ·) β-conglycinin (s); (—) myosin + β-conglycinin (M); (- · -) M-m-s (calculated).

However, on heating for 0.5 h at any temperature in the range 75–100 °C, followed by cooling, density gradient centrifugation indicated the presence of aggregated material, as well as components remaining near the top of the tube (Figure 3). Data provided by the analytical ultracentrifuge showed that some 7S material remained at temperatures up to 85 °C, and that a very small amount of 2S material was also present at each temperature. The sedimentation coefficient of the aggregate increased with temperature as shown in Figure 4.

The composition of the aggregated β-conglycinin was also dependent on temperature of incubation. Figure 5

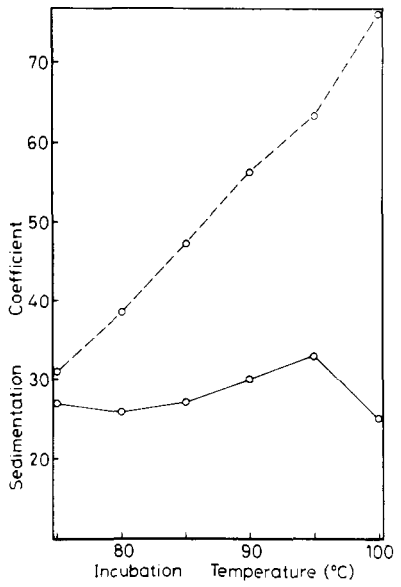

Figure 4. Sedimentation coefficient (in phosphate buffer at 20 °C) of rapidly sedimenting material obtained after heating β-conglycinin alone (○- - -○) and mixtures of β-conglycinin with myosin (○—○).

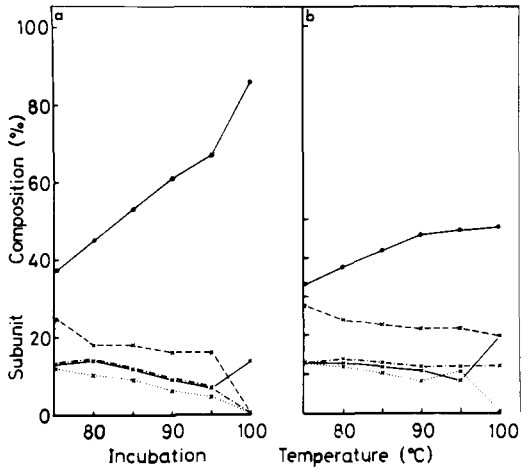

Figure 5. Apparent subunit composition of soy protein portion of aggregates formed from β-conglycinin alone (a) and mixtures of β-conglycinin and myosin (b). Subunits A, B, C, D, and E are as shown in Figure 1. Apparent subunit compositions were derived from peak areas in densitometer scans. The peak area for each subunit is expressed as a percentage of the total area for all soy protein peaks in the same densitometer scan: (A) (×· · ·×); (B) (×- - -×); (C) (×- · -×); (D) (×—×); (E) (•—•).

shows apparent subunit compositions derived from areas of peaks in densitometer scans obtained after electrophoresis on sodium dodecyl sulfate–polyacrylamide gels. With the increase in temperature, the composition changed progressively from one resembling the whole β-conglycinin toward that of the first peak eluted from DEAE-Sephadex A-50. At 100 °C, the results are complicated by the decomposition of band A to bands D and E.

Heat-Induced Aggregation of Myosin. Following exposure of myosin at concentrations in the region of 1 mg/ml to temperatures in the range 50–100 °C for 30 min, density gradient centrifugation resulted in some protein (10–20% of total) at the bottom of the tube ($s_{20} > 80$ S), some material at the top of the tube ($s_{20} < 10$ S), and some material of intermediate sedimentation coefficient (Figure 3). Sodium dodecyl sulfate gel electrophoresis showed that the material at the top of the tube was rich in myosin light chains, while the faster sedimenting material was rich in myosin heavy chains. Previous workers (e.g., Dreizen and Richards, 1973) have shown that dissociation into light and heavy chains begins at 40 °C.

Table I. Specific Viscosities of Mixtures of Myosin and β-Conglycinin[a]

β-Conglycinin Concn (%)	Calcd values for nonassociating mixtures, using eq 1	2	3	4	5	Exptl values
0.04	0.216	0.223	0.218	0.217	0.218	0.219
0.08	0.222	0.237	0.225	0.224	0.226	0.227
0.17	0.230	0.262	0.237	0.234	0.239	0.237
0.33	0.246	0.307	0.259	0.254	0.263	0.262

[a] Concentration of myosin was 0.1% in each solution of β-conglycinin in phosphate buffer. Solutions were mixed and allowed to stand overnight at 2 °C, prior to measurement of flow times at 25 °C.

Mixtures of Myosin and β-Conglycinin. When myosin and β-conglycinin were mixed at temperatures of 2 and 25 °C, no evidence was found, by the criteria above, for any complex formation by viscometry (Table I), gel chromatography, or density gradient centrifugation (Figure 3). Table I lists specific viscosities for several concentrations of β-conglycinin mixed with myosin. Equation 1 gives values too low, while eq 2 predicts higher values than were observed experimentally for the specific viscosities of the mixtures. Equations 3, 4, and 5 yield values in reasonable agreement with the experimental ones. Since the experimental values lie within the range covered by eq 1–5, these data provide no evidence for complex formation under these conditions.

Following exposure to 50 °C for 30 min, the distribution of protein after centrifugation in a sucrose density gradient was different for a mixture of myosin and β-conglycinin from that expected for a noninteracting mixture. This is shown in Figure 3 which indicates protein in solution or suspension in the density gradient (but not material centrifuged to the bottom of the tube). In the mixture there appeared material of sedimentation coefficient 26 S, which was not present in either the myosin or β-conglycinin heated separately. The appearance of this material was accompanied by a corresponding diminution in the amount of myosin at the bottom of the tube. However, sodium dodecyl sulfate gel electrophoresis gave no indication of any β-conglycinin subunits in the 26S fraction. Furthermore, on standing for 1 week at 2 °C, the sedimentation properties reverted to those of a noninteracting mixture, with the myosin aggregate of sedimentation coefficient 26 S returning to the bottom of the tube. Hence, the heating of myosin at 50 °C in the presence of β-conglycinin temporarily diminished the extent of myosin aggregation.

After treatment at 75 °C, the specific viscosities at some concentrations of mixtures of myosin and β-conglycinin fell outside the range of values predicted by eq 1–5 (Figure 6). Furthermore, at temperatures in the range 75 to 100 °C the sedimentation properties of the mixture differed considerably from those of myosin and β-conglycinin heated separately (Figure 3). Centrifugation of the mixture gave one broad band of rapidly sedimenting material, shown by sodium dodecyl sulfate gel electrophoresis to contain both myosin heavy chains and β-conglycinin subunits (Figure 7). The mean sedimentation coefficient of this material did not exhibit the steep increase with temperature which was observed for the aggregate formed on heating β-conglycinin alone (Figure 4). In contrast to the results obtained at 50 °C, the sedimentation properties

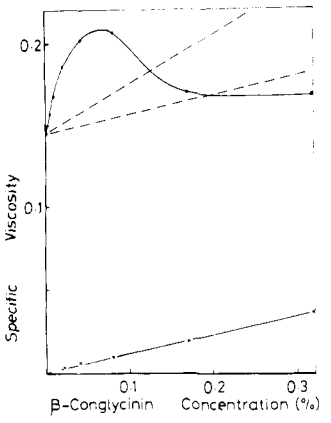

Figure 6. Specific viscosities of β-conglycinin (×—×) and mixtures of myosin (0.1%) with β-conglycinin (•—•) at the concentrations shown in phosphate buffer. All samples were incubated in a water bath at 75 °C for 30 min. After storage overnight at 2 °C, flow times were measured at 25 °C. The dashed lines enclose the range of values predicted for mixtures by eq 1-5 in the text.

of these samples remained unchanged on storage for 1 week at 2 °C.

Figure 5 shows the variation with temperature in the apparent subunit composition of the soy protein fraction of the rapidly sedimenting material formed from the mixture of myosin and β-conglycinin. Subunits A, B, and C participate in this material to a greater extent than in the aggregate formed from β-conglycinin heated alone.

DISCUSSION

Although at low temperatures there was no evidence for any complex formation between myosin and β-conglycinin, higher temperatures (75–100 °C) appeared to induce some form of interaction indicated by (1) an increase in specific viscosity of the mixture, (2) a decrease in sedimentation coefficient, and (3) a change in composition of the soy protein component of the aggregate. It is possible that myosin and β-conglycinin may aggregate separately, and that the changes in sedimentation coefficient and viscosity after heat treatment could have been due to transient contacts between myosin and β-conglycinin without the formation of a permanent complex. Such was the result after heating at 50 °C, where no soy protein subunits were observed in the 26S material. However, after treatment at temperatures of 75 °C and higher, the rapidly sedimenting material, containing both myosin heavy chains and soy protein subunits, gave rise to only one broad band in the sucrose density gradient, without any apparent division into separate components. Furthermore, fractions taken from the leading and trailing edges of the density gradient band containing the faster sedimenting material appeared to have the same content of myosin heavy chains and β-conglycinin subunits. Since it is unlikely that separate aggregates would have the same range of sedimentation coefficients, this suggests that complexes incorporating both myosin heavy chains and β-conglycinin subunits are formed.

The above results, together with those of Yamamoto et al. (1973), demonstrate that interactions between myosin and soy protein can be induced under suitable conditions. Further investigation is required to determine the role of any such interactions in the more complex systems of manufactured meat products.

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The author expresses his appreciation to Heather Gee and Lyndon Kurth for skillful technical assistance.

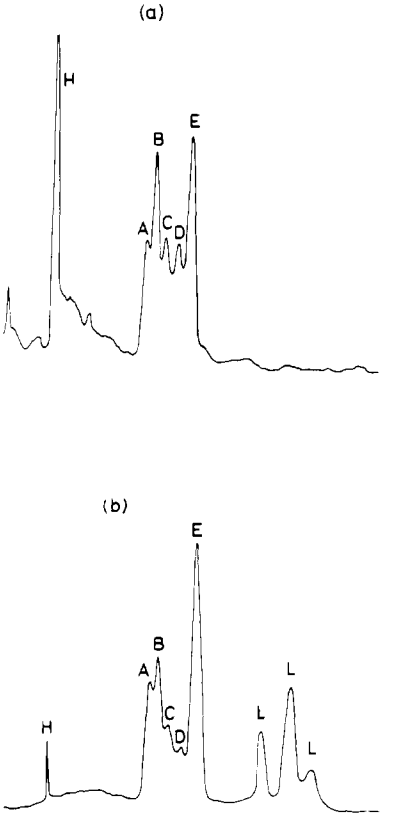

Figure 7. Densitometer scans of sodium dodecyl sulfate-polyacrylamide gels. Migration is from left to right toward anode. A mixture of myosin (1 mg/ml) and β-conglycinin (2 mg/ml) in phosphate buffer was placed in a 75 °C water bath for 0.5 h, cooled at room temperature, stored overnight at 2 °C, and centrifuged on a sucrose density gradient as described in the text: (a) rapidly sedimenting material (27S); (b) material remaining in band near top of the tube; H, myosin heavy chains; L, myosin light chains; A,B,C,D,E, β-conglycinin subunits as in Figure 1.

Analytical ultracentrifuge data were provided by C. J. Leeder and D. J. Winzor of the Biochemistry Department, University of Queensland.

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Processing Damage to Lysine and Other Amino Acids in the Manufacture of Blood Meal

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Four vat-dried and five ring-dried blood meals were analyzed for their total and reactive lysine contents, and for their potency in bioassays for lysine. The crude protein in the former contained, on the average, 9% less total lysine and 26% less fluorodinitrobenzene (FDNB)-reactive lysine than did the ring-dried blood meals. Using rats, chicks, and turkey poults for growth assays the potency of vat-dried blood meals as sources of lysine ranged from 0 to 43%, and that of ring-dried samples ranged from 80 to 97%, of the corresponding total lysine values. The vat drying resulted in an equally severe reduction in bioavailability of methionine, and the digestibility of the protein as a whole was greatly reduced. The ring-drying procedure, involving short-time, high-temperature heating, resulted in a product of reasonably good quality. Blood was not particularly sensitive to heat damage in controlled heating tests. Mixing blood with either corn meal, wheat bran, or starch prior to drying resulted in greater damage.

A considerable amount of blood is available as a by-product at abattoirs. Some goes to waste, but much is dried down to blood meal for animal feeding, and it has also been suggested that it could be recovered directly for the fortification of human diets (review by Happich, 1975). Its high protein and particularly high lysine content would make it a valuable supplementary protein in pig and poultry diets if the protein had a high digestibility and the lysine had a degree of availability equivalent to that in most other high-protein feedstuffs. In practice, however, it has been regarded as only poorly digestible and unpalatable (Morrison, 1956; Cullison, 1975). Winter (1929) reported that, while the nitrogen of specially prepared dried blood had a digestibility of 94% for pigs, the corresponding value for commercial blood meal was only 72%. Kratzer and Green (1957) reported that bioassays, using chicks and turkeys, gave values equivalent to only 60% of the total lysine content of commercial, vat-dried blood meals. In vat-drying, blood is dried by stirring in a steam-jacketed cylinder at a temperature of up to 165 °C for 10–12 h.

This investigation began with total and biologically available lysine assays of blood meal prepared by a newer process, ring drying, in which the blood is coagulated by steam and the sludge dried rapidly in a closed ring system in a current of very hot air (400–410 °C). Conventional (i.e., vat-dried) blood meals were used for comparison, and these gave such low values in the lysine assays that further tests were made of their digestibility and value in supplying the sulfur amino acids. Using fresh blood, the influences of controlled heating and carrier on fluorodinitrobenzene (FDNB)-reactive lysine were also studied.

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EXPERIMENTAL SECTION

Test Materials. The vat-dried and ring-dried blood meals were commercial samples of U.S. manufacture for which exact processing details were not obtainable. (Some analytical values for samples coded X949 and X953 have already been reported by Hurrell and Carpenter (1975)).

The fresh blood used was from steers. Portions were mixed with equal weights of either corn meal or corn starch, and a further portion with half its weight of wheat bran. These mixtures, and the blood itself, were divided up and either freeze-dried or dried in shallow dishes in air ovens set at 50 °C (for 48 h) or 100 °C (for 24 h). For a further experiment, portions of freeze-dried blood were adjusted to 18% moisture content, packed in glass ampules, sealed, and heated at controlled temperatures. Ampules containing crystalline hemoglobin (Sigma Chemical Co., St. Louis, Mo.) were heated at the same time.

Chemical Analyses. Analyses for total lysine were carried out essentially by the procedure of Spackman et al. (1958), using an ion-exchange column. Methionine and cystine were determined after preliminary oxidation with performic acid to methionine sulfone and cysteic acid, respectively (Moore, 1963). Fluorodinitrobenzene (FDNB)-reactive lysine was determined by the direct procedure of Carpenter (1960) as modified by Booth (1971).

Animal Assays. The turkeys used in the lysine assays were Large White males. Day-old poults were placed on a practical stock diet for 3 days, and the basal assay diet supplemented with 0.4% lysine for a further 4 days. They were then weighed and distributed on the basis of weight into groups of eight turkeys each. Three groups were assigned to each treatment. The experimental feeding period was 10 days. The basal diet contained (in percent) sesame meal 30, corn gluten meal 26, corn starch 34.55, corn oil 4, dicalcium phosphate 2.5, calcium carbonate 1.5, salt 0.5, fermentation residue product 0.25, taurine 0.025,