ULTRACENTRIFUGE STUDIES ON SOYA BEAN PROTEIN

by

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INTRODUCTION

OSBORNE AND CAMPBELL[1] proposed the name glycinin for the protein fraction which precipitated when a solution of soya bean protein in 10% sodium chloride was dialysed against water. From a 10% NaCl extract of the meal JONES AND CSONKA[2] claimed to obtain five protein fractions by ammonium sulphate precipitation. The fraction precipitating at 55% saturation with ammonium sulphate resembled the glycinin of OSBORNE AND CAMPBELL. HARTMAN AND CHENG[3] showed that glycinin could be prepared by adjusting the pH of an aqueous extract of soya bean meal to 4.5. Other workers[4] have shown that the composition of glycinin depended on the method of preparation. DANIELSSON[5] examined the protein from glycin soya along with other seed globulins in the ultracentrifuge. A 1 *M* NaCl extract of the ground seeds was saturated to 70% with ammonium sulphate, the precipitate dissolved in 0.2 *M* NaCl and precipitated twice by dialysis. In buffer of ionic strength $I = 0.3$ and pH 7 he showed that the protein thus prepared contained two components of sedimentation constants (s) = 13.1 and 8.0 S (Svedberg units). An electrophoretic investigation of soya bean protein was carried out by BRIGGS AND MANN[6] who showed the presence of at least seven electrophoretic components in an aqueous extract of the meal, 75% of the protein being a fast fraction containing three components. These authors prepared glycinin by various methods and showed that each preparation gave a different electrophoretic pattern. They also demonstrated that the ammonium sulphate fractions of JONES AND CSONKA[2] were heterogeneous electrophoretically. An electrophoretically homogeneous component was claimed to have been precipitated by cooling a concentrated, aqueous extract of the meal at 0° C for several hours. No change in the electrophoretic pattern of the proteins was obtained by varying the meal: liquor ratio in the extractions.

The present investigation is mainly concerned with an ammonium sulphate fractionation of the complicated protein system present in soya bean meal. Two of the sedimenting components present in the protein have been isolated in a reasonable state of purity and preliminary ultracentrifuge studies of the effect of various ionic environments on these components are reported. For comparison with previous work glycinin prepared in various ways and the cold precipitated fraction of BRIGGS AND MANN[6] have also been examined.

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EXPERIMENTAL

Apparatus

Ultracentrifugal measurements were made in a Spinco ultracentrifuge, Model E, at a speed of *ca.* 50,700 r.p.m. (200,000 *g*). Sedimentation was followed by means of the diagonal Schlieren optical system. In calculating sedimentation constants no allowance has been made for possible lowering of the rotor temperature as a result of adiabatic contraction during acceleration[7]. The sedimentation constants in all cases have been reduced to the viscosity and density of water at 20° C.

Materials

Buffer salts and ammonium sulphate were A.R. or of equivalent purity.

The de-oiled soya bean meal was supplied by the Clyde Soya Company.

Preparation of proteins

Using a meal: liquor ratio of 1:10, soya bean meal was extracted at room temperature with 10% (w/v) aqueous NaCl for six hours. The liquor, after centrifugation, was filtered through paper pulp. Ammonium sulphate fractions were then obtained from the filtrate by appropriate adjustments of the ammonium sulphate concentration. After 85% saturation no more protein was precipitated by further addition of ammonium sulphate or trichloracetic acid. The precipitate in every case was redissolved in 10% NaCl and reprecipitated at the appropriate saturation with ammonium sulphate.

For comparison with the results of previous workers, glycinin was prepared by the method of (a) OSBORNE AND CAMPBELL[1] and (b) HARTMAN AND CHENG[3]. In method (a) 6 g of meal were extracted with 100 ml 10% NaCl, and the protein precipitated from the extract by saturation to 85% with ammonium sulphate. The precipitated protein, after centrifugation, was dissolved in 10% NaCl and the glycinin precipitated by dialysis against distilled water. In method (b) 6 g of meal were extracted with 100 ml water and the glycinin precipitated by acidifying to pH 4.5 with *N* sulphuric acid.

The cold precipitated fraction of BRIGGS AND MANN[6] was isolated by extracting 20 g of soya bean meal with 100 ml water, leaving the extract overnight at 0° C and centrifuging the precipitate in the cold. No further purification of the precipitate (as described by these authors) was carried out.

Unless otherwise stated the protein in each case was dissolved in phosphate buffer, $I = 0.5$, pH = 7.8 and dialysed with stirring against large volumes of the same buffer before examination in the ultracentrifuge. To all the solutions of protein in buffer a little toluene was added to prevent bacterial growth.

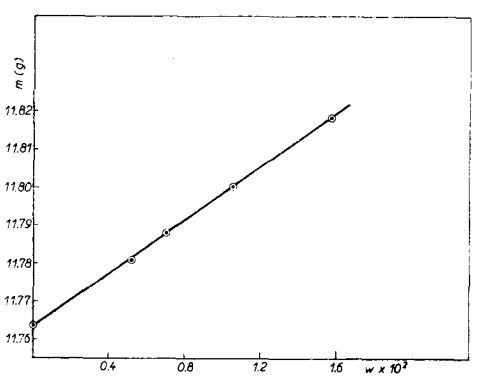

Fig. 1. m (mass of a given volume of solution of soya bean protein for which the solute weight fraction is w) against w.

Protein concentrations were determined by micro-Kjeldahl nitrogen determinations using the value 17% for the percentage nitrogen in the protein.

Partial specific volume

The partial specific volume $\bar{v}$ of the total soya bean protein (extracted with 10% *w/v* sodium chloride and precipitated at 85% saturation with ammonium sulphate) was determined from the equation[8],

$$(1 - \bar{v}\varrho) = \frac{1 - w}{m}\,\frac{dm}{dw}$$

where ϱ = density of the solution for which the solute weight fraction is w, and m = mass of a given volume of the solution. m was determined with a pyknometer for a range of protein concentrations. The solvent used was phosphate buffer $I = 0.1$, pH = 8. From Fig. 1 in which m is plotted against w, the value $\bar{v} = 0.71 \pm 0.004$ was obtained.

RESULTS

Total extract

Fig. 2(a) gives the sedimentation diagram of a 10% NaCl extract of meal which was dialysed against phosphate buffer ($I = 0.5$, pH = 8). At least four sedimenting components are present with s constants of 15, 11, 7 and *ca.* 2 S. The same components in the same relative proportions were obtained irrespective of the meal: liquor ratio.

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This is not surprising in view of the statement of BRIGGS AND MANN[6] that the electrophoretic pattern did not change with variation in the meal: liquor ratio. The protein which precipitated from the 10% NaCl extract by saturation to 85% with ammonium sulphate when dissolved in phosphate buffer gave the diagram of Fig. 2(b) no appreciable difference in the relative areas from Fig. 2(a) being observed.

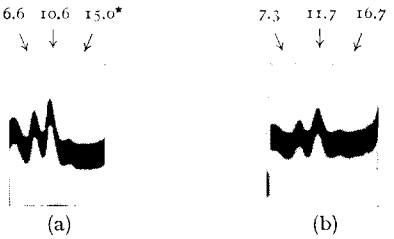

Fig. 2. Sedimentation diagrams of (a) 10% NaCl extract of soya bean meal dialysed to $I = 0.5$, pH = 7.8; protein conc. = 1.2 g/100 ml; (b) 0/85 ammonium sulphate fraction at $I = 0.5$, pH = 7.8; protein conc. = 0.77 g/100 ml.

* Numbers above peaks in this and subsequent diagrams refer to rounded sedimentation constants in Svedberg units. Direction of sedimentation in every case is from left to right.

Earlier preparations

The sedimentation diagram and constants of glycinin prepared by the method of OSBORNE AND CAMPBELL[1] are shown in Fig. 3(a) while Fig. 3(b) gives sedimentation data for glycinin as prepared by HARTMAN AND CHENG[3]. In neither case is the protein homogeneous, and differences in the relative areas of the components between the two diagrams can be observed. The cold precipitated protein of BRIGGS AND MANN[6] gave the sedimentation diagram shown in Fig. 3(c). Although the protein was not subjected to the further purification described by these authors and would therefore be contaminated by supernatant liquor, it is apparent that the s_{11} component* is present in the highest concentration. It therefore seems reasonable to conclude that the electrophoretically pure component of BRIGGS AND MANN[6] gives the s_{11} component in the ultracentrifuge.

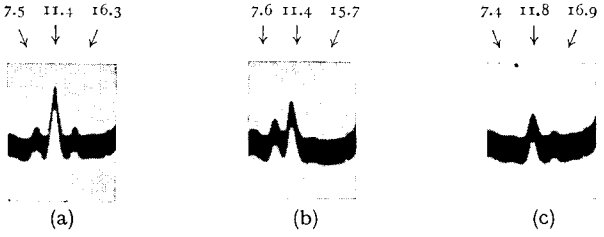

Fig. 3. Sedimentation diagrams of (a) glycinin of OSBORNE AND CAMPBELL[1]; protein conc. = 1.46 g/100 ml; (b) glycinin of HARTMANN AND CHENG[3]; protein conc. = 0.89 g/100 ml; (c) cold precipitated fraction of BRIGGS AND MANN[6]; protein conc. = 0.49 g/100 ml, $I = 0.5$, pH = 7.8 buffer used in each case.

Ammonium sulphate fractions

The protein precipitating from a 10% NaCl extract at different saturation limits with ammonium sulphate was examined in the ultracentrifuge in phosphate buffer, the

* A species of sedimentation constant 11 Svedberg units is termed the s_{11} component.

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sedimentation diagrams and constants being shown in Fig. 4. Included also in Fig. 4 are the amounts of protein precipitated at each stage, these being given as a percentage of the total precipitated protein. These values were obtained by dissolving the protein

Fraction	0/50	50/55	55/60	60/65	65/70	70/85
% of total precipitated protein	1.0%	24.3%	11.2%	16.8%	11.0%	35.1%
Sedimentation diagrams and constants	7.6 11.7	7.3 11.6	7.0 11.1 15.5 18.5	7.5 10.5 14.8	7.3 10.8 14.7	7.0 11.3
Protein conc. in g/100 ml	0.43	0.81	1.42	2.10	1.90	0.65

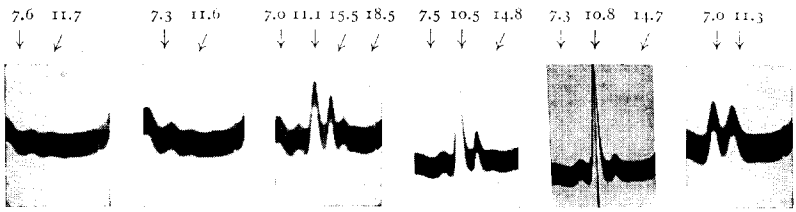

Fig. 4. Sedimentation diagrams of ammonium sulphate fractions from a 10% NaCl extract of soya bean meal $I = 0.5$, pH = 7.8.

in phosphate buffer and removing the ammonium sulphate by dialysis; the solutions were then made up to standard volumes and micro Kjeldahl nitrogen estimations were carried out. It will be observed from Fig. 4 that the s_{11} component precipitates preferentially in the 60/65 and 65/70 fractions and the s_7 component in the 70/85 fraction, although both these components as well as some very slowly sedimenting material are present in all six fractions examined. In order to purify the s_7 component, the 70/85 fraction was redissolved in 10% NaCl and saturated to 65% with ammonium sulphate. This caused 80% of the protein to precipitate. The supernatant liquor was then saturated to 85% with ammonium sulphate and the precipitated protein in phosphate buffer gave, in the ultracentrifuge, the diagram shown in Fig. 5 in which the s_7 component is present to the extent of 70%. This fraction is hereafter designated the 70/85/1 fraction. The s_{11} component occurs in the 65/70 fraction to the extent of 85%. Thus, by this fractionation, two components (s_{11} and s_7) were obtained in a reasonable state of purity and the two fractions in which they occurred, the 65/70 and 70/85/1 fractions, were therefore subjected to a further examination. It is possible that increased purification of the s_{11} and s_7 components would be achieved by further reprecipitations of these fractions but this purification has not yet been attempted.

Fig. 5. Sedimentation diagram of the 70/85/1 soya bean protein fraction at $I = 0.5$, pH = 7.8 protein conc. = 1.32 g/100 ml.

65/70 Fraction

Effect of variation in ionic strength. At constant pH (7.9 ± 0.1) and constant protein concentration (0.9 g/100 ml) the ionic strength of a solution of the 65/70 fraction

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was varied from 1.0 to 0.1 by dialysis. No precipitation of protein occurred during dialysis. Sedimentation diagrams and constants are given in Fig. 6. No appreciable change in the sedimentation pattern was visible between ionic strengths 1.0 and 0.35. At $I = 0.1$, however, association appeared to occur since a considerable amount of an ill-defined s_{19} component appeared. An appreciable increase in the sedimentation constant of the main peak was observed with decrease in ionic strength. The s_7 component which is clearly defined at $I = 0.5$ is less apparent at $I = 0.1$, owing to its association at the lower ionic strength to a component of sedimentation constant close to the value of 11 (see later). At $I = 0.2$, the proportion of the fast, ill-defined component is less than at $I = 0.1$.

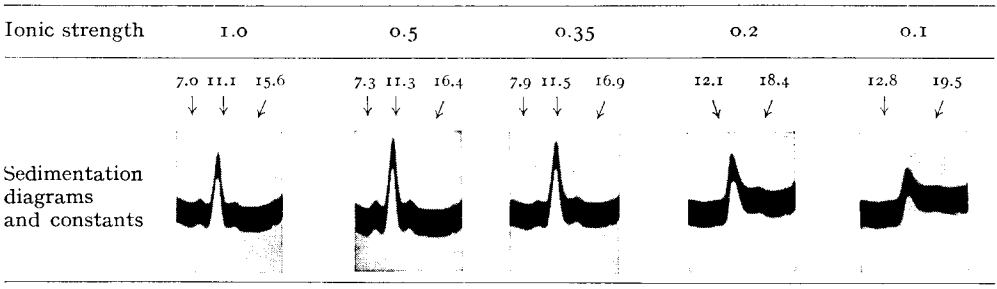

Fig. 6. Sedimentation diagrams of the 65/70 soya bean protein fraction at pH 7.9 (± 0.1) and protein conc. = 0.9 g/100 ml.

Changes in ionic strength were effected by dialysis over a period of several hours. In order to estimate the speed of the processes involved, experiments were performed in which the ionic strength was raised from 0.1 to 0.5 by the addition of solid NaCl and lowered from 0.5 to 0.1 by diluting a concentrated protein solution with water. The ultracentrifuge run was immediately performed in both cases and the sedimentation patterns obtained were characteristic of the final ionic strength, no further changes occurring. Thus the changes were complete within the first hour and were also reversible. No appreciable change in the sedimentation pattern at either $I = 0.5$ or $I = 0.1$ was observed with (a) change in protein concentration or (b) time.

Effect of variation in pH. At $I = 0.5$ no change in the sedimentation diagram was observed within the pH range 6.2–8. When the pH was lowered by dialysis to 5.2, precipitation of the protein occurred immediately the solution was introduced into the ultracentrifuge cell.

At $I = 0.1$ and constant protein concentration, the effect of pH on the sedimentation diagram within the range 6.9 to 9.5 is shown in Fig. 7. At pH values below

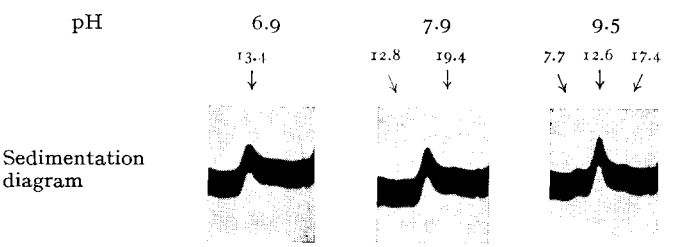

Fig. 7. Sedimentation diagrams of the 65/70 soya bean protein fraction at $I = 0.1$ and protein conc. = 0.9 g/100 ml.

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6.9 some protein precipitated during dialysis while pH values above 9.5 were considered too high to be safely used in the ultracentrifuge cell. It will be observed from Fig. 6 that no marked changes are evident within the pH range examined, although the proportion of fast, polydisperse material increased with lowering of pH. Moreover at $I = 0.1$, pH = 9, some dissociation of the s_{12} peak to an s_8 component appears to occur. No change in these sedimentation diagrams with time was observed.

70/85/1 Fraction

Effect of ionic strength variation. The sedimentation diagrams of the 70/85/1 fraction at pH 7.9 (± 0.1) and various ionic strengths are given in Fig. 8. At $I = 0.5$ the protein concentration was twice that at the other ionic strengths but no change in the relative areas at $I = 0.5$ was observed when the concentration was one quarter of that of Fig. 7.

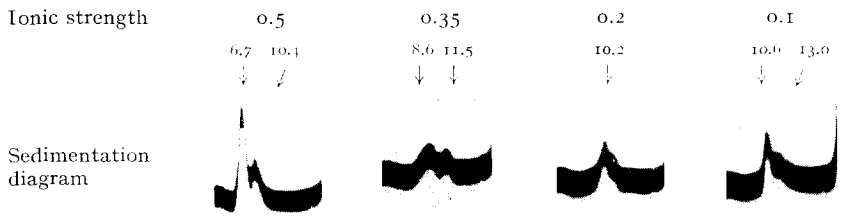

Fig. 8. Sedimentation diagrams of the 70/85/1 soya bean protein fraction at pH = 7.9 (± 0.1) and protein conc. = 0.65 g/100 ml (except at $I = 0.5$ for which protein conc. = 1.32 g/100 ml).

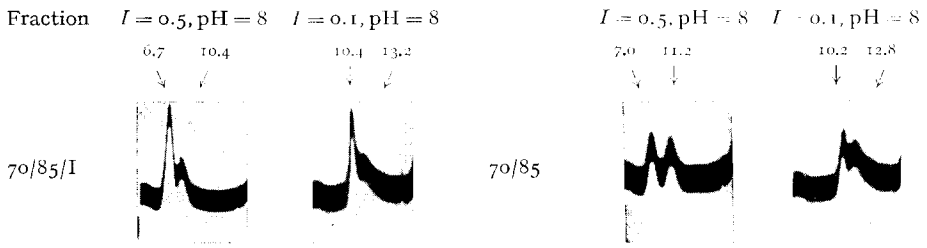

Fig. 9. Comparison of sedimentation diagrams of 70/85/1 and 70/85 soya bean protein fractions at $I = 0.5$ and $I = 0.1$; protein conc. for 70/85/1 fraction at $I = 0.5$ and 0.1 = 1.32 g/100 ml, protein conc. for 70/85 fraction = 0.65 g/100 ml at $I = 0.5$ and 1.3 g/100 ml at $I = 0.1$.

It will be observed that on lowering the ionic strength from $I = 0.5$ to $I = 0.1$ the s_7 component disappears and is replaced by an $s_{10.5}$ component. The s_{13} material at $I = 0.1$ is most probably derived from the s_{11} component at $I = 0.5$. This is clear when the 70/85/1 and 70/85 fractions are compared at $I = 0.5$ and 0.1 (Fig. 9). In each case the proportions of $s_7 : s_{11}$ at $I = 0.5$ are similar to the proportions of $s_{10.5} : s_{13}$ at $I = 0.1$. In Fig. 9 the protein concentrations of the 70/85/1 fraction at $I = 0.5$ and 0.1 are the same. Assuming the same value for the frictional ratio f/f_0 for each sedimenting component, the sedimentation constants 6.7 and 10.4 correspond to molecular weights of *ca.* 105,000 and 205,000 respectively. Thus it appears that the s_7 component dimerises as the ionic strength is lowered from $I = 0.5$ to $I = 0.1$ or even to $I = 0.2$. At $I = 0.35$ the more slowly sedimenting peak was considerably broader than those obtained at the other ionic strengths and the s^0_{20} value was intermediate between the values 7 and 10.5 obtained at $I = 0.5$ and 0.1 respectively. It seems possible that at $I = 0.35$ some rapid

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reversible type of reaction (*e.g.* $2\ s_7 \rightleftharpoons s_{10.5}$) is involved. As in the case of the 65/70 fraction, the processes occurred within one hour and were completely reversible for $I = 0.1 \rightarrow 0.5$ and vice versa.

Effect of pH variation. No change in the sedimentation diagram at $I = 0.5$ was observed within the pH range 6.2–8. As with the 65/70 fraction, precipitation of protein occurred in the ultracentrifuge cell at $I = 0.5$, pH = 5.2. Variation in pH at $I = 0.1$ did not produce marked changes in the sedimentation diagrams (Fig. 10). No evidence for the dissociation of the $s_{10.5}$ component to s_7 was observed. The increased polydispersity of the faster sedimenting material with lowering of pH from 9.5 to 6.9 is probably to be accounted for by the increased association of the 65/70 impurity present (see earlier).

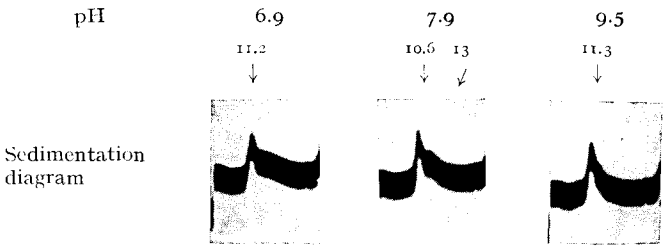

Fig. 10. Sedimentation diagrams of 70/85/1 soya bean protein fraction at $I = 0.1$ and protein conc. = 0.65 g/100 ml.

DISCUSSION

It is obvious that the term glycinin as defined by earlier workers on soya bean protein refers to a mixture of proteins, at least four different sedimenting components being present in the mixture.

The sedimentation constants of the two main soya bean protein components at $I = 0.5$, pH = 8 reported here are considerably lower than the values of 13.1 and 8.0 S obtained by DANIELSSON[5] at $I = 0.3$, pH = 7. When a mixture of arachin and soya bean protein at $I = 0.5$, pH = 8 was examined in the ultracentrifuge, a separation of arachin from the s_{11} soya component occurred. Since at $I = 0.5$, pH = 7.8 the sedimentation constant of arachin is *ca.* 13 S[9], it follows that under these conditions the value for the soya component must be appreciably less than this figure. It seems likely therefore, that the discrepancies in sedimentation constants between DANIELSSON's results and those reported here, are to be explained by the different ionic conditions employed. The variations observed in the sedimentation constant of a particular component at any given ionic strength and pH in the present investigation are probably caused by differences in protein concentration.

Although none of the sedimenting components of soya bean protein has been isolated pure in this work, yet ammonium sulphate fractionation has given the two main components in a sufficient state of purity for further examination. The states of aggregation of both these components are susceptible to changes in ionic environment and to a considerably lesser extent, to changes in pH. In both cases high ionic strengths favour the dissociated state and in this respect these components resemble the 65/85 conarachin fraction of the groundnut globulins[9].

A comparison of the two soya bean protein components with 65/85 conarachin shows

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interesting similarities and differences. Thus with all three proteins the changes which occur with variation in ionic strength take place within one hour. With conarachin, light-scattering measurements showed that they occurred within one minute[9]. No light-scattering measurements, however, were made with soya bean protein in this investigation. Moreover at $I = 0.5$ no differences in the sedimentation diagrams of 65/85 conarachin or the soya bean components were observed with change of pH within the range 6–8 but aggregation occurred in each case when the pH was lowered to the isoelectric region. Whereas in the case of conarachin at $I = 0.1$, considerable changes in the sedimentation patterns were observed with variation in pH new sedimenting components appearing at pH 6.3, the changes with both soya bean components under similar conditions were very slight, no new, discrete components being observed.

SUMMARY

An ammonium sulphate fractionation of the protein in a 10% NaCl extract of soya bean meal has been carried out. Examination of the fractions in the ultracentrifuge at $I = 0.5$ pH = 7.8 has revealed the presence of at least five sedimenting components.

Two of these components have been obtained in a reasonable state of purity. In both cases association occurred by lowering of ionic strength, the reactions being reversible and occurring within one hour. The effect of pH change within the range 6.9–9.5 was much less appreciable.

The glycinin of previous workers has been shown to contain at least four sedimenting components.

RÉSUMÉ

Les protéines d'un extrait par NaCl à 10% de la farine de soja ont été fractionnées par le sulfate d'ammonium. L'examen des fractions à l'ultracentrifugeuse, à $I = 0.5$ et pH = 7.8, a révélé la présence d'au moins cinq constituants différents.

Deux de ces constituants ont été obtenus dans un état de pureté convenable. Pour les deux la diminution de la force ionique provoque une association réversible et se produisant en une heure. La variation du pH entre 6.9 et 9.5 à beaucoup moins d'influence.

La glycinine précédemment décrite par d'autres auteurs s'est révélée contenir au moins quatre constituants.

ZUSAMMENFASSUNG

Das Protein, das mit 10% NaCl aus Sojabohnenmehl extrahiert wird, wurde mit Ammoniumsulfat fraktioniert. Die Prüfung der Fraktionen in der Ultrazentrifuge bei $I = 0.5$ und pH = 7.8 zeigte die Anwesenheit von wenigstens 5 sedimentierenden Komponenten.

Zwei dieser Komponenten wurden einigermassen rein erhalten. In beiden Fällen trat bei Erniedrigung der Ionenstärke Assoziation ein, wobei die Reaktionen reversibel waren und eine Stunde dauerten. Die Wirkung einer pH-Veränderung innerhalb des pH-Bereichs 6.9 bis 9.5 war viel weniger ausgesprochen.

Es wurde gezeigt, dass das Glycinin vorhergehender Untersucher 4 sedimentierende Komponenten enthält.

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