Solubility Characteristics of Globulins from *Phaseolus* Seeds in Regard to Their Isolation and Characterization

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Gel electrophoretic evidence is presented to compare the relative efficiency of several extraction procedures in obtaining seed globulin fractions from the French bean, *Phaseolus vulgaris*, having no cross-contamination. Acidic conditions were shown to be particularly effective. The isoelectric point of the globulin 1 (G1) fraction was found to be at pH 4.4–5.6, and that of the globulin 2 (G2) fraction was at pH 3.7. Differences in solubility characteristics for these fractions in respect to protein and salt concentration, temperature, and ionic species are detailed.

The globulins of legume seeds are major sources of food protein, and are also valuable materials for studies on the biosynthesis of protein in plants (McLeester *et al.*, 1973). An understanding of the parameters controlling the solubility of globulins will help to provide a basis for the development of procedures for the isolation of pure legume seed globulins, thereby permitting critical biochemical and nutritional (van Megen, 1974) characterizations.

Some 50–75% of the protein in legume seeds requires appreciable salt concentrations for solubilization, and hence is designated globulin (Osborne, 1924). Two globulin fractions, legumin and vicilin, were prepared from pea (*Pisum sativum*) seeds by Osborne (1896), and similar fractions have been shown to occur throughout the family Leguminosae (Danielsson, 1949a). The methods used to obtain these two globulin fractions generally make use of their differential solubility. Typically, the globulins are extracted into a salt solution buffered to approximately pH 7.0, and subsequently fractionated by changing the ionic strength or the pH of the extract. However, despite various approaches on this basis, a complete separation of the two globulin fractions has not been obtained (Wetter and McCalla, 1949; Danielsson, 1949a; Graham and Gunning, 1970; Millerd *et al.*, 1971). The isoelectric precipitation technique developed by Danielsson (1949a,b, 1950) has been most effective in the separation of the two globulin components from pea seeds on the basis of analytical ultracentrifugation and moving-boundary electrophoresis. However, using the same plant material and criteria for purity, Brand and Johnson (1958) were unable to completely separate the two globulin components by isoelectric procedures. Difficulty was also found in preparing pure samples of vicilin and legumin from seeds of *Vicia faba* using this technique (Graham and Gunning, 1970). The use of sedimentation coefficients as criteria for the purity of globulins should be supported by evidence from other techniques (Sun *et al.*, 1974).

We have previously described an acidic extraction procedure which is successful in completely separating two globulin fractions, G1 and G2, from the cotyledons of French bean (*Phaseolus vulgaris*) seeds (McLeester *et al.*, 1973). The G1 fraction appears to be identical with glycoprotein II described by Pusztai and Watt (1970). The use of acidic procedures has been questioned on the basis that they may induce changes in the state of the protein extracted (Wright and Boulter, 1973). While association–dissociation changes in the G1 protein do occur under various pH conditions (Pusztai and Watt, 1970; Sun *et al.*, 1974), they are reversible; the G2 protein is stable under acidic conditions. In this article, we present additional evidence that acidic extraction conditions permit clear separation of two globulin fractions from French bean seeds, and that these fractions are identical with fractions obtained under alkaline conditions except for the lack of cross-contamination. We also detail solubility characteristics of the two purified globulin fractions under various individual and combined effects of salts, pH, protein concentration, and temperature.

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EXPERIMENTAL TECHNIQUES

Plant Materials and Isolation of Globulins. Globulin fractions were extracted from dry seeds of *Phaseolus vulgaris* L. cv. Tendergreen purchased from Olds Seed Co., Madison, Wis. Five methods for globulin extraction were compared. (1) *The ascorbate–NaCl procedure* was as described previously (McLeester *et al.*, 1973; Sun *et al.*, 1974) except that the G1 protein was precipitated by dilution of the clear extract with 5 (rather than 1) vol of distilled H_2O. (2) *The HCl–NaCl procedure* was similar to the ascorbate–NaCl method except that the ascorbate was omitted, and the homogenate was maintained at pH 3.5 by addition of 5 *N* HCl. (3) *The NaCl procedure* was similar to the acidic procedures, but utilized 0.5 *M* NaCl as the extraction solution and the pH of the homogenate (pH 6.1) was not adjusted. (4) *The alkaline–NaCl procedure*, where 1 g of white seed flour obtained by grinding for 90 sec in a Micro-mill (Laboratory Apparatus, Co., Cleveland, Ohio) was passed through a 0.5-mm sieve (Sun *et al.*, 1974) and stirred in 20 ml of 0.5 *M* NaCl containing 0.1 *M* Hepes [1-(*N*-2-hydroxyethylpiperazin-*N'*-yl)ethanesulfonic acid] buffer, pH 7.4, for 1 hr (0–4°). This material was then centrifuged (4°) at 30,000*g* for 30 min and the supernatant dialyzed overnight against H_2O (resistivity greater than 10^6 ohms/cm^2) at 4° (100 ml of extract:4 l. of H_2O). The precipitate from this dialysis was pelleted by centrifugation at 30,000*g* for 30 min and designated the first globulin (G1) precipitate. The supernatant was further dialyzed against two changes of 4 l. of H_2O at 4° for 2 days. The precipitate from this dialysis was collected by centrifugation and designated the second globulin (G2) precipitate. (5) *The isoelectric precipitation procedure* followed techniques described by Bailey and Boulter (1970, 1972).

Analytical Procedures. Protein was quantified by the method of Lowry *et al.* (1951). The procedures for the dissociation of proteins and analytical electrophoresis of the dissociated proteins on sodium dodecyl sulfate (SDS) gels (5%) have been described previously (McLeester *et al.*, 1973). Conductivity of the protein and NaCl mixtures was measured by a Conductivity Bridge, Model RC 16B2 (Industrial Instruments Inc., Cedar Grove, N. J.).

Solubility Studies. Globulin protein isolated by the ascorbate–NaCl procedures was dispersed in water or dissolved in an appropriate concentration of NaCl solution (see legends of figures for the ratio of protein to solution). For the solubility experiments, solid NaCl was added to

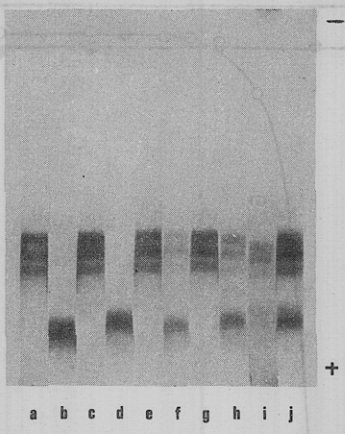

Figure 1. Acrylamide gel electrophoresis of G1 and G2 proteins extracted from *Phaseolus vulgaris* cotyledons by different methods. Separation was on continuous SDS gels (5% w/v of monomer) after dissociation at 100° for 2 min in a solution containing 6 *M* urea, 1.5% (w/v) SDS, and 1.5% 2-mercaptoethanol. The G1 and G2 fractions obtained by the extraction procedures detailed in the Experimental Techniques section are shown for: (a,b) ascorbate–NaCl; (c,d) HCl–NaCl; (e,f) NaCl; (g,h) alkaline–NaCl; (i,j) isoelectric precipitation. The G1 fraction is shown in a, c, e, g, and i while the G2 fraction is seen in b, d, f, h, and j.

give the required concentration, or the pH was adjusted with 5 *N* HCl or 5 *N* NaOH. Aliquots of 0.5 ml were withdrawn and centrifuged (22.5°) for 30 min at 40,000*g*, and the protein in the supernatant measured. For studying the effect of temperature on solubility, the mixture was brought to a desired temperature level, and the sample rapidly filtered under suction, the protein content of the filtrate being measured. For the effect of different ionic species, globulin protein was added to 1.0 ml of salt solution until undissolved protein was observed. After centrifugation at 40,000*g* for 30 min, protein in solution in the supernatant was determined.

The experimental data for all figures were repeated on protein samples from a different batch of seed; in all cases results differed by less than ±4%. We chose not to buffer the solutions because buffer ions combine with globulins, resulting in markedly different solubility properties and adding confusion to interpretation of the data. For example, it was not possible to add sufficient ions to buffer the G2 fraction protein while keeping it insoluble due to low salt. Conversely, it made little sense to add a new ionic species in an attempt to maintain pH during an experiment to follow the dependence of solubility on the concentration of a given salt.

RESULTS

Electrophoretic Evidence for Purity of Globulin Fractions. Polypeptide components of the globulin fractions isolated by various methods were obtained by treatment with SDS–urea–mercaptoethanol and separated by SDS–gel electrophoresis (Figure 1). The ascorbate–NaCl extraction procedure yielded two globulin fractions, G1 and G2, with entirely different gel electrophoretic patterns. The different polypeptide components of these fractions (gels a and b, Figure 1) clearly demonstrate the effectiveness of this acidic procedure in separating them from each other. Essentially the same results were obtained using an HCl–NaCl extract (gels c and d, Figure 1). However, when NaCl solution alone was used as an extraction medium, it was found that although the G1 fraction was obtained free from G2 proteins (gel e, Figure 1), the G2 fraction was contaminated by G1 protein (gel f, Figure 1). The first protein precipitate resulting from dialysis of the alkaline NaCl extract was found to contain mostly G1 protein (gel g, Figure 1), while the second precipitate was shown to be a mixture of G1 and G2 proteins (gel h, Figure 1). Isoelectric precipitation (procedure 5) yielded protein fractions which were found to be cross-contaminated (gels i and j, Figure 1).

Table I. Comparison of Amounts of Globulins Prepared from French Bean Cotyledons by Different Extraction Methods[a]

Fractions	Ascorbate–NaCl	HCl–NaCl	NaCl	Alkaline–NaCl	Isoelectric precip.
G1	7.8	8.1	7.0	7.5	1.3
G2	2.5	2.3	4.1	2.5	4.6
Total	10.3	10.4	11.1	10.0	5.9

[a] Details of the extraction methods are given in the Experimental Techniques section. The globulin amounts are expressed as percentages of dry weight of seed flour.

Relative Amounts of Globulin Fractions. The amounts of protein in the two globulin fractions extracted by the HCl–NaCl procedure are similar to those from the ascorbate–NaCl procedure (Table I). A shift of the globulin protein from the G1 fraction to the G2 fraction was found when the NaCl extraction procedure was used, indicating contamination of the G2 fraction by G1 protein. This was confirmed by the gel electrophoretic patterns of the G2 fraction extracted by this method (gel f in Figure 1). The total amount of globulin extracted by the two acidic NaCl procedures, by NaCl alone, and by the alkaline NaCl procedure varied by about 10%, while that by the isoelectric precipitation technique was only half of these values. The decrease in the total globulin prepared by the isoelectric precipitation procedure could be accounted for by the marked decrease in the amount of protein recovered in the G1 fraction, which was only 16% of the G1 protein isolated by the acidic procedures. The amount of protein in G2 fraction isolated by this method was 1.84 times greater than that by the two acidic isolation procedures. Loss of G1 protein and the contamination of the G2 fraction by G1 protein during isoelectric precipitation resulted in a low recovery of the G1 protein (Table I), and the presence of G1 protein in the G2 fraction (gel j, Figure i).

Effect of NaCl Concentration on the Solubility of Isolated Globulin Fractions. The influence of NaCl on the solubility of G1 and G2 proteins isolated by the ascorbate–NaCl procedure from *Phaseolus vulgaris* seed is shown in Figure 2. G1 protein started to dissolve at 0.1 *M* NaCl, increased in solubility as more salt was added, and completely dissolved at 0.2 *M*. No "salting out" effect was seen up to 1.5 *M*. A considerable amount of G2 protein was soluble in deionized water (1×10^6 ohms/cm^2); evidently this protein has appreciable solubility at very low salt concentrations. An increase in solubility with increasing salt concentration was observed, and the G2 protein completely dissolved at 0.05 *M* NaCl.

As the salt concentration was raised from 0 to 1.0 *M* NaCl, the acidity of the G1 solution increased from 5.0 to 4.5, and that of the G2 solution from 4.6 to 4.2. The pH values of the solutions were not adjusted, and represent the acidity of the native proteins. However, it was noted that increasing the NaCl concentration from 0.1 to 0.4 *M* resulted in a 95% increase in solubility with only a 0.1 pH unit decrease. Thus, the solubility change followed from an increase in salt concentration rather than a pH change; nevertheless, pH is also very important, as shown below.

Effect of NaCl and Lack of Protein–Protein Interaction on the Solubility of a Mixture of G1 and G2 Globulins. The influence of NaCl on the solubility of a mixture

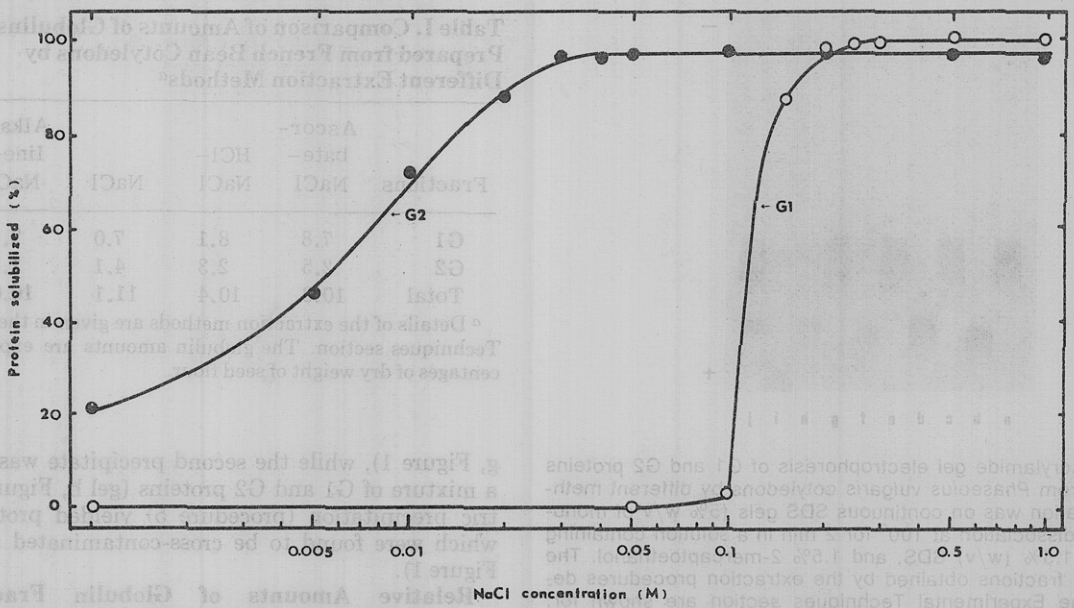

Figure 2. Solubility of G1 and G2 proteins as a function of NaCl concentration (separate experiments). For G1 protein (O) 500 mg was added to 250 ml of water, and for G2 protein (●) 100 mg was added to 200 ml of water. Thus, 100% protein solubilized was equivalent to 2 mg/ml for the G1 experiment and 0.5 mg/ml for the G2 experiment.

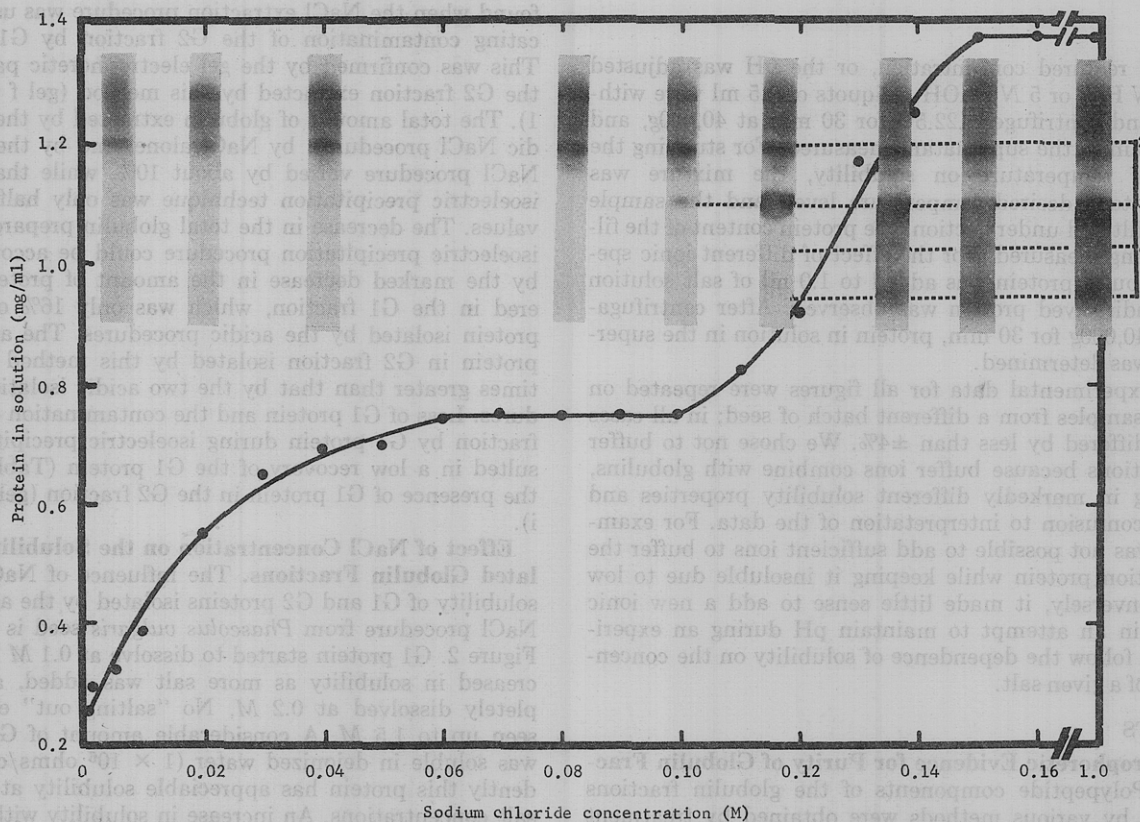

Figure 3. Differential solubility of a mixture of the G1 and G2 globulin fractions. G1 fraction protein (120 mg) and G2 fraction protein (150 mg) were added to 200 ml of distilled water (resistivity 1.1 × 10^6 ohm cm^{-2}) and the mixture continually stirred. Solid NaCl was added to give the required concentrations, allowance being made for the volume withdrawn for each sample. Two minutes after each addition of NaCl 2 ml of solution was taken, undissolved protein sedimented by centrifugation, and the protein concentration of the supernatant measured. The positions of the photographs showing the *disc electrophoretic patterns* of the soluble protein samples correspond to their appropriate salt concentrations, except that those for 0.12 and 0.14 *M* NaCl are displaced to the left of their experimental points. The broken lines and *the scale to the right* of the diagram denote the relative mobility values for bands typically found only in the G1 fraction.

of G1 and G2 proteins is shown in Figure 3. The curve shows a two-step solubility for the mixture (pH 4.6). Gel electrophoretic analysis showed that solubilization of the G2 fraction at low salt concentrations occurred over the first step of the curve, while G1 protein did not go into solution below 0.1 *M* NaCl. The regions of the curve indicating a proportional increase in protein solubility with salt concentration were verified by the corresponding increases in the intensity of the protein bands. Both G1 and G2 proteins were present in the high salt region of the curve (Figure 3, gel pictures) since G2 proteins are completely soluble in sodium chloride concentrations greater

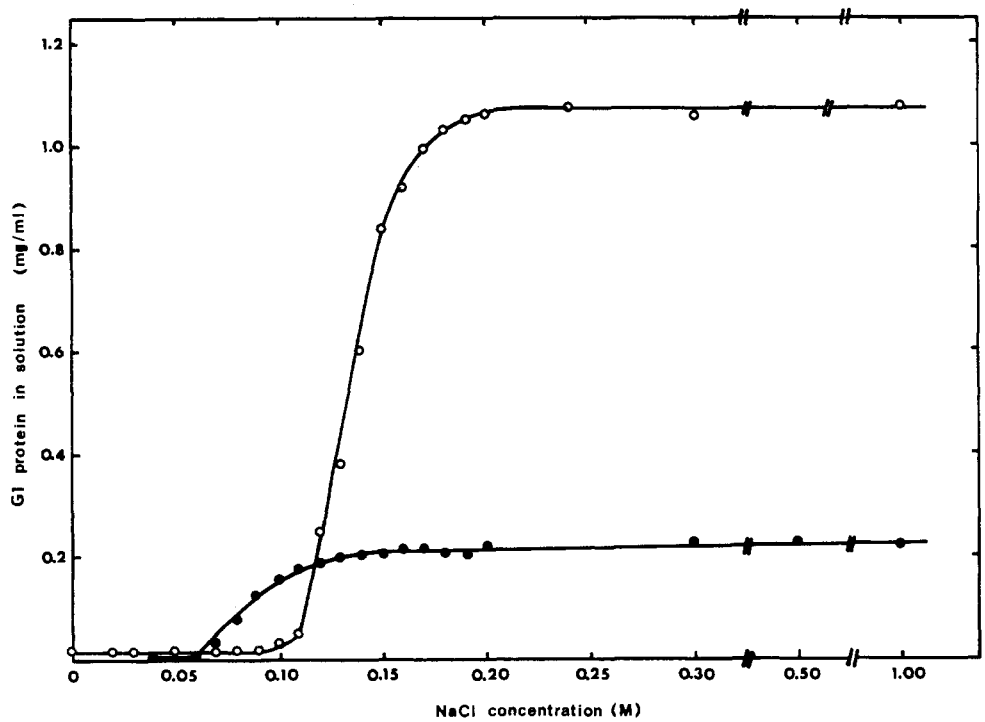

Figure 4. The influence of NaCl and protein concentration on the solubility of G1 protein. The solubility of G1 protein with NaCl concentration was determined for samples originally containing 0.2 mg/ml of water (●) or 1.1 mg/ml of water (○).

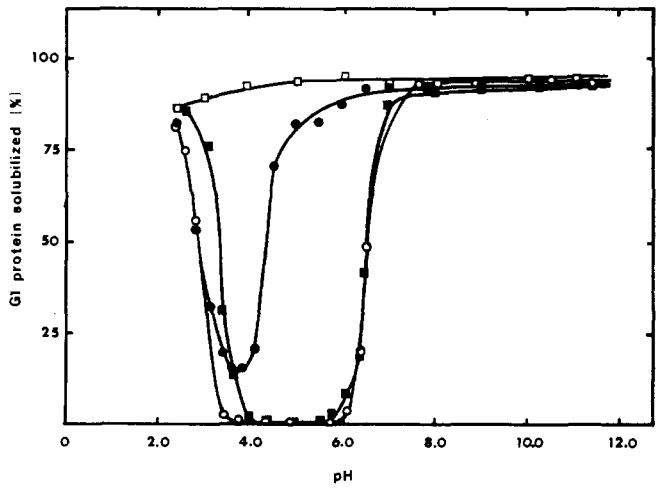

Figure 5. The influence of pH and NaCl concentration on the solubility of G1 protein. G1 globulin, 400 mg, was added to 200 ml of water (■), 0.1 *M* (○), 0.2 *M* (●), or 0.5 *M* (□) NaCl. The protein in solution (100% = 2 mg/ml) was determined over the range pH 2.4–11.4.

Table II. A Comparison of Solvent Power of Different Salts on the G1 Protein

Salts	Protein solubilized by salt solns of $I = 0.125$, mg/ml	Final pH of protein soln
Na_2SO_4, K_2SO_4	0.02	4.2
$NaNO_3$, KNO_3, NaCl, KCl	0.03–0.05	4.0
$(NH_4)_2SO_4$	0.03	4.2
NH_4NO_3	0.49	4.0
$MgSO_4$	2.40	4.0
$MgCl_2$, $Mg(NO_3)_2$, $Ca(NO_3)_2$	2.40–2.68	3.8
$Na_3C_6H_5O_7$	2.78	6.8

than 0.05 *M*. The salt concentrations required for the solubility of a mixture of the two globulin components are essentially the same as that for the two globulins alone (Figure 2), indicating no coacervation of the G1 and G2 proteins under these conditions.

Combined Effects of NaCl and Protein Concentration. The solubility of G1 protein at two concentrations (0.2 and 1.1 mg/ml) was compared (Figure 4). At 0.1 *M* NaCl approximately 70% of the G1 protein at 0.2 mg/ml was solubilized; surprisingly, the higher protein concentration (1.1 mg/ml) showed no detectable solubility at this salt concentration. The data are shown for solutions of pH 4.1 and 3.9, the higher protein concentration being more acid because of the acidic nature of this protein. However, adjustment of the pH of the low protein concentration to that of the high protein concentration did not alter the solubility profile from that shown in Figure 4. Clearly, higher protein concentrations require more ions for solubilization to commence than do lower protein concentrations.

Effect of Different Ionic Species on Solubility. The relative ability of various salts to solubilize G1 protein is listed in Table II. At similar ionic strengths, sodium citrate was 100 times more effective in dissolving the G1 protein than was either sodium or potassium chloride. The high protein solubility in citrate was due to the higher final pH of the protein solution in this salt (pH 6.8) than in the other salts used; only 0.01 mg dissolved per milliliter after adjustment of the pH to 4.0 with HCl. Divalent cations are generally more effective than monovalent ions in dissolving the G1 protein.

Effect of pH on Solubility. G1 protein was found essentially insoluble over the range pH 4.4–5.6 in solutions of low ionic strength (Figure 5). Changes in pH beyond this region brought about a rapid increase in the solubility of G1 protein. Maximum solubility of the G1 protein (about 94%) was observed at pH 11.4. In contrast, the G2 fraction was least soluble at pH 3.7 (Figure 6), and as the pH dropped below or increased above this value, there was a rapid increase in solubility of the G2 protein. At pH 2.2, the lowest value studied, the percentage of G2 protein sol-

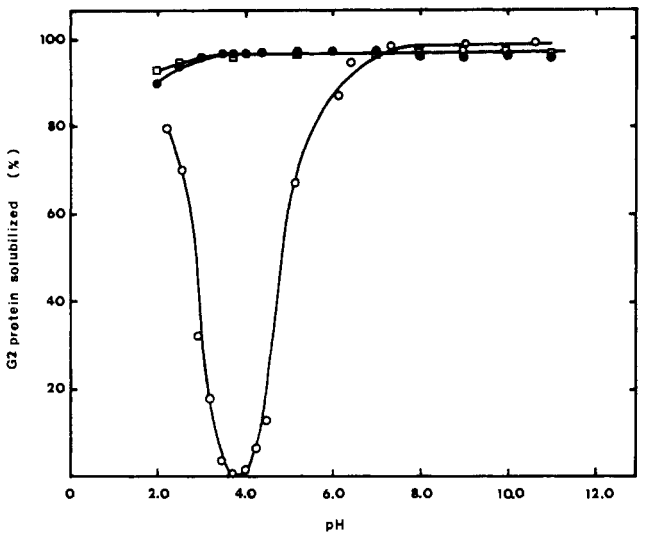

Figure 6. The influence of pH and NaCl concentration on the solubility of G2 protein. G2 globulin, 250 mg of protein, was added to 200 ml of water (○), 0.08 *M* (●), or 0.2 *M* (□) NaCl. The protein in solution (100% = 1.25 mg/ml) was determined over the range pH 2.2–10.6.

ubilized was 80% while at the highest pH (10.6) 98% of the G2 fraction could be solubilized. The acidity values at which minimal solubility occurred are considered to be the isoelectric points for G1 (pH 4.4–5.6) and G2 (pH 3.7) proteins.

Combined Effects of pH and NaCl on Solubility. Little change in solubility of G1 protein in response to pH changes was seen as the ionic strength was increased from 0 to 0.1 *M* NaCl (Figure 5). However, at an ionic strength of 0.2 *M* (NaCl) or greater, the solubility of G1 protein increased markedly over the pH range 4.0–5.6. The minimum solubility in the presence of 0.2 *M* NaCl was at pH 3.6–3.8, although at this pH 13% of the protein remained soluble. When the salt concentration was increased to 0.5 *M* NaCl, changes in pH did not affect the solubility of G1 protein. It is clear from Figure 6 that the effect of pH on the solubility of G2 proteins practically disappeared in the presence of relatively low salt (0.08 *M* NaCl) concentrations.

Combined Effects of Temperature and NaCl. When G1 protein was added to a 0.05 *M* NaCl solution it did not dissolve (see Figure 2), and increasing the temperature of the mixture from 0 to 65° did not solubilize a significant amount of protein (Figure 7). However, when the NaCl concentration was raised to 0.08 *M* (an ionic strength close to the point for the commencement of solubility; see Figure 2), an appreciable increase in the solubility of G1 protein was seen when the temperature was raised, 12% of the G1 protein being solubilized at 70°. The conductivity of both protein–salt solutions was recorded and increases in conductivity were seen in both cases as the temperature was raised. In 0.08 *M* NaCl, protein solubilization started at 6.4 mmhos and increased with conductivity; at 18.5 mmhos (70°) 12% of the protein was solubilized. However, in the case of a lower salt concentration (0.05 *M* NaCl) no significant amount of protein was solubilized as the conductivity of the solution increased from 3.1 to 10.5 mmhos with temperature.

DISCUSSION

The distinct polypeptide patterns obtained for G1 and G2 fractions obtained by acidic extraction (Figure 1) provide convincing evidence for the efficiency of using these conditions for separation. The G1 fraction exhibits characteristics of the globulin fraction defined as legumin by Osborne and Campbell (1898), and the G2 fraction those of vicilin. This correlation has been questioned (Wright and Boulter, 1973) but it can be clearly seen that material extracted from *Phaseolus vulgaris* under alkaline conditions used for authentic samples of legumin and vicilin from *Vicia faba* (Bailey and Boulter, 1970, 1972) contains the same peptide bands as those in the acidic material, while the vicilin fraction has bands which are common to the legumin fraction (gels i and j, Figure 1). A convincing demonstration of the separation of the two fractions under acidic conditions is shown in Figure 3 where a two-step solubility curve was obtained. Additional experiments

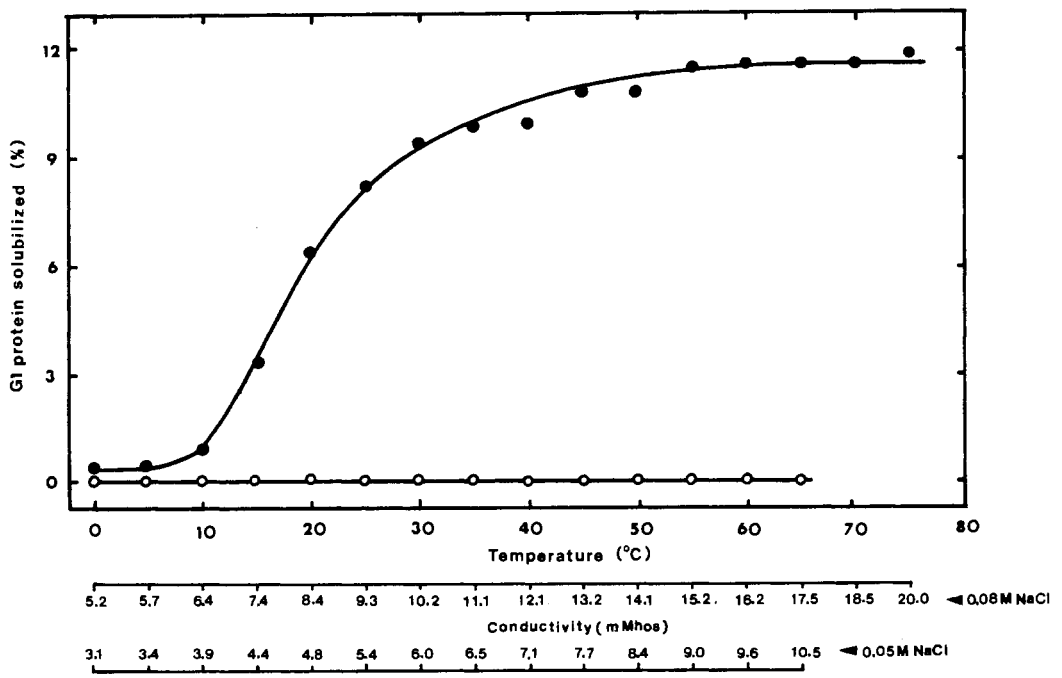

Figure 7. Relationship of temperature, NaCl concentration, and conductivity on the solubility of G1 protein. G1 protein, 500 mg, was added to 250 ml of 0.05 *M* NaCl (○) or 0.08 *M* NaCl (●). The protein in solution (100% = 2 mg/ml) was determined over the temperature range 0–75°, and the corresponding conductivity measured.

(data not shown) confirm that the G1 protein contains 2.9% (g/100 g of protein) neutral sugar and 0.6% hexosamine, and appears to be identical with glycoprotein II, a major protein of *Phaseolus vulgaris* seeds (Pusztai and Watt, 1970).

Without doubt, alkaline procedures are ineffective in separating globulin fractions from *Phaseolus* seed, and we have shown (Sun *et al.*, 1974) that reversible dissociation of the G1 fraction to a monomeric (7.1 S) form at alkaline pH values results in its co-sedimentation with G2 protein (6.6 S). It is also clear that procedures effective in separating one globulin fraction from another in one legume species may be ineffective in doing so in other species; the terms legumin and vicilin lose their validity when applied to protein fractions which are procedurally similar, but differ in peptide composition (McLeester *et al.*, 1973).

The reasons for poor separation under alkaline conditions become clearer when the varying effects of ionic strength and species, protein concentration, and temperature are taken into account. Thus, at high protein concentrations, high salt concentrations are required for solubility (Figure 4). However, at high salt concentrations pH has little influence on the solubility of these proteins. This is seen in Figures 5 and 6 where virtually complete precipitation of the G1 and G2 fractions occurred over certain pH ranges at very low NaCl levels, while little precipitation occurred at higher levels. This reduction of the pH effect by increasing ionic strengths has been observed in *Phaseolus* (Osborne, 1893) and soybean protein (Smith and Circle, 1938; van Megen, 1974), and explains the low yield of G1 and the heavy contamination of the G2 fraction by G1 protein prepared by isoelectric precipitation procedures (gels i and j in Figure 1; Table I). At an ionic strength of 0.2 *M* NaCl, lowering the pH to 4.7 precipitates only 70% of the G1 globulin; the other 30% remains in solution (Figure 5), and will subsequently precipitate along with the G2 fraction on further dialysis. The isolation of the G1 and G2 fractions by the alkaline NaCl procedure encounters the same problem of the combined effects of pH and salt, and the situation is further complicated by the additional presence of buffering ions which readily bind to the free acidic glutamate and aspartate radicals of the globulins, altering the solubility characteristics of the protein. Conversely, both fractions are soluble at pH 3.5 in 0.5 *M* NaCl, but dilution with distilled water lowers the ionic strength of the solution, and at about 0.08 *M* NaCl (pH 3.7, 4°), depending upon the amount of protein present, essentially all of the G1 protein is precipitated. Further reduction of the ionic strength by dialysis precipitates the G2 fraction. While pH is the controlling factor under these conditions, the choice of ion used in maintaining the pH can be important, and for *Phaseolus* ascorbate is preferable to HCl because of its antioxidant properties (Polter and Mueller-Stoll, 1970).

In addition to the interactions of ionic strength and pH, the effect of temperature must be considered, and this can be complex as there is no general rule describing the effect of temperature on protein solubility. Many proteins increase their solubility with temperature, some in dilute, others in concentrated salt solution, while the solubility of some proteins decreases sharply when the temperature is raised (for review, see Taylor, 1953). No effect of temperature on the solubility of G1 protein was found in the presence of 0.05 *M* NaCl. However, at an ionic strength close to that limiting solubility of G1 (0.08 *M* NaCl), an increase in solubility with rising temperature was observed (Figure 7). Conductivity may be the controlling parameter for solubility under these conditions; conductivity is highly temperature dependent, and an attempt was made to correlate the solubility of G1 protein with the conductivity of the NaCl solution (Figure 7). The reverse effect, a decrease in conductivity with lowered temperature and subsequent decrease in protein solubility, may be the basis for the cold insoluble fraction obtained from several legumes (Briggs and Mann, 1950; Ghetie and Buzila, 1962, 1964a,b).

The basis for the excellent separation of G1 and G2 globulins in acidic conditions is clear from these studies. Both G1 and G2 proteins are completely soluble in the 0.5 *M* NaCl extraction medium: dilution by addition of 5 vol of water results in a final concentration of 0.08 *M* NaCl, where G1 is at least 98% precipitated and G2 completely soluble (Figure 2). Futhermore, this precipitation is at a pH close to the isoelectric points of these proteins (Figures 5 and 6), and at which the G1 protein is in its associated, tetrameric, state (Sun *et al.*, 1974).

It would be convenient if general rules for solubility of globulin fractions from legume seeds could be established. However, while the present data are in accord with the well-established major division of globulin fractions on the basis of solubility in sodium chloride, they underscore the interacting influences of parameters such as pH, ionic strength and composition, and temperature on the globulin fractions. These influences appear likely to make the solubility characteristics of the proteins from different legume species unique to each species, although general trends clearly exist. The present documentation of solubility characteristics for globulins from *Phaseolus* should be of value in further characterizing these important proteins.

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LITERATURE CITED

Bailey, C. J., Boulter, D., *Eur. J. Biochem.* **17**, 460 (1970).
Bailey, C. J., Boulter, D., *Phytochemistry* **11**, 59 (1972).
Brand, B. P., Johnson, P., *Trans. Faraday Soc.* **54**, 1911 (1958).
Briggs, D. R., Mann, R. L., *Cereal Chem.* **27**, 243 (1950).
Danielsson, C. E., *Biochem. J.* **44**, 387 (1949a).
Danielsson, C. E., *Acta Chem. Scand.* **3**, 41 (1949b).
Danielsson, C. E., *Acta Chem. Scand.* **4**, 762, (1950).
Ghetie, V., Buzila, L., *Acad. Repub. Pop. Rom., Stud. Cercet. Biochim.* **5**, 65 (1962).
Ghetie, V., Buzila, L., *Acad. Repub. Pop. Rom., Stud. Cercet. Biochim.* **7**, 47 (1964a).
Ghetie, V., Buzila, L., *Acad. Repub. Pop. Rom., Stud. Cercet. Biochim.* **7**, 367 (1964b).
Graham, T. A., Gunning, B. E. S., *Nature (London)* **228**, 81 (1970).
Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J., *J. Biol. Chem.* **193**, 265 (1951).
McLeester, R. C., Hall, T. C., Sun, S. M., Bliss, F. A., *Phytochemistry* **12**, 85 (1973).
Millerd, A., Simon, M., Stern, H., *Plant Physiol.* **48**, 419 (1971).
Osborne, T. B., Report Connecticut Agricultural Experiment Station, 1893, pp 186–210.
Osborne, T. B., *J. Amer. Chem. Soc.*, **18**, 583 (1896).
Osborne, T. B., Ed., "The Vegetable Proteins," 2nd ed, Longmans, Green and Co., London, 1924.
Osborne, T. B., Campbell, G. F., *J. Amer. Chem. Soc.* **20**, 410 (1898).
Polter, C., Mueller-Stoll, W. R., *Z. Naturforsch B.* **25**, 695 (1970).
Pusztai, A., Watt, W. B., *Biochim. Biophys. Acta* **207**, 413 (1970).
Smith, A. K., Circle, S. J., *Ind. Eng. Chem.* **30**, 1414 (1938).
Sun, S. M., McLeester, R. C., Bliss, F. A., Hall, T. C., *J. Biol. Chem.* **249**, 2118 (1974).
Taylor, J. F., *Proteins* **1**, 1 (1953).
van Megen, W. H., *J. Agr. Food Chem.* **22**, 126 (1974).
Wetter, L. R., McCalla, A. G., *Can. J. Res.* **27**(C), 96 (1949).
Wright, D. J., Boulter, D., *Phytochemistry* **12**, 79 (1973).

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