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Effects of polysaccharides upon the functional properties of 11 S globulin of broad beans

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The effect of polysaccharides upon the conformational stability, emulsifying properties and thermal gelation ability of the 11 S globulin (legumin) from broad beans under conditions of both protein–polysaccharide incompatibility and complex formation has been investigated. Under conditions of incompatibility (pH 7·6 for carboxyl-containing and neutral polysaccharides) the conformational stability of the legumin is not changed by the presence of the polysaccharides. At the same time a significant decrease in the emulsifying threshold and an increase in the stability of oil-in-water emulsions, stabilized by the protein, as well as some decrease in the gelation threshold in the presence of polysaccharides have been observed. Under conditions of legumin–polysaccharide complex formation (pH 4·2–6·0, low ionic strength for carboxyl- and sulphate-containing polysaccharides) a significant decrease in the temperature and enthalpy of legumin denaturation as well as a decrease in the minimum biopolymer concentration required for emulsions formation and an increase in emulsion stability have been observed. As a rule, legumin–polysaccharide complexes were poorly soluble even at a substantial excess of polysaccharide. Their solubility which was independent of the method of mixing was a limiting factor for preparing gels and stable foams on using these complexes.

INTRODUCTION

The high nutritional value and potential availability of seed storage proteins makes them of interest to the food industry (Tolstoguzov, 1978; Wright, 1983; Tolstoguzov *et al.*, 1985; Schmandke, 1988). During processing of these proteins it is necessary to take into account the effect on their functional properties (Tolstoguzov, 1978; Schmandke, 1988). The functional properties of soybean proteins are widely known (Kinsella, 1979). The functional properties of broad bean proteins (e.g. solubility and gelling ability) are lower compared with soybean proteins (Wright, 1983; Schmandke, 1988). This investigation was performed to improve the functional properties of broad bean proteins by the inclusion of polysaccharides. It is known that several polysaccharides are able to improve protein emulsifying properties (Ledward, 1979; Tokaev *et al.*, 1987; Tolstoguzov *et al.*, 1985; Kiosseoglou & Doxastakis, 1988). These effects are usually attributed to the formation of polyelectrolyte protein–polysaccharide complexes (Ledward, 1979; Tokaev *et al.*, 1987; Tolstoguzov *et al.*, 1985). However, there is a lack of data on the effects of polysaccharides upon other functional properties of food proteins including their conformational stability and gelling ability.

In this paper, the results of a study of the effect of polysaccharides upon conformational stability, emulsifying and thermal gelling ability of the 11 S globulin from broad beans are considered. Various polysaccharides including neutral, anionic (carboxyl- and sulphate-containing), linear and branched have been used to study the different types of protein–polysaccharide interaction in solution, resulting in both thermodynamic incompatibility and complex formation (Hidalgo & Hansen, 1969; Gurov *et al.*, 1974, 1977; Antonov *et al.*, 1979*a*, *b*, *c*).

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EXPERIMENTAL

Broad bean legumin was isolated by the liquid-liquid phase separation method (Suchkov *et al.*, 1990). Homogeneity of the legumin preparation was higher than 90% according to sedimentation velocity data. Methylcellulose, dextran T-500 (Loba Chemie, Austria), carboxymethylcellulose (Koch Light, UK), sodium alginate (Fluka, Switzerland), pectin (Serva, FRG), arabic gum (Merck, FRG), kappa- and iota-carrageenans (Sigma, USA) and dextran sulphate (Pharmacia, Sweden) were used without additional purification.

Stock solution of protein and polysaccharide were prepared in bidistilled water at pH 7·6 at a concentration of about 1% (an accurate concentration was calculated from the dried residue weight). For the preparation of protein-polysaccharide mixtures the protein stock solution was added gradually to the polysaccharide one with thorough agitation. The pH of the mixture was adjusted with 0·1 M HCl.

The composition of the legumin-polysaccharide mixed solutions is characterized by the ratio of legumin weight concentration (C_L) to polysaccharide weight concentration (C_P): $q = C_L/C_P$.

Thermograms were recorded over the temperature range 10–130°C at a heating rate of 1°C/min and excess pressure of 2·5 bar, using a differential adiabatic scanning microcalorimeter 'DASM 4' (Biopribor, USSR). A temperature corresponding to the thermogram maximum was taken as the protein denaturation temperature. A base line of thermogram in the denaturation range and a denaturation enthalpy were calculated by the spline interpolation and numerical integration methods, respectively.

Sedimentation velocity experiments were carried out at 50 000 rpm using the 317OB ultracentrifuge (MOM, Hungary).

Oil-in-water emulsions were prepared using the ultrasonic disintegrator 'UZDN-1U 4.2' (USSR) at a frequency of 22 kHz. Sonication was carried out at in the temperature range 5–10°C for 10 min. Under these conditions the conformational state of the protein is not altered as it has been proved by differential scanning microcalorimetry and sedimentation velocity measurements. Perfluordecalin (boiling temperature 138–142°C, density 1·92 g/ml, solubility in water 2×10^{-8} g/ml) was used as the non-polar phase. The non-polar phase was added into a solution of protein or protein-polysaccharide at a rate of 0·1 ml/min during sonication. The non-polar phase content in the emulsions was 20% of the aqueous phase volume. The emulsions were centrifuged at 1000 rpm for 10 min and the volume of the separated non-polar phase (V_V) was measured. The dependence of $\Phi_0 = 100\, V_e/V_0$ on protein concentration was plotted, where $V_e = (V_0 - V)$ and V_0 is the total volume of the non-polar phase in the system.

The lower emulsifying threshold (LET) was chosen as a measure of the emulsifying ability of the protein (T. Ya. Bogracheva, pers. comm., 1990). LET is equal to the minimum protein concentration at which $\Phi_0 = 100\%$.

The gravitational stability of emulsions prepared with a protein concentration of 1% in the aqueous phase was studied under centrifugation at 3000 rpm for 5 min and characterized by the volume fraction of the emulsion (Φ_E) in the system.

The gelation threshold (GT) was used as a measure of the proteins' gelling ability (Grinberg *et al.*, 1985*a*, *b*). GT is equal to the minimum protein concentration at which the formation of a protein gel is observed. Values of legumin GT were determined after heating samples of legumin or legumin/polysaccharide solutions in sealed ampoules at a temperature of 90°C for 30 min.

RESULTS AND DISCUSSION

1. Conformational stability

The thermogram of the protein at pH 7·6 has a single heat absorption peak. This picture is not altered in the presence of neutral or carboxyl-containing polysaccharides. On the other hand, the thermogram of the protein in the presence of sulphate-containing polysaccharides has two peaks (peaks 1 and 2). The values of the denaturation temperature of the protein for some legumin-polysaccharide systems in neutral medium at $q = 20$ are given in Table 1.

It is known that neutral and carboxyl-containing polysaccharides are incompatible with legumin at neutral pHs (Semenova *et al.*, 1991). In the case of thermodynamic incompatibility of the protein and polysaccharide, one could expect an increase in the denaturation temperature of the protein due to excluded volume effects (Schellman, 1978; Winzor & Wills,

Table 1. Denaturation temperature (°C) of legumin in the presence of polysaccharides (pH 7·6, $q = 20$)

Polysaccharides	$I = 0.01$		$I = 0.4$	
	Peak 1	Peak 2	Peak 1	Peak 2
Dextran	74·9	—		
Sodium alginate	75·1	—		
Pectin	74·3	—		
Carboxymethylcellulose	75·4	—		
Methylcellulose	74·0	—		
Arabic gum	74·3	—		
Dextran sulphate	76·3	63·0	90·3	—
Iota-carrageenan	77·9	61·0	90·8	—
Kappa-carrageenan	76·4	64·0	93·1	—
Free legumin	76·0	—	93·0	—

I Ionic strength.

1986). Nevertheless, it is obvious from Table 1 that the neutral and carboxyl-containing polysaccharides under investigation do not affect the conformational stability (the denaturation temperature) of legumin. This presumably implies that the increase in the molecular volume of the legumin resulting from its denaturation is insignificant. It is relevant to note that the denaturation of some small globular proteins is not accompanied by any substantial change in their molecular volume (Nicoli & Benedek, 1976).

In the case of protein/sulphate-containing polysaccharide systems, the denaturation temperature for peak 1 coincides with that of free protein. At the same time, the denaturation temperature for peak 2 is 12–15° lower (Table 1). Peak 2 is not observed at a concentration of sodium chloride in the system of more than 0·4 M.

The composition of the protein/sulphate-containing polysaccharide systems in neutral medium was studied by sedimentation velocity. It has been shown that at low salt concentrations and when $q = 20$, sedimentation profiles of these systems contain two peaks. The first peak corresponds to a component with a sedimentation coefficient of about 11 S and the second to a component with a sedimentation coefficient of about 30 S. The slowly sedimenting component is obviously free protein and the rapidly sedimenting component is probably a protein–polysaccharide complex. The fast component is not observed at a concentration of sodium chloride in the system more than 0·4 M. This points to dissociation of the protein–polysaccharide complex at high salt levels.

Thus, one can assume that the protein forms electrostatic complexes with sulphate-containing polysaccharides even at pH values well above the pH of the protein isoelectric point (IEP, pH ≈ 4·8).

The composition of the protein/dextran sulphate system under conditions of protein–polysaccharide complex formation, as well as the conformational stability of the protein in its complexes with dextran sulphate, were studied at various values of the parameter q. The plots of the sedimentation coefficients of the components of this system, as well as the characteristics of legumin stability in complexes as functions of q, are shown in Fig. 1. At small q values the system consists of only one component with a mean sedimentation coefficient of about 2 S (Fig. 1(a)). This value is close to the value of sedimentation coefficient of dextran sulphate. One can assume that this component is a protein/dextran sulphate complex with limited binding of the protein to the polysaccharide matrix. The hydrodynamic behaviour of such a complex is mainly determined by the hydrodynamic properties of the polysaccharide. The conformational stability of the bound protein in the complex is significantly lower than that of free protein (Figs 1(b) and (c)).

The mean sedimentation coefficient of protein/dextran sulphate complexes rises monotonically as the

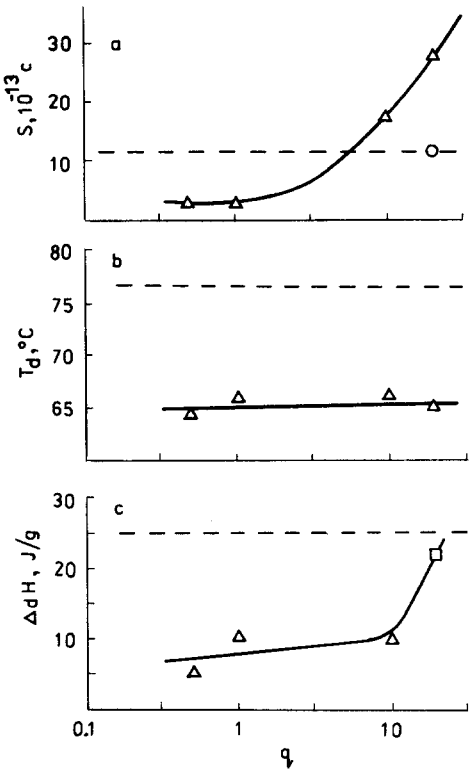

Fig. 1. The sedimentation coefficient (a), denaturation temperature (b) and specific denaturation enthalpy (c) of legumin in the presence of dextran sulphate at pH 7·6 as a function of the parameter q (○, free legumin; △, bound legumin; □, integral characteristic). The dashed lines correspond to the values of denaturation characteristics for legumin in the reference buffer.

parameter q increases (Fig. 1(a)). This suggests the formation of a heavier complex, i.e. an increase in protein binding. However, the change in the protein content in the complexes does not affect the stability of the bound protein (Figs 1(b) and (c)).

It should be noted that a second component appears on sedimentation of protein/dextran sulphate systems at $q = 20$. The sedimentation coefficient of this component is equal to that of the protein (Fig. 1(a)). This implies that free protein is present in the system along with the complexes. Accordingly, the denaturation peak for free protein is detected on the thermogram.

A decrease in the conformational stability of the protein in complexes with dextran sulphate is probably a consequence of the preferential binding of the denatured form of this protein to the polysaccharide matrix. The stability of bound protein does not depend upon its binding density. Therefore, the preferential binding of the denatured form of the protein is realized similarly in complexes with various composition. This is possible if the binding density is low even when there is an excess of protein in the system. This conclusion seems to be reasonable since protein and dextran sulphate are both negatively charged at pH 7·6. In this case, only weak non-cooperative complexing between

protein and polysaccharide is possible. One can assume that the complex formation is the result of localised electrostatic interactions.

As a rule, carboxyl-containing polysaccharides do not form stable complexes with proteins at pH values above their isoelectric point. However, these polysaccharides can form electrostatic complexes with some proteins in acid medium (pH < IEP) at low ionic strengths (Hidalgo & Hansen, 1969; Ganz, 1974; Ledward, 1979). The authors have studied the behaviour of protein/sodium alginate and protein/pectin over the pH range 4·2–7·6. For comparison, a similar investigation has been carried out on the protein/dextran sulphate system considered above.

Broad bean legumin is practically insoluble at low ionic strengths over the pH range 4·2–7·0 (Wright, 1983). In the presence of pectin, alginate and dextran sulphate one can prepare dilute legumin solutions under these conditions at $q = 1$. This increase in legumin solubility directly suggests the formation of soluble legumin–polysaccharide complexes. The complex formation in the systems under investigation has been also proved by sedimentation velocity data (Fig. 2).

Let us consider the change of quarternary structure of the protein in acid medium. At low ionic strengths the protein is soluble in this medium at pH < 4·2.

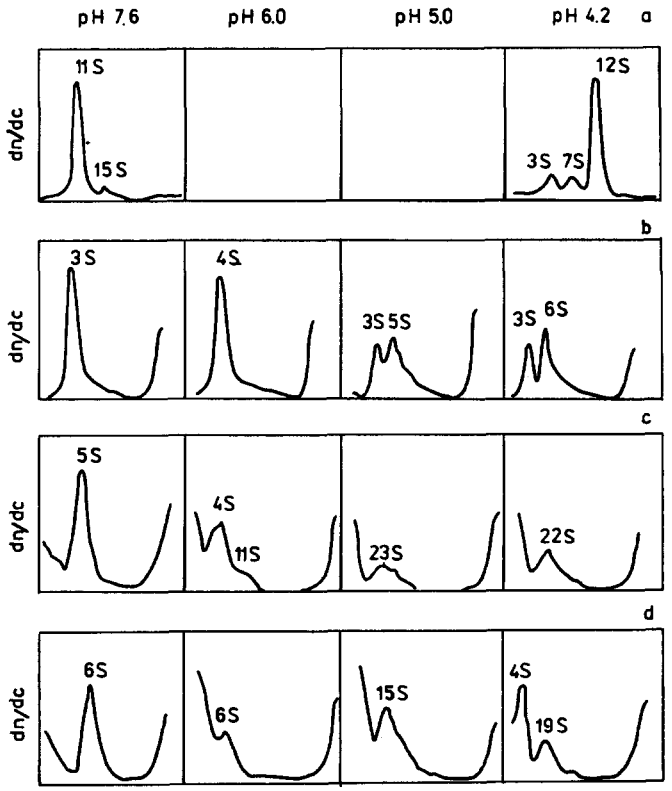

Fig. 2. Sedimentation patterns for the legumin/polysaccharide systems at various pH values (C_L = 0·5%; q = 1): (a) legumin; (b) legumin/dextran sulphate; (c) legumin/sodium alginate; (d) legumin/pectin.

According to sedimentation velocity data (Fig. 2(a)) at pH 4·2 the protein is represented in solution by three components: 12 S (dodecamer), 7 S (hexamer) and 3 S (dimer). Consequently, protein dissociates into hexamers and dimers under these conditions.

Sedimentation velocity of profiles of protein/dextran sulphate systems at various pH values are shown in Fig. 2(b). As has already been mentioned, protein was bound non-cooperatively to dextran sulphate at pH 7·6 and $q = 1$. At pH < 5·0 the sedimentation composition of this system was changed markedly. The main sedimentation peak separated into two components with sedimentation coefficients of about 3 S and 6 S. Both 3 S and 6 S components appear to be legumin–dextran sulphate complexes with different contents of bound protein. The coexistence of two types of complexes with substantially different compositions is peculiar to the systems showing cooperative binding (Gurov *et al.*, 1978). Thus, changing pH from 7·6 to 4·2 in the protein–dextran sulphate system results in a transfer from non-cooperative protein binding to cooperative binding.

The sedimentation velocity data for the protein/alginate and protein/pectin systems at pH values from 7·6 to 4·2 are given in Figs 2(c) and (d). The sedimentation patterns of these systems were very complicated. Nevertheless, two main features could be distinguished at pH < 6·0. In all cases, a fast polydispersed component with a mean sedimentation coefficient from 11 S to 23 S and a pronounced 'tail' near the cell bottom were observed. The fast component appears to be soluble protein–polysaccharide complexes. The 'tail' is attributed probably to precipitation of a significant amount of material during ultracentrifugation. Thus, a wide spectrum of products including soluble complexes and colloidal particles is formed as a result of the protein–polysaccharide interaction in the systems under consideration. It should be emphasized that these systems were colloidal rather than true solutions. As a rule they separated simultaneously into a transparent supernatant and a white precipitate within three days. Data on the conformational stability of the protein in the systems under investigation at pH values from 7·6 to 4·2 are shown in Figs 3–5.

At pH 4·2 the thermogram of free protein shows a main peak with a denaturation temperature of about 78°C and a shoulder at 60° (Fig. 3(a)). In accordance with the data on the protein quarternary structure (Fig. 2(a)), this shoulder may be attributed to the denaturation of the protein hexamer rather than the dimer (Danilenko *et al.*, 1987).

In the case of the protein/dextran sulphate system (Fig. 3(b)) a single heat absorption peak is observed over the whole pH range (4·2–7·6). This can be attributed to denaturation of protein bound to dextran sulphate.

The behaviour of protein/pectin and protein/alginate

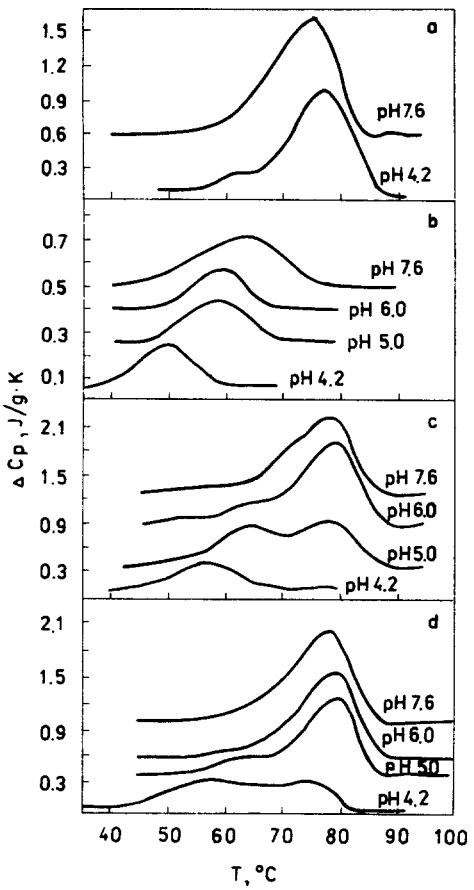

Fig. 3. Thermograms of the legumin in the legumin/polysaccharide systems at various pH values (C_L = 0·5%; q = 1): (a) legumin; (b) legumin/dextran sulphate; (c) legumin/sodium alginate; (d) legumin/pectin.

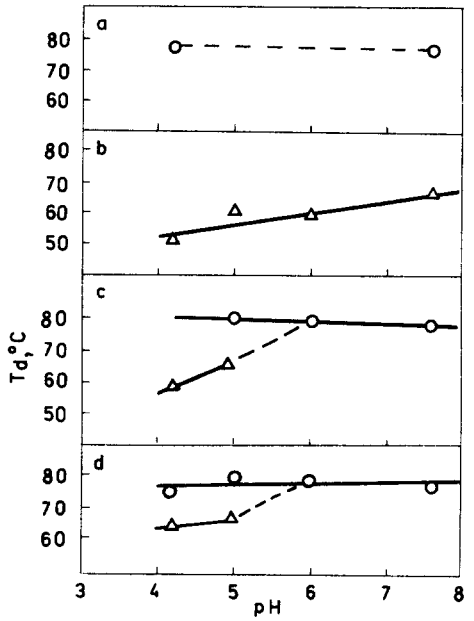

Fig. 4. Denaturation temperature of legumin in the legumin/polysaccharide systems as a function of pH (C_L = 0·5%; q = 1): (a) legumin; (b) legumin/dextran sulphate; (c) legumin/sodium alginate; (d) legumin/pectin (○, free legumin; △, bound legumin).

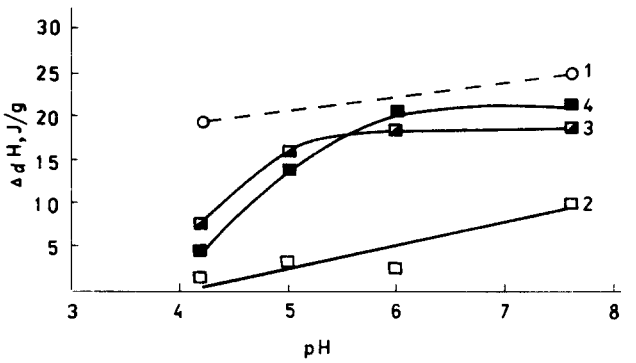

Fig. 5. Specific denaturation enthalpy of legumin in the legumin/polysaccharide systems as a function of pH (C_L = 0·5%; q = 1): 1, legumin; 2, legumin/dextran sulphate; 3, legumin/sodium alginate; 4, legumin/pectin.

systems at pH < 6·0 was similar (Figs 3(c) and (d)). Two heat absorption peaks were observed in the thermograms of these systems. The high and low temperature peaks are attributed to denaturation of free and bound protein, respectively. As the pH value was lowered the denaturation peak of the free protein decreased, whereas the denaturation peak of the bound protein increased.

The plots of denaturation temperature and enthalpy of legumin against pH (Figs 4 and 5) for all systems under investigation point to a substantial decrease in conformational stability of legumin due to complex formation with the polysaccharide matrix.

2. Emulsifying properties

In order to characterize the effect of the polysaccharide on the emulsifying properties of the protein, a legumin/pectin system was chosen. The choice of this system was dictated by the practical importance of pectin as well as the possibility of studying the effect of polysaccharide on protein emulsifying properties under conditions of thermodynamic incompatibility and complex formations of proteins with polysaccharides using the same system. It was shown that protein and pectin were incompatible at pH 7·6 and formed protein-polysaccharide complexes at pH 4·2. Therefore, the emulsifying properties of the protein/pectin system were investigated under these conditions.

The dependencies of the parameter Φ_0 on the protein concentration for protein alone and in the presence of pectin at pH 7·6 and 4·2 are shown in Fig. 6.

The LET value for protein at pH 7·6 and 4·2 coincided and were equal to 0·15 ± 0·02%. This result presumably indicates that the emulsifying ability of protein does not depend on the sign of the protein charge in the pH range studied.

In the presence of pectin in the system a significant decrease in the LET of the protein was observed. In this case, values of LET for protein at pH 7·6 and 4·2 were

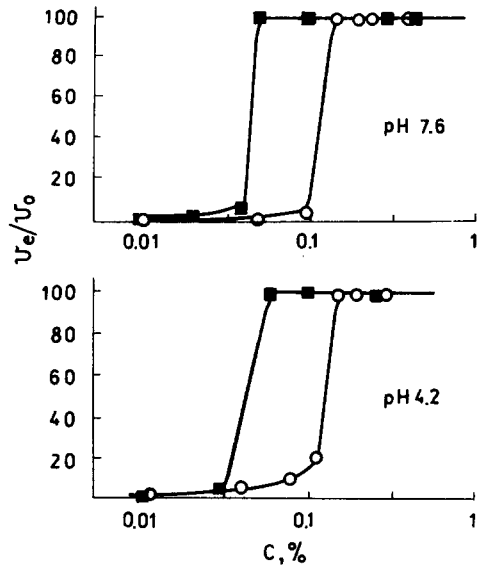

Fig. 6. Plots of the parameter Φ_0 against legumin concentration for emulsions stabilized by legumin (○) and legumin/pectin mixtures (■) at pH 7·6 and pH 4·2 (q = 1).

approximately the same and equal to 0·05 ± 0·02% (Fig. 6).

The LET of a protein depends on both the adsorption properties of the protein and the rate of coalescence of dispersed particles in the emulsion. Therefore, it is necessary to take into account the effect of the polysaccharide on the coalescence rate as well as the adsorption of the protein.

The main factor determining the coalescence rate appears to be the continuous phase viscosity of the emulsion. The higher the viscosity, the lower the rate. Kiosseoglou and Doxastakis (1988) have shown that the coalescence rate of dispersed particles in emulsions stabilized by soybean protein decreased significantly on the addition of sodium alginate, methylcellulose and carboxymethylcellulose. This was attributed to an increase in the viscosity of the continuous phase by the polysaccharide; thus, the decrease in the LET value of the protein in the presence of pectin can be partly regarded as a result of the increase in the continuous phase viscosity.

The second cause of the decrease in the LET value of the protein in the presence of pectin is probably an effect of the polysaccharide on the adsorption of the protein at the oil/water interface.

At pH 7·6 legumin is incompatible with pectin. Therefore, at this pH protein seems to be excluded by pectin from the aqueous phase volume of the emulsion. In other words, the thermodynamic incompatibility of protein with polysaccharide causes increasing protein adsorption at the interface.

At pH 4·2 legumin complexes with the pectin. In this case an adsorption layer at the interface is built mainly by legumin–pectin complexes. It has been shown that strong interfacial films are formed by protein–polysaccharide complexes at lower protein concentrations than is the case with protein alone (Gurov & Nuss, 1986). It is possible that the complexed protein has a greater interactive radius with the interface than the free protein; thus, complex formation with the polysaccharide appears to improve the adsorption properties of the protein.

Table 2. The stability of emulsions ($100\Phi_E$) stabilized by legumin (C_L = 0·5%) in the presence of pectin

System	pH 7·6	pH 4·2
Legumin	40	67
Legumin/pectin, q = 1	84	96

The data on the gravitational stability of emulsions stabilized by the protein and its mixture with pectin are given in Table 2. The stability of emulsions stabilized by legumin at pH 4·2 was higher than that at pH 7·6. As regards the emulsions stabilized by the protein–pectin mixtures, their stability was higher than that of emulsions stabilized by protein alone both at pH 4·2 and pH 7·6. It should be emphasized that the emulsions stabilized by legumin–pectin complexes were of very high stability. The main factor determining increasing gravitational emulsion stability in the presence of polysaccharides is obviously an increase in continuous phase viscosity. The surface activity of emulsifier is of minor importance since the gravitational stability is generally measured at emulsifier concentration well above the LET value.

3. Gelation properties

Broad bean legumin forms transparent thermotropic gels at pH 7·6 as well as at pH 4·2. The values of legumin GT at these pH values were estimated to be 13·00 ± 0·25% and 11·00 ± 0·25%, respectively (Table 3). These values considerably exceed the GT value of the 11 S globulin of soybeans (Bikbov *et al.*, 1985). The relatively high GT value of legumin has been explained by the fact that a significant fraction of the polypeptide chains of this protein does not participate in the formation of the gels' three-dimensional network (Bronich *et al.*, 1988).

Table 3. Thermal gelation concentration threshold of legumin in the presence of polysaccharides

System	pH	q	GT ± 0·25 (%)
Legumin	7·6		13·0
	4·2		11·0
Legumin/pectin	7·6	30	10·3
		100	10·7
Legumin/dextran sulphate	7·6	20	10·5

Table 4. Morphology of the legumin/polysaccharide systems

Polysaccharide	C_L (%)	q	pH	Morphology
	Incompatibility region			
Pectin	10	<14	7·6	Liquid two-phase system
	Complexing region			
Dextran sulfate	1	20	<6·0	Precipitate
	1	2	3–6	Colloidal solution
	1	1	3–6	Opalescent solution
	5	<1	4·2	Highly opalescent solution
	6	1	4·2	Precipitate
Pectin, sodium alginate	0·5	<1	4·2–7	Colloidal solution
	1	<1	4·6–6	Precipitate
Gum arabic	0·5	<1	6·5–7	Colloidal solution
	0·5	<1	4·2–6	Precipitate
Kappa-carrageenan	0·5–1	<4	6·5	Precipitate
	0·5	20	4·2–6	Precipitate
Iota-carrageenan	0·5–1	<4	7·6	Precipitate
	0·5	20	4·2–6	Precipitate

To produce protein gels containing polysaccharides it was necessary to prepare sufficiently concentrated legumin/polysaccharide solutions. However, homogeneous solutions of the mixed system can only be prepared at low concentrations of the polysaccharides ($\ll$1%). An increase in polysaccharide content resulted in phase separation into two liquid phases, one of which was rich in protein whilst the other was rich in the polysaccharide (Table 4). In the case of a single-phase system, the addition of pectin resulted in a slight decrease in the legumin GT value (Table 3). This fact is presumably attributed to an increase in aggregation of denatured protein molecules due to increasing protein activity because of the excluded volume effect.

The study of gelation of legumin-polysaccharide complexes was of especial interest. As the authors have mentioned above, the formation of electrostatic complexes of legumin with dextran sulphate, sodium alginate and pectin leads to the inhibition of protein precipitation in the vicinity of the legumin IEP. Therefore, the possibility of preparing concentrated solutions of these complexes using a titration method for both sulphate- and carboxyl-containing polysaccharides in the pH range 7·0–4·2 has been studied (Table 4). In these experiments the parameter q, the ionic strength, and the pH of the initial solutions have been varied. Only in the case of the legumin/dextran sulphate systems have sufficiently concentrated solutions of complexes been prepared and their gelation properties characterized. At pH 7·6 the GT value of legumin/dextran sulphate mixture was estimated to be 10·50 ± 0·25% (Table 3). Consequently, the effect of complexing (in legumin/dextran sulphate system) and that of incompatibility (in legumin/pectin system) upon the gelation ability of legumin were comparable. However, the legumin/dextran sulphate system is only of scientific importance since it cannot be used in food systems.

For the other systems studied, even at a significant excess of the polysaccharide the concentration region for the formation of soluble legumin-polysaccharide complexes was limited to a very low protein concentration. For instance, in the case of legumin/pectin and legumin/sodium alginate systems it was possible to prepare the solutions of complexes with concentration below 1%. However, as it has been noted above, these systems were colloidal rather than true solutions and therefore unstable.

Therefore, although thermally gelled legumin-polysaccharide complexes are of interest to the food industry it is very difficult to prepare these gels because of the extremely low solubility of legumin-polysaccharide complexes. The possibility to use these complexes as stabilizers of food foams is also limited as stable food foams can be produced only at high concentrations of the stabilizer.

CONCLUDING REMARKS

The data obtained suggest that it is unlikely that major improvement of protein functionality can be achieved by the addition of polysaccharides. Under the conditions of thermodynamic protein-polysaccharide incompatibility the effect of the polysaccharide on the functional properties of protein is presumably insignificant

because of the low solubility of the polysaccharide in the concentrated protein solutions. On the other hand, where complexes are formed there are only soluble complexes in very dilute protein-polysaccharide solutions with a significant excess of polysaccharide ($q < 1$). The most promising approach is the use of protein-polysaccharide complexes prepared in dilute solutions to stabilize food emulsions in the weakly acid pH region (in the vicinity of IEP of protein). This approach would be of especial interest in the case of partly denatured proteins which are practically insoluble at this pH.

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