Functional Properties of Glycosylated Derivatives of the 11S Storage Protein from Pea (*Pisum sativum* L.)

Alain Baniel,† Danièle Caer,‡ Bernard Colas,‡ and Jacques Gueguen*,†

Laboratoire de Biochimie et Technologie des Protéines, Institut National de la Recherche Agronomique, La Géraudière, BP 527, 44026 Nantes Cedex 03, France, and Laboratoire de Biochimie II, Centre de Recherche de Biologie et Physicochimie Cellulaires, Faculté des Sciences, 2 Rue de la Houssinière, 44072 Nantes Cedex 03, France

The viscosity, solubility, foaming, and emulsifying properties of glycosylated derivatives of pea legumin were compared with those of the unmodified protein. These properties reflected conformational changes induced by covalent attachment of glycosyl residues (galactose, lactose, galacturonic acid). The neoglycoproteins were more viscous than the native protein, reflecting a greater hydrodynamic volume and increased protein–water interactions. The solubility of the legumin was also improved by glycosylation, especially with galactose. Galacturonic acid derivatives had pH_i shifted toward more acidic values. Likewise, foaming capacity as well as the stability of foams and emulsions was improved by glycosylation, and the degree of improvement in their properties depended on the type of carbohydrate bound to the protein. Neutral sugars favored the foaming properties, while the charged carbohydrates improved the emulsifying properties.

INTRODUCTION

Vegetable proteins are used in food technology because of their interesting role as functional agents. Functionality of macromolecules is an expression of their physicochemical properties in the food. In the past 10 years, great interest has been focused on the relationships between the structural and physicochemical properties of proteins and their functional properties.

Current hypotheses suggest that surface hydrophobicity is one of the characteristics of a protein most likely to define its surface behaviors and consequently its foaming and emulsifying properties (Graham and Phillips, 1979; Nakai, 1983; Li Chan et al., 1984; Nakai et al., 1986). Other authors concluded that to prevent flocculation and increase resistance to coalescence, the interfacial layer has to be as thick as possible, heavily hydrated, and charged (Graham and Phillips, 1976; Becher, 1977; Dagorn Scaviner et al., 1987; Gueguen et al., 1990).

Glycosylation is expected to affect the hydrophilic/hydrophobic balance and net charge of proteins and to favor the water–protein interactions. Previous studies on bovine serum albumin (Gray, 1974; Schwartz and Gray, 1977; Lee and Lee, 1980), β-lactoglobulin (Waniska and Kinsella, 1984a,b; Kitabatake et al., 1985), and casein (Lee et al., 1979; Canton and Mulvihill, 1983; Courthaudon et al., 1989) have shown a better solubility of these neoglycoproteins. Few have been done on their surface behaviors.

To our knowledge, there are no published studies on the influence of glycosylation on the functional properties of seed storage proteins. That was investigated for 11S globulin (legumin) from pea (*Pisum sativum* L.), which is one of the major proteins in these seeds. Like most 11S proteins, which are widely distributed in leguminous species, pea legumin possesses an oligomeric structure characterized by an arrangement of six subunits αβ (Plietz et al., 1983) and a molecular weight of around 360 000 (Derbyshire et al., 1976; Casey, 1979). Most of the papers dealing with the influence of chemical modification on the functional properties of 11S type proteins were concerned with the effect of acylation (Schwenke et al., 1983; Prahl and Schwenke, 1986; Kim and Rhee, 1990; Gueguen et al., 1990). As generally observed for the 11S globulins, high degrees of succinylation induce dissociation and drastic conformational changes for pea legumin (Schwenke et al., 1990). Functional properties were found to be dependent on the degree of dissociation and unfolding of the protein and on its net negative charge which are related to the level of modification. The simultaneous occurrence of these phenomena results in difficulty in explaining the structure–function relationships (Gueguen et al., 1990). On the other hand, Caer et al. (1990) stated that the glycosylated derivatives of legumin kept their packed oligomeric structure. Only a slight opening of the quaternary structure as well as an increase of the α-helix content was exhibited. Therefore, because of the limited changes in the quaternary and tertiary structures of legumin after glycosylation, the structure–function relationship could be better understood if the protein is in its oligomeric conformation. The objective of this study was an examination of how changes in the hydrophilic/hydrophobic balance and net charge influence the solubility, viscosity, and surface behaviors of the glycosylated legumin. This study, on the potentialities of glycosylation to improve the functional properties of storage vegetable proteins, should also concern the specific interest of many storage proteins naturally glycosylated in leguminous seeds (Chrispeels, 1983; Wright, 1987).

MATERIALS AND METHODS

Materials and Chemicals. Legumin was prepared from pea seeds (*P. sativum* L.) and purified by successive chromatographic separations as described by Gueguen et al. (1984) and Larre and Gueguen (1986).

Sodium cyanoborohydride and 2,4,6-trinitrobenzenesulfonic acid were purchased from Aldrich Chemical Co. Carbohydrates and galacturonic acid were obtained from Merck, and *n*-dodecane (99%) was obtained from Sigma.

Glycosylation of Protein. The glycosylation of legumin was carried out by reductive alkylation as described by Caer et al.

* To whom correspondence should be addressed.
† Institut National de la Recherche Agronomique.
‡ Centre de Recherche de Biologie et Physicochimie Cellulaires.

0021-8561/92/1440-0200$03.00/0

(1990), using sodium cyanoborohydride as reductive agent. In a typical experiment, the reaction was performed at 37 °C for 120 h in 50 mL of 0.1 M sodium phosphate buffer (pH 8) containing 400 mg of legumin (22.2 μM), sodium cyanoborohydride (0.587 M), and various amounts of carbohydrate or galacturonic acid. Lactose and galactose concentrations in the mixture were established in the present study from our previous data (Caer et al., 1990). A medium degree of modification of the amino groups was chosen, i.e., 40% for galactose and 20% for lactose. The initial carbohydrate/lysine molar ratio was 12.3 for galactose (36.04 mM) and 6.0 for lactose (17.6 mM). For galacturonic acid, 58.6 mM was used to reach the maximum degree of modification (around 20% of the amino groups).

After the reaction, the solutions were dialyzed at 4 °C against 0.1 M sodium phosphate buffer (pH 8) for 24 h and then desalted by eluting the sample on a Trisacryl GF05LS column with a diluted ammonia solution (pH 8). Then the desalted proteins were freeze-dried.

The amount of carbohydrates attached to legumin by ketoamine linkage was measured according to the method of Gallop et al. (1981). By periodate oxidation of the carbohydrate moieties, formaldehyde is released and quantitated by the Hantzsch reaction (Nash, 1953). As it was verified that only the amino groups were modified under these experimental conditions (Caer et al., 1990), the degree of glycosylation was expressed as the number of alkylated amino groups in percentage of the total amino groups in legumin. According to the sequence published by Lycett et al. (1984), 144 total amino groups (132 lysyl residues + 12 N terminal) was used for the calculation, although some differences between amino acid sequence of the various $\alpha\beta$ subunits exist.

Intrinsic Viscosity. Intrinsic viscosities ($[\eta]$) were determined at 25 °C in 0.1 M sodium phosphate buffer (pH 8, containing 0.2 M NaCl) with an automatic Ostwald viscosimeter (capillarity of 0.36 mm; solvent flow time, 267.51 s) using protein concentrations in the range 2–15 mg/mL.

Solubility. The solubility of the unmodified and glycosylated legumin samples was determined according to the method of Coffmann and Garcia (1977) modified as follows: 5 mg of freeze-dried samples were dispersed in 4 mL of either distilled water or 0.2 M NaCl solution. Then the pH value was adjusted in the range 2–11.5 by adding 0.1 N HCl or 0.1 N NaOH. Distilled water or NaCl solution was added to a final volume of 5 mL. After 60 min of stirring, using a rotating stirrer at room temperature, the pH of the protein solutions was measured and the samples centrifuged for 15 min at 34000*g*. The protein concentration in the supernantants was determined according to the method of Lowry et al. (1951) and expressed as a percentage of initial total protein concentration.

Emulsifying Properties. The emulsifying properties were expressed as the emulsifying activity index (EAI) and the emulsion stability. The emulsions were prepared as described by Dagorn-Scaviner et al. (1987) by homogenizing 10 mL of *n*-dodecane with 30 mL of a protein solution (1.3 mg/mL of 0.1 M sodium phosphate buffer, pH 8, 0.2 M NaCl), using a Polytron PT 10 homogenizer (30 s at 20 000 rpm).

Emulsifying Activity Index. The EAI was estimated according to the method of Pearce and Kinsella (1978). The turbidity T ($T = 2.303\ A/l$; A, absorbance of the emulsion; l, 1 cm, path length of the cuvette) of the emulsion, diluted 250-fold with a solution of 0.1% SDS–0.1 M NaCl, pH 7, was measured at 500 nm. EAI is defined as

$$\mathrm{EAI} = 2T/\phi C$$

where ϕ is the volume fraction of the oil phase (here $\phi = 0.25$) and C the protein concentration in the diluted aqueous phase. EAI, expressed in m^2/g, is related to the stabilized interfacial area per unit weight of protein.

Emulsifying Stability. Emulsion stability was determined by following the flocculation–creaming process during aging of the emulsion, as described by Dagorn-Scaviner et al. (1987). Emulsion was poured into a 10-mL graduated vessel. Emulsion destabilization was followed by plotting the volume of separated aqueous phase V_t (mL) vs time (t). According to Dagorn-Scaviner et al. (1987) the flocculation–creaming process can be considered a succession of first-order kinetics which can be visualized by plotting ln $(V_e/V_e - V_t))$ vs t, V_e being the equilibrium value of V_t after 24 h. Each kinetic step is characterized by its rate constant β and its duration Δt. The equilibrium volume fraction, ϕ_e, of the organic phase in the creamed phase is calculated by

$$\phi_e = 2.5/((10 - V_e) + 2.5)$$

Foaming Properties. Foaming properties were expressed as foaming capacity and foam stability (Dagorn-Scaviner, 1986; Gueguen et al., 1990). Foaming was induced by bubbling a stream of nitrogen (2.57 mL/s) during 15 s through fritted glass into 8 mL of protein solutions (0.1 M sodium phosphate buffer, pH 8, 0.2 M NaCl). Then, the conductivity of the resulting foam was recorded. Foam capacity was evaluated by the initial conductivity (C_i, μS/cm). Foam stability was expressed as $t_{1/2}$, the time necessary to obtain a conductivity of $C_i/2$ measured on the experiment curve, and the foam stability index (FSI), defined by Kato et al. (1983) as $\mathrm{FSI} = C_0\ \Delta t/\Delta C$ (min), where C_0 is the conductivity at zero time obtained by the extrapolation of the linear part of the C vs t plot and $\Delta C/\Delta t$ is the slope of this linear region.

The $t_{1/2}$ is related to drainage which happens just after foam formation. FSI describes the stability of the foam when most of the drainage has occurred and is chiefly related to coalescence.

As low amounts of glycosylated proteins were available, the reproducibility of emulsifying and foaming properties was estimated with native legumin on five repetitions and the relative error coefficient calculated on each parameters. These coefficients were used for the other samples.

RESULTS

Degree of Glycosylation. Fifty-three, twenty-four, and twenty-seven moles of galactose, lactose, and galacturonic acid were bound per mole of legumin, corresponding to degrees of glycosylation of 37%, 17%, and 19%, respectively. Because the total weight in lactosyl and galactosyl residues bound per mole of protein is very close, the influence on functional properties of number of occupied sites and of length of the linked carbohydrate molecule could be discussed. Besides, the degree of glycosylation reached for galacturonic acid, which is maximum (Caer et al., 1990), is satisfactory to approach the influence of the charge.

Viscosity. Intrinsic viscosity of the unmodified legumin sample (4.3 mL/g) was very similar to those reported by Schwenke et al. (1986) for the 12S globulin from rapeseed (4.5 mL/g) and sunflower (4.0 mL/g). In a recent study, we found a higher intrinsic viscosity (5.5 mL/g) (Schwenke et al., 1990) because of the removal, by gel filtration, of aggregates from the protein preparation just before the viscosimetric measurements. In the present work the whole legumin preparation was used.

An increase in intrinsic viscosity was exhibited by glycosylated samples, which varied with the type of carbohydrates bound (Table I). Binding of galactose or galacturonic acid to legumin induced a lower increase than bound lactose. The values of Huggins' constant (k') were considerably lower for the glycosylated samples, indicating a weakening of molecular interactions. The very high value of k' for the unmodified sample confirms the presence of aggregates. In contrast, the low values of k' in galacturonic acid derivatives and further lowering of k' in lactosylated derivatives indicate that these aggregates tend

Table I. Intrinsic Viscosity[a]

	native legumin	lactosylated derivative	galacturonic acid derivative	galactosylated derivative
$[\eta]$	4.3	7.7	6.3	6.7
k'	10.4	0.59	1.82	3.4

[a] $[\eta]$, intrinsic viscosity in mL/g; k', Huggins' constant.

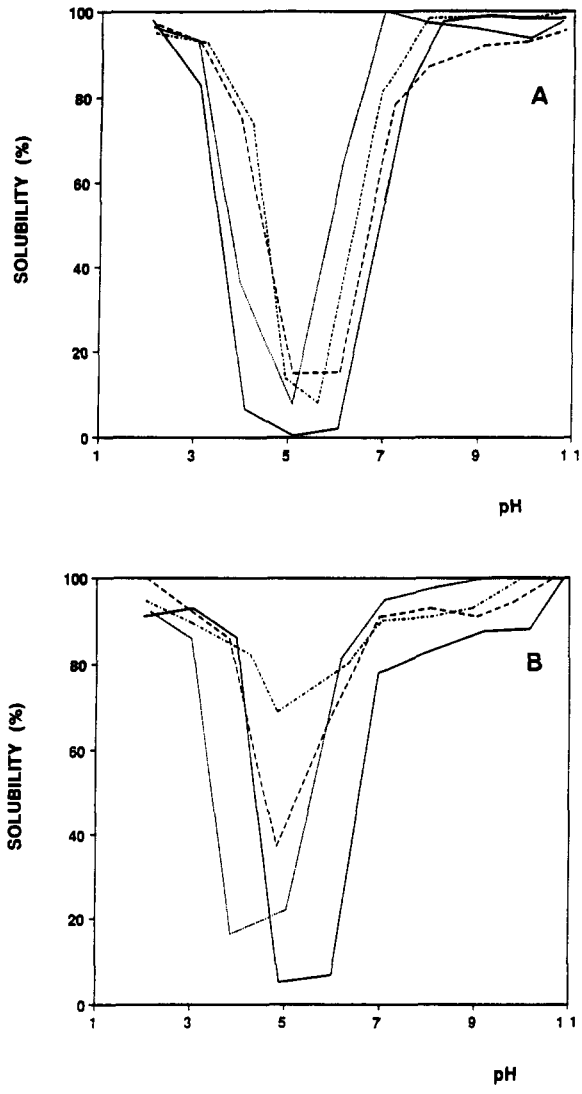

Figure 1. Influence of pH on the solubility of native and glycosylated legumin: (—) unmodified; (– · –) galactosylated; (– –) lactosylated; (···) galacturonic acid derivative. (A) Samples dispersed in distilled water; (B) samples dispersed in 0.2 M NaCl.

to disappear with glycosylation. The value of k' in galactosylated samples was intermediate between the two other glycosylated derivatives and unmodified legumin. These results agree with the electrophoretic patterns of glycosylated legumins under nondenaturing conditions published by Caer et al. (1990). We showed the presence of a minor band corresponding to aggregates, especially in unmodified and in galactosylated legumin samples, which tends to disappear when the amount of lactose and galacturonic acid bound to the protein is increased.

Solubility. Legumin solubility in water is generally increased by glycosylation between pH 2 and 8, but glycosylated samples still exhibited, like unmodified legumin, a very deep V profile for the solubility curve. However, solubility was drastically improved by galactosylation and lactosylation in the pH range 4–4.5 and by galacturonic acid binding in the pH range 5.5–6 (Figure 1A). In 0.2 M NaCl, lactosylated and galactosylated samples exhibited a very high solubility (Figure 1B). Whatever the pH, the solubility was above 75% for galactosylated derivatives. Moreover, the solubility, in the pH_i region around pH 5, is significantly increased as observed by Courthaudon et al. (1989) for glycosylated caseins. The shift of the isoelectric point toward acidic pH, due to the blockage of ϵ-amino–lysyl groups by the carbohydrates, is reinforced for galacturonic acid derivatives because of the addition of new carboxylic groups. This explained the lower solubility of these samples in the acidic pH range.

Emulsifying Properties. The EAI, which denotes the ability of the protein to help the dispersion of the oil phase and to provide a sufficient coating of the interfacial area (Dagorn et al., 1987), is not significantly modified by glycosylation. The EAI of unmodified as well as glycosylated legumin samples is approximately 80 m^2/g, while 106 m^2/g was found for bovine serum albumin (BSA) used as standard protein (Table II). On the other hand, glycosylated legumin samples exhibited better emulsifying stabilities than unmodified protein (Figure 2).

The analysis of each step of the destabilization kinetics is reported in Table II. For the four protein concentrations studied (0.4–3 mg/mL), an increase in the duration of each step and a decrease in the rate constants were exhibited by glycosylated protein, which varied with the type of carbohydrates bound. From the higher to the lower values of β_1, the legumin samples were classified in the following order: unmodified > galactosylated > lactosylated > galacturonic acid derivatives.

Covalent binding of galacturonic acid to legumin was the most efficient modification to decrease the flocculation–creaming process, probably because of the repulsive effects induced by the added negative charges.

Besides, at low concentration (0.4 mg/mL), the volume fractions of the *n*-dodecane in the creamed phase (ϕ_e) were lower. This effect, explained by higher hydration of the interfacial layers due to an increase of protein–water interactions by glycosylation, was masked for higher protein concentrations except for lactosylated legumin.

Foaming Properties. Kinetics of foam collapse were compared for native and glycosylated legumin, using BSA as standard protein (Figure 3). Initial conductivity (C_i), as a measure of foam capacity, is significantly improved by glycosylation (Table III) (about twice as high as the unmodified protein) whatever the carbohydrate type and the protein concentration. The higher values were obtained for the lactosylated legumin.

$t_{1/2}$, as a measure of foam stability, is related to the ability of the protein to adsorb quickly at the interface area and to decrease the drainage just after the foam formation. On the other hand, FSI, which describes the resistance of the foam to collapse, is connected to mechanical and rheological properties of interfacial layers. Lactosylated and galactosylated derivatives exhibited higher $t_{1/2}$ and FSI values compared to those of unmodified legumin and BSA (Table III). For the high concentrations, binding of galacturonic acid did not significantly improve foam stability compared to that of unmodified legumin.

DISCUSSION

The effect of glycosylation on the functional properties of pea legumin was discussed on the basis of the structural alterations. Spectroscopic data showed that accessibility of tyrosyl residues was slightly increased by glycosylation, indicating a slight opening of the globular structure (Caer et al., 1990). These changes in the quaternary and tertiary structures induced a decrease of the aggregation phenomena. For lactosylated protein and, furthermore, for galacturonic acid derivatives, a partial dissociation into 8S and 3S components occurred. Secondary structure was also modified, glycosylation increasing the percentage of helical structure from 9% to about 20% and decreasing β structure from 46% to 20%.

Table II. Emulsifying Properties[a]

protein	EAI, m^2/g	concn, mg/mL	V_e, mL	ϕ_e	first step Δt_1, min	first step β_1, 10^{-4}/s	second step Δt_2, min	second step β_2, 10^{-4}/s
BSA	106 ± 3.9	0.4	4.6	0.46 ± 0.02	8	15.0 ± 0.45	5	23.0 ± 1.56
		1	5.2	0.52 ± 0.02	21	4.7 ± 0.14	15	7.2 ± 0.49
		1.5	5.0	0.50 ± 0.02	17	6.0 ± 0.18	16	9.0 ± 0.61
		3	4.5	0.45 ± 0.02	21	5.5 ± 0.17	13	7.2 ± 0.49
native legumin	87 ± 3.2	0.4	6.8	0.78 ± 0.03	2	52.0 ± 1.56	1	102.0 ± 6.93
		1	5.4	0.54 ± 0.02	3	43.3 ± 1.30	5	30.0 ± 2.04
		1.5	5.2	0.52 ± 0.02	5	25.0 ± 0.75	5	20.0 ± 1.36
		3	4.6	0.46 ± 0.02	4	10.0 ± 0.30	4	25.0 ± 1.70
lactosylated derivative	83 ± 3.1	0.4	5.0	0.50 ± 0.02	3	42.0 ± 1.26	5	37.0 ± 2.51
		1	4.6	0.46 ± 0.02	8	15.0 ± 0.45	7	20.0 ± 1.36
		1.5	4.9	0.49 ± 0.02	17	7.0 ± 0.21	13	11.0 ± 0.75
		3	3.6	0.39 ± 0.02	15	6.7 ± 0.20	9	10.0 ± 0.68
galacturonic acid derivative	nd	0.4	4.9	0.49 ± 0.02	4	23.0 ± 0.69	4	25.0 ± 1.70
		1	4.9	0.49 ± 0.02	12	10.8 ± 0.32	6	16.7 ± 1.13
		1.5	5.0	0.50 ± 0.02	16	6.7 ± 0.20	12	10.5 ± 0.71
		3	4.4	0.45 - 0.02	18	5.5 ± 0.17	11	8.3 ± 0.56
galactosylated derivative	81 ± 3.0	0.4	5.4	0.54 ± 0.02	3	37.0 ± 1.11	4	35.0 ± 2.38
		1	4.1	0.42 ± 0.02	7	26.7 ± 0.80	8	10.0 ± 0.68
		1.5	4.1	0.42 ± 0.02	8	9.8 ± 0.29	6	16.7 ± 1.13
		3	nd	nd	nd	nd	nd	nd

[a] EAI, emulsifying activity index, measured at 1.3 mg/mL protein concentration; concn, protein concentration; V_e, aqueous-phase volume at the equilibrium; ϕ_e, volume fraction of organic phase in the creamed phase; Δt_i, duration of each step; β, rate constant for each step.

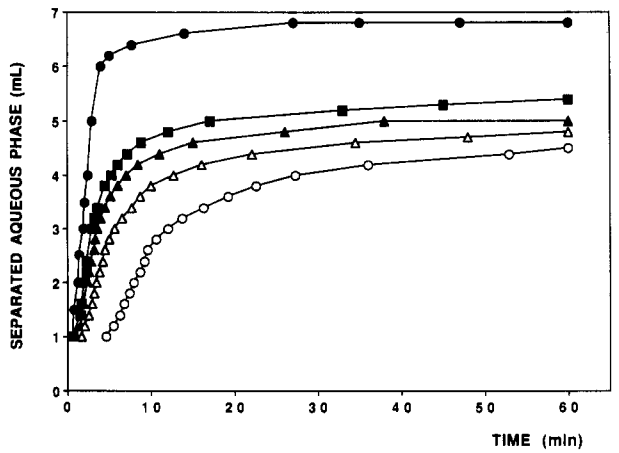

Figure 2. Kinetics of emulsion destabilization (0.1 M sodium phosphate buffer, pH 8, 0.2 M NaCl; protein concentration, 0.4 mg/mL): (○) bovine serum albumin; (●) unmodified; (■) galactosylated; (▲) lactosylated; (△) galacturonic acid derivative.

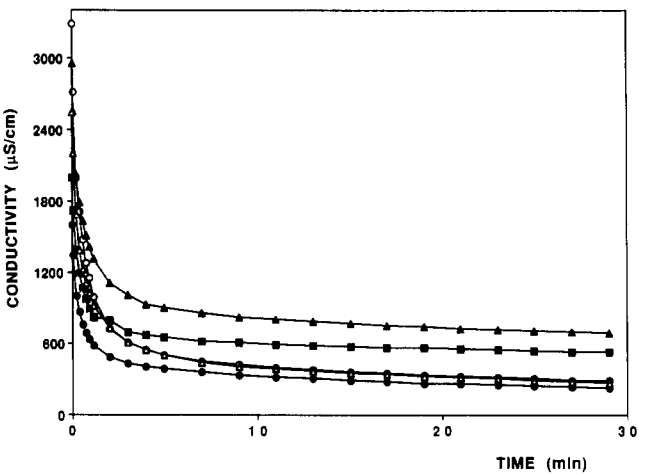

Figure 3. Kinetics of foam destabilization (0.1 M sodium phosphate buffer, pH 8, 0.2 M NaCl; protein concentration, 2 mg/mL): (○) bovine serum albumin; (●) unmodified; (■) galactosylated; (▲) lactosylated; (△) galacturonic acid derivative.

Higher intrinsic viscosities measured for glycosylated derivatives are due to the slight opening of the globular structure which induces greater hydrodynamic volume and increases hydrophilic interactions between glycosylated proteins and water. That was observed by Waniska and Kinsella (1984a) with maltosyl and glucosaminyl derivatives of β-lactoglobulin. A similar effect on intrinsic viscosity of succinylated legumin, at low degree of succinylation (20–60%), was observed by Schwenke et al. (1990) and interpreted as due to a partial dissociation of the oligomeric protein into subunits. We should expect that neoglycoproteins possess a larger hydrodynamic volume than succinylated legumin because of the higher hydrophilic character of carbohydrates compared to succinyl group. Since it was not the case, greater intrinsic viscosity seems to be more related to structure modifications than to direct interactions between the bound residues and water. This was also observed by Le Meste et al. (1989), who studied, by electron spin resonance and sorption isotherms, the influence of glycosylation on hydration properties of caseinates. According to these authors, the water bound to the protein was very similar for glycosylated and control caseinates. An increase in the water holding capacity for modified samples was related to the swelling of the protein after glycosylation.

The size of the glycosyl residue also influences intrinsic viscosity. The higher value was reached for lactosylated legumin, whereas similar ones were obtained for galacturonic acid derivative and galactosylated legumin. In accordance with the theory, intrinsic viscosity is higher for the greater length of side chains.

Glycosylation also appears to be very efficient for improving solubility of legumin, especially in the presence of salt. Similar results were obtained by Courthaudon et al. (1989) on glycosylated caseins. The salting-in effects related to changes in the charge equilibrium of the protein, due to alkylation of amino groups, are influenced by the degree of glycosylation and the type of glycosyl residues. For example, binding of galacturonic acid lowered the value of pH_i and improved solubility; this effect is, however, limited by the rather low level of legumin modification. On the other hand, although binding of neutral carbohydrates did not modify so much the isoelectric point, it

Table III. Foaming Properties[a]

protein	concn, mg/mL	C_i, μS/cm	$t_{1/2}$, s	FSI, min
BSA	0.5	2025 ± 113.4	24.6 ± 2.5	88.0 ± 2.6
	1	2480 ± 138.8	25.2 ± 2.5	86.6 ± 2.6
	2	3280 ± 183.7	27.6 ± 2.8	83.0 ± 2.4
native legumin	0.5	750 ± 42.0	14.4 ± 1.4	54.9 ± 1.6
	1	1080 ± 60.5	19.0 ± 1.9	42.8 ± 1.3
	2	1600 ± 89.6	30.0 ± 3.0	83.1 ± 2.5
lactosylated derivative	0.5	2100 ± 117.6	22.2 ± 2.2	78.6 ± 2.4
	1	2425 ± 135.8	40.0 ± 4.0	91.5 ± 2.8
	2	2950 ± 165.2	54.4 ± 5.4	168.8 ± 5.1
galacturonic acid derivative	0.5	2050 ± 114.8	27.6 ± 2.8	103.9 ± 3.1
	1	2400 ± 134.4	30.6 ± 3.1	74.0 ± 2.2
	2	2540 ± 142.2	30.3 ± 3.0	79.0 ± 2.4
galactosylated derivative	0.5	1600 ± 89.6	30.0 ± 3.0	65.0 ± 2.0
	1	2475 ± 136.6	34.2 ± 3.4	136.0 ± 4.1
	2	2000 ± 112.0	41.5 ± 4.2	226.4 ± 6.8

[a] Concn, protein concentration; C_i, initial conductivity; $t_{1/2}$, time corresponding to $C_i/2$; FSI, foam stability index ($C_0\Delta t/\Delta C$).

markedly increased solubility in presence of salt. Moreover, the level of hydrophilicity, which is varying with the type and amount of carbohydrate residues bound to the protein, seems not be the major parameter to explain the difference in neoglycoprotein solubility. For a close amount of bound hydroxyl groups, legumin galactosylated at 37% exhibited a higher solubility than protein lactosylated at 17%. Consequently, a greater solubility of galactosylated sample should be explained, first, by a higher level of blocked lysyl residues, which increases the net negative charge of the protein, and second, by a larger distribution of hydrophilic residues bound at the surface of the protein, which may favor water–protein interactions and weaken, by steric effects, the electrostatic attraction forces between the legumin $\alpha\beta$ subunits.

Despite better solubility, the emulsifying activity (EAI) of glycosylated derivatives was not improved as expected. As shown by high intrinsic viscosity for modified proteins, glycosylation increased the hydrodynamic volume of proteins and should consequently slow down the diffusion rate of molecules to the interface. Moreover, dissociation of glycosylated derivatives into subunits is too limited (Caer et al., 1990) to have a significant effect on protein flexibility for modifying its ability to anchor at water/oil interface. On the other hand, anchorage at the water/air interface seems to be easier, considering the higher foaming capacities of neoglycosproteins. The influence of the polarity of interface on the mechanism of protein adsorption could be related to the increased surface hydrophilicity of glycosylated legumins which should partly suppress the presence of loops in the oil phase. These results are in good agreement with those of Nakai and co-workers (Nakai et al., 1980; Townsend and Nakai, 1983). They showed that surface hydrophobicity has a positive effect on emulsifying activity but is not correlated with foaming capacity. Besides, a high viscosity was associated with an optimum foaming capacity.

Stability of emulsions, improved with neoglycoproteins, has to be discussed taking into account modifications in interfacial layer characteristics. A greater helix content and the opening of the quaternary structure should improve the ability of these neoglycoproteins to form stable interfacial layers. The lower volume fractions of *n*-dodecane in the creamed phase (ϕ_e) obtained for the lower protein concentration (0.4 mg/mL) suggest that destabilization of the quarternary structure, by glycosylation, favors the hydration of adsorbed proteins. The efficiency of glycosylated legumin in retarding emulsion breaking is related to a greater thickness of the interfacial layer, mainly due to the enhanced hydration. For higher bulk protein concentration, this effect is partially masked; in these conditions the higher hydration of the creamed phase is more related to an increase of the interfacial protein concentration than to specific behaviors of neoglycoproteins. As judged by the constant rate of destabilization (β_1), the presence of negative charges in the case of galacturonic acid derivative may inhibit flocculation by additional effects of repulsion between the droplets. This phenomenom was also observed by Gueguen et al. (1990) for succinylated 12S rapeseed globulin.

Similar behaviors could be stated to explain the improved foam stability by neoglycoproteins. However, the role of the charges in the stabilizing process was less beneficial in foams compared to in emulsions. This was shown from the lower stability of foams prepared with the higher concentrations of galacturonic acid derivatives. Townsend and Nakai (1983) observed that foam stability markedly decreased at high charge density. They suggested that intermolecular repulsions between protein molecules at the interface could have a destabilization effect on foams.

In conclusion, the glycosylation appears to be of great importance in improvement of the functional properties of 11S type seed storage proteins. Neoglycoproteins are largely more soluble than native protein and exhibit a higher viscosity and better surface behaviors. The improvement of functional properties is mainly due to the partial opening of the quaternary structure and to an increase of net negative charge. It was found to be also dependent on the degree of glycosylation and on the type of glycosyl residues. The neutral carbohydrates are more adapted to improve solubility and foaming properties, whereas charged sugars exhibited better emulsifying properties.

This study proves the great interest in developing methods of glycosylation adapted to the food industry. Further work will explore the potentialities of some enzymatic reactions.

LITERATURE CITED

Becher, P. *Emulsions: Theory and Practice*; Krieger Publishing: Huntington, NY, 1977.

Caer, D.; Baniel, A.; Subirade, M.; Gueguen, J.; Colas, B. Preparation and Physicochemical Properties of Glycosylated Derivatives of Pea Legumin. *J. Agric. Food Chem.* **1990**, *38*, 1700–1706.

Canton, M. C.; Mulvihill, D. M. In *Physicochemical Aspects of Dehydrated Protein-rich Milk Products*, Proceedings of IDF Symposium; Statens Forsogsmejeri: Hillerod, Denmark, 1983; pp 339–353.

Casey, R. Immunoaffinity Chromatography as a Means of Purifying Legumin from *Pisum* (Pea) Seeds. *Biochem. J.* **1979**, *177*, 509–520.

Chrispeels, M. J. Biosynthesis, Processing and Transport of Storage Proteins and Lectins in Cotyledons of Developing Seeds. In *Seed Storage Proteins*, Proceedings of a Royal Society Discussion Meeting; Fowden, L., Miflin, B. J., Eds.; Royal Society: London, 1983, pp 309–322.

Coffmann, C. W.; Garcia, V. V. Functional Properties and Amino Acid of a Protein Isolate from Mung Bean Flour. *J. Food Technol.* **1977**, *12*, 473–484.

Courthaudon, J. L.; Colas, B.; Lorient, D. Covalent Binding of Glycosyl Residues to Bovine Casein: Effects on Solubility and Viscosity. *J. Agric. Food Chem.* **1989**, *37*, 32–36.

Dagorn-Scaviner, C. Thesis, Université de Nantes, France, 1986.

Dagorn-Scaviner, C.; Gueguen, J.; Lefebvre, J. Emulsifying Properties of Pea Globulins as Related to their Adsorption Behaviors. *J. Food Sci.* **1987**, *52*, 335–341.

Derbyshire, E.; Wright, D. J.; Boulter, D. Legumin and Vicilin Storage Proteins of Legume Seeds. *Phytochemistry* **1976**, *15*, 3–24.

Gallop, P. M.; Fluckiger, R.; Hanneken, A.; Mininsohn, M. M.; Gabbery, K. H. *Chemical* Quantitation of Hemoglobin Glycosylation: Fluorimetric Detection of Formaldehyde Released upon Periodate Oxidation of Glycoglobin. *Anal. Biochem.* **1981**, *117*, 427–432.

Graham, D. E.; Phillips, M. C. The Conformation of Proteins at Interfaces and their Role in Stabilizing Emulsions. In *Theory and Practice of Emulsion Technology*; Smith, A. L., Ed.; Academic Press: London, 1976, pp 75–98.

Graham, De. E.; Phillips, M. C. Proteins at Liquid Interfaces. III. Molecular Structures of Adsorbed Film. *J. Colloid Interface Sci.* **1979**, *70*, 427–439.

Gray, G. R. The Direct Coupling of Oligosaccharides to Proteins and Derivatized Gels. *Arch. Biochem. Biophys.* **1975**, *163*, 426–428.

Gueguen, J.; Vu, A. T.; Schaeffer, F. Large-scale Purification and Characterization of Pea Globulins. *J. Sci. Food Agric.* **1984**, *35*, 1024–1033.

Gueguen, J.; Bollecker, S.; Schwenke, K. D.; Raab, B. Effect of Succinylation on Some Physicochemical and Functional Properties of the 12S Storage Protein from Rapeseed (*Brassica napus* L.). *J. Agric. Food Chem.* **1990**, *38*, 61–69.

Kato, A.; Takahashi, A.; Matsudomi, N.; Kobayashi, K. Determination of Foaming Properties of Proteins by Conductivity Measurements. *J. Food Sci.* **1983**, *48*, 62–65.

Kim, K. S.; Rhee, J. S. Effect of Acetylation on Emulsifying Properties of Glycinin. *J. Agric. Food Chem.* **1990**, *38*, 669–674.

Kitabatake, N.; Cuq, J. L.; Cheftel, J. C. Covalent Binding of Glycosyl Residues to β-lactoglobulin: Effects on Solubility and Heat Stability. *J. Agric. Food Chem.* **1985**, *33*, 125–130.

Larre, C.; Gueguen, J. Large Scale Purification of Pea Globulins. Comparison between Six Anion Exchangers in Medium-Pressure Liquid Chromatography. *J. Chromatogr.* **1986**, *361*, 169–179.

Lee, H. S.; Sen, L. C.; Clifford, A. J.; Whitaker, J. R.; Feeney, R. E. Preparation and Nutritional Properties of Caseins Covalently Modified with Sugars. Reductive Alkylation of Lysine with Glucose, Fructose or Lactose. *J. Agric. Food Chem.* **1979**, *27*, 1094–1098.

Lee, R. T.; Lee, Y. C. Preparation and some Biochemical Properties of Neoglycoproteins Produced by Reductive Amination of Thioglycosides Containing an ω-aldehydroaglycon. *Biochemistry* **1980**, *19*, 156–163.

Le Meste, M.; Colas, B.; Blond, G.; Simatos, D. Influence of Glycosylation on the Hydration Properties of Caseinates. *J. Dairy Res.* **1989**, *56*, 479–486.

Li Chan, E.; Nakai, S.; Wood, D. F. Hydrophobicity and Solubility of Meat Proteins and their Relationship to Emulsifying Properties. *J. Food Sci.* **1984**, *49*, 345–350.

Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein Measurement with the Folin Phenol Reagent. *J. Biol. Chem.* **1951**, *193*, 265–275.

Lycett, G. W.; Croy, R. R. D.; Shirsat, A. H.; Boulter, D. The Complete Nucleotide Sequence of a Legumin Gene from Pea (*Pisum sativum* L.) *Nucleic Acids Res.* **1984**, *11*, 4493–4506.

Nakai, S. Structure-Fonction Relationships of Food Proteins with Emphasis on the Importance of Protein Hydrophobicity. *J. Agric. Food Chem.* **1983**, *31*, 676–683.

Nakai, S.; Ho. L.; Helbig, N.; Kato, A.; Tung, M. A. Relationship Between Hydrophobicity and Emulsifying Properties of Some Plant Proteins. *Can. Inst. Food Sci. Technol. J.* **1980**, *13*, 23–27.

Nakai, S.; Li Chan, E.; Hayakawa, S. Contribution of Protein Hydrophobicity to its Functionality. *Nahrung* **1986**, *30*, 327–336.

Nash, T. The Colorimetric Estimation of Formaldehyde by Means of the Hantzsch Reaction. *Biochem. J.* **1953**, *55*, 416–421.

Pearce, K. N.; Kinsella, J. E. Emulsifying Properties of Proteins: Evaluation of a Turbidimetric Technique. *J. Agric. Food Chem.* **1978**, *26*, 716–723.

Plietz, P.; Damaschun, H.; Müller, J. F.; Schwenke, K. D. The Structure of 11S Globulins from Sunflower and Rape Seed. A Small Angle X-Ray Scattering Study. *Eur. J. Biochem.* **1983**, *130*, 315–320.

Prahl, L.; Schwenke, K. D. Functional Properties of Plant Proteins. Part 7. Rheological Properties of Succinylated Proteins Isolates from Faba Beans (*Vicia Faba* L.). *Nahrung* **1986**, *30*, 311–318.

Schwartz, B. A.; Gray, G. R. Proteins Containing Reductively Aminated Disaccharides. *Arch. Biochem. Biophys.* **1977**, *181*, 542–549.

Schwenke, K. D.; Rauschal, E. J.; Robowsky, K. D. Functional properties of plant proteins. Part 4. Foaming properties of modified proteins from faba beans. *Nahrung* **1983**, *27*, 335–350.

Schwenke, K. D.; Linow, K. J.; Zirwer, D. Modification of the Oligomeric Structure of 11S Globulins from Sunflower (*Helianthus annus* L.) and Rapessed (*Brassica napus* L.) Seeds by Succinylation. *Nahrung* **1986**, *30*, 263–270.

Schwenke, K. D.; Zirwer, D.; Gast, K.; Görnitz, E.; Linow, K.-J.; Gueguen, J. Changes of the Oligomeric Structure of Legumin from Pea (*Pisum sativum* L.) after Succinylation. *Eur. J. Biochem.* **1990**, *194*, 621–627.

Townsend, A.; Nakai, S. Relationships between Hydrophobicity and Foaming Characteristics of Food Proteins. *J. Food Sci.* **1983**, *48*, 588–594.

Waniska, R. D.; Kinsella, J. E. Physico-chemical Properties of Maltosyl and Glucosaminyl Derivatives of β-lactoglobulin. *Int. J. Pept. Protein Res.* **1984a**, *23*, 467–476.

Waniska, R. D.; Kinsella, J. E. Preparation of Maltosyl, β-cyclodextrinyl, Glucosaminyl and Glucosamineoctaosyl Derivatives of β-lactoglobulin. *Int. J. Pept. Protein Res.* **1984b**, *23*, 573–580.

Wright, D. J. The Seed Globulins. In *Developments in Food Protein 5*; Hudson, J. F., Ed.; Elsevier Applied Science: London, 1987; pp 81–157.

Received for review November 6, 1991. Accepted November 14, 1991.