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Original article

Condensation of glycosidic and aromatic structures on amino groups of β-lactoglobulin B *via* reductive alkylation. Solubility and emulsifying properties of the protein derivatives

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Summary — Nucleophilic primary amino groups of bovine β-lactoglobulin B were modified with different aldehydes by an addition-elimination reaction commonly named "reductive alkylation". Subsequently resulting imines (Schiff bases) were reduced, producing secondary amines derivatives of this protein. Two kinds of ligands, differing by their hydrophobicity, were coupled to this protein: 81% and 69% of β-lactoglobulin amino groups were modified with glucose and maltose, respectively; 31% and 44% of β-lactoglobulin amino groups were modified with vanillin and benzaldehyde, respectively. Reversed-phase HPLC and electrophoresis of modified β-lactoglobulin indicated that, in the case of glycosylation, the condensation was rather homogeneous, while the modification with aromatic substituents was quite heterogeneous. Water solubility of glycosylated β-lactoglobulins did not differ much from that of β-lactoglobulin, on the whole pH range. By contrast, aromatic derivatives exhibited a very low solubility near the isoelectric point. Emulsifying activity and emulsion stability were also checked on the whole pH range. Glucose-modified β-lactoglobulin had improved emulsifying properties whatever the pH, as compared to native protein. Emulsifying activity of aromatic derivatives was improved near the isoelectric point. Maltosylated-, vanillin-modified- and benzaldehyde-modified- β-lactoglobulin exhibited higher emulsion stability in the acidic pH region but lower emulsion stability in the alkaline pH range, as compared to native β-lactoglobulin.

β-lactoglobulin / reductive alkylation / solubility / emulsifying property / glucidic ligand / aromatic ligand

Résumé — Condensation de motifs glucidiques et aromatiques sur la β-lactoglobuline B par alkylation réductrice de ses groupements aminés. Solubilité et propriétés émulsifiantes des dérivés. *Les groupements nucléophiles amine primaire de la β-lactoglobuline ont été modifiés avec des aldéhydes par une réaction d'addition-élimination communément appelée "alkylation réductrice". Les groupements imine produits (bases de Schiff) ont été ensuite réduits, ce qui a amené à la formation de dérivés amine secondaire. Deux sortes de ligands, différant par leur hydrophobicité, ont été greffés sur cette protéine : 81% et 69% des groupements aminés de la β-lactoglobuline ont été modifiés par le glucose et le maltose; 31% et 44% des groupements aminés de cette protéine ont été touchés par les ligands aromatiques (vanilline et benzaldéhyde). L'analyse de ces dérivés par chromatographie haute performance en phase inverse et par électrophorèse a indiqué que les dérivés glycosylés étaient modifiés de façon plutôt homogène, alors que les dérivés aromatiques avaient subi une transformation beaucoup plus hétérogène. La solubilité dans l'eau des dérivés glycosylés de la β-lactoglobuline était peu différente de celle de la protéine native. Par contre, les déri-*

vés aromatiques étaient très peu solubles aux environs du point isoélectrique. L'activité émulsifiante et la stabilité des émulsions ont été déterminées sur toute la gamme de pH. La β-lactoglobuline modifiée par le glucose avait de meilleures propriétés émulsifiantes que la protéine native à tous les pH. L'activité émulsifiante des dérivés aromatiques était améliorée près du point isoélectrique. La β-lactoglobuline modifiée par le maltose, la vanilline ou le benzaldéhyde formait des émulsions plus stables aux pH acides que la β-lactoglobuline native, mais moins stables aux pH alcalins.

β-lactoglobuline / alkylation réductrice / solubilité / propriété émulsifiante / ligand glucidique / ligand aromatique

INTRODUCTION

The functional properties of proteins, *eg* solubility, foaming and emulsifying activities, are affected by the physicochemical and structural characteristics of the protein molecule (Kinsella, 1976). Numerous attempts have been made to improve these properties in order to tailor new protein products convenient for industrial use. Chemical modification of side-chain amino acids has been proposed and tested as one of the ways to make up functional and nutritional qualities of edible proteins (Feeney, 1977; Kinsella and Shetty, 1979; Chobert *et al*, 1987, Feeney, 1987). The chemical grafting of substituents, and particularly glycosylation, has been tried as a potential means to increase the polarity of the proteins and consequently their solubility and heat stability (Marshall and Rabinowitz, 1976). Covalently-attached carbohydrates may induce desirable functional properties to proteins. Therefore, they have been used to modify various proteins and milk proteins in particular. Waniska and Kinsella (1984a, b) have prepared and characterized glycosylated derivatives of β-lactoglobulin by using the cyclic carbonate method according to Doane *et al* (1967). Kitabatake *et al* (1985) have covalently attached gluconic and melibionic acids to amino groups of β-lactoglobulin by using a water-soluble carbodiimide for the activation of carboxyl groups, and studied the effect of this modification on solubility and heat stability of this protein. Lee *et al* (1979) have used the reductive alkylation reaction described by Means and Feeney (1968) for the coupling of reducting oligosaccharides to casein with sodium cyanoborohydride in aqueous solution and checked the nutritive value of these substituted proteins. Recently, Courthaudon *et al* (1989) used the reductive alkylation procedure to bind different carbohydrates on lysyl residues of bovine whole casein and described the effects on solubility and viscosity of this protein.

β-lactoglobulin is a well-characterized protein (McKenzie, 1971; Creamer *et al*, 1983; Sawyer *et al*, 1985; Papiz *et al*, 1986) which exhibits good solubility but relatively weak emulsifying properties. The aim of our study was to apply reductive alkylation on β-lactoglobulin in order to bind either reducing sugars (glucose and maltose) or hydrophobic aldehydes (benzaldehyde and vanillin) and to determine the comparative effects of these ligands on solubility and emulsifying properties of β-lactoglobulin.

MATERIALS AND METHODS

Materials

Organic solvents used for HPLC were from Carlo Erba, Italy. All other reagents were of analytical grade. Buffers and solvents for HPLC

were filtered through Millipore 0.45 μm filters (Millipore Corp, Bedford, MA) and degassed under vacuum before use.

Sodium cyanoborohydride and vanillin were from Merck (Germany), benzaldehyde was obtained from Labosi (France). Glucose, maltose and 2,4,6-trinitrobenzenesulfonic acid (TNBS) were purchased from Sigma Chemical Co (St Louis, MO). Phenyl isothiocyanate (PITC), 6 N hydrochloric acid, and amino acid standards were from Pierce Chemical Co (Oud-Beijerland, Netherlands). Rapesesed oil was from Carrefour (France).

Preparation of β-lactoglobulin

Bovine β-lactoglobulin B was prepared as described by Mailliart and Ribadeau Dumas (1988) from the milk whey of a cow homozygous for this protein. The purity of the preparation was checked by polyacrylamide gel electrophoresis (for details see "electrophoresis" section).

Reductive alkylation and binding of ligands

Carbohydrates (glucose and maltose) and aromatic ligands (vanillin and benzaldehyde) were covalently bound to β-lactoglobulin after reductive alkylation of its lysyl residues according to Lee *et al* (1979) with modifications, as described below.

For the coupling of carbohydrates, β-lactoglobulin (500 mg) was dissolved in 5 ml of 0.2 mol.l^{-1} potassium phosphate buffer, pH 8.0. The β-lactoglobulin solution was mixed with 1.68 mmol of either glucose or maltose, 75 mg of $NaCNBH_3$ and 0.1% (w/v) NaN_3 and kept at 37 °C for 120 h. NaCl was added to give a final concentration of 0.1 mol.l^{-1}.

The weak-apparent solubility of aromatic ligands has constrained us to modify some of the experimental conditions for their coupling. β-lactoglobulin (500 mg) was dissolved in 20 ml of the above mentioned phosphate buffer. Vanillin was previously solubilized in ethanol (alcohol final concentration 20% v/v). Since higher reactivity of benzaldehyde and vanillin was expected, a shorter reaction time of 2 h only was chosen. All the other conditions were as described in the case of carbohydrates. An appropriate control was prepared in the same manner in the absence of the carbonyl reagent.

Modified β-lactoglobulins were separated from the reagents and the uncoupled ligands on a Trisacryl GF 05 column (IBF, France) against 0.1 mol.l^{-1} NaCl, then dialyzed against H_2O and freeze-dried. The obtained proteins were named: glc-β-lg for glucose-modified β-lactoglobulin, mal-β-lg for maltose-modified β-lactoglobulin, van-β-lg for vanillin-modified β-lactoglobulin and bald-β-lg for benzaldehyde-modified β-lactoglobulin.

Measure of the extent of modification

The degree of substitution was determined by quantifying the decrease in free amino groups with TNBS (Adler-Nissen, 1979) and by measuring the loss in lysine by amino acid analysis.

Amino acid analysis

After acid hydrolysis in the presence of 6 N HCl for 24 h at 110 °C in a Pico-Tag Station (Waters), amino acids were derived with PITC according to Bidlingmeyer *et al* (1984) and quantified by reversed phase-HPLC (RP-HPLC) on a Pico-Tag C-18 column (3.9 mm id x 15 cm, Waters). Dried samples were dissolved in 95% 2 mmol.l^{-1} Na_2HPO_4, pH 7.4/5% acetonitrile. The HPLC column was equilibrated in solvent A (94% 0.14 mol.l^{-1} CH_3COONa + 3.59 mmol.l^{-1} triethylamine, pH 6.4/6% acetonitrile). Elution was performed with a gradient from solvent A to solvent B (40% H_2O/60% acetonitrile) with a flow rate of 1 ml/min. The column and solvents were thermostated at 38 °C. The effluent absorbancy was measured at 254 nm. The HPLC equipment consisted of an auto-sampling injector model 231 (Gilson, France), assisted by a chromatography work station Maxima 820, an APC IV computer and a pin writer P6 (NEC Corporation, Boxborough, USA); 2 solvent delivery systems model 510, a temperature control system and a variable wavelength UV monitor model 455 (all from Waters Associates, Milford, MA, USA).

Electrophoresis

Non-dissociating electrophoresis was performed according to Hames (1981) with a stacking gel realized according to Laemmli (1970). The acrylamide concentration was 4.2% in the stacking gel and 10% in the running gel.

HPLC profiles of native and modified β-lactoglobulins

RP-HPLC of native and modified β-lactoglobulins was performed according to Pearce (1983) with some modifications. The samples, 20 µl of a 1 mg/ml protein solution in eluant A (0.15 mol.l^{-1} NaCl adjusted at pH 2.5) were chromatographied at a 1 ml/min flow rate on a Nucleosil C 18 (SFCC) column, with a linear gradient from eluant A to eluant B (acetonitrile) in 40 min. The HPLC equipment was the same as described in the "amino acid analysis" section.

Solubility

Native and modified β-lactoglobulins were dispersed in distilled water (0.1% w/w) by mixing them with a shaker. The pH was adjusted from 1.0 to 10.0 by using concentrated HCl or NaOH in order to limit dilution. After an equilibration period of 10 min at room temperature (23 °C), a portion of each solution was used to determine emulsifying properties. The remainder was centrifuged for 15 min at 4 °C (centrifuge Sigma 201) at 5 000 rpm (2 700 g). The protein content in the supernatant was determined either by the method of Lowry et al (1951) for the glycosylated derivatives or by the method of Smith *et al* (1985) using bicinchoninic acid for the aromatic derivatives; in both cases, native β-lactoglobulin was used as a standard. The solubility was expressed as a percentage of total protein concentration, except for the vanillin and benzaldehyde derivatives, where it was expressed in relative solubility (the reason why is explained under section Results "solubility").

Emulsifying activity

Three milliliters of 0.1% protein solution at desired pH and 1 ml of rapeseed oil (ϕ, volume fraction of the dispersed phase = 0.25) were shaken together and homogenized at 20 000 rpm for 30 s at room temperature (Kinematica GmbH Polytron equipped with a Reco 20 T speed and time control system). Emulsifying activity of the β-lactoglobulins was evaluated by spectroturbidity according to Pearce and Kinsella (1978), with slight modifications. The aliquots were immediately taken from the emulsion and diluted 500-fold into 0.1% (w/v) SDS in 0.1 mol.l^{-1} NaCl, pH 7.0. The tubes were inverted 3 times to obtain homogeneous mixtures, then absorbance at 500 nm was recorded. Identical 1-cm path-length glass cuvettes were used and rinsed with a jet of distilled water and dried between 2 determinations. Absorbancies of duplicate aliquots of each emulsion were measured and the individual values plotted. The emulsifying activity was expressed as its emulsifying activity index (EAI).

EAI = $2T/\phi c$, where T : turbidity = 2.3 A/l (A : absorbance at 500 nm and l = light path = 1 cm = 10^{-2} m), ϕ = oil phase volume = 0.25 and c is the concentration of protein (0.1%) before the emulsion is formed.

Emulsion stability

The stock emulsions prepared above were kept at room temperature for 24 h then heated at 80 °C for 30 min. After cooling to room temperature and stirring, turbidity was again measured as above (EAI, 80 °C). The emulsion stability was calculated by the formula :

$$\Delta\, EAI\,\% = \frac{EAI - EAI_{80\,°C}}{EAI} \times 100$$

The smaller the value of Δ *EAI* %, the better the stability.

RESULTS AND DISCUSSION

Analyses of the modified β-lactoglobulins

The extent of modification of lysyl residues, calculated from the results of the TNBS method and the amino acid analysis is presented in table I. There are 16 amino groups per β-lactoglobulin molecule (Piez *et al,* 1961) which are susceptible to reductive alkylation (15 lysyl residues and N-terminal residue). The glycosylated derivatives exhibit a high number of lysyl residues modified, 12 and 10 for glc-β-lg and mal-β-lg, respectively. On the opposite, van-β-lg and bald-β-lg are less modified (4 and 7 lysyl residues, respectively). This is probably due to the short reaction time chosen for the coupling of aromatic ligands. The results obtained by the TNBS method agree with those obtained by amino acid analysis. The number of NH_2 groups modified obtained by the TNBS method indicates that, in the case of glc-β-lg, mal-β-lg and van-β-lg, the NH_2 terminal group is modified by the ligand. On the contrary, in the case of bald-β-1lg, the same number (7 –NH_2) of modified groups obtained by both methods indicate that its NH_2 terminal group is not modified.

Native and modified β-lactoglobulins were submitted to non-dissociating electrophoresis (fig 1). The control sample, submitted to the alkylation procedure but in the absence of the carbonyl reagent, exhibits the same electrophoresis pattern as native β-lactoglobulin (data not shown). From this result it can be implied that no significant unspecific modification of β-lactoglobulin is occurring in the applied reaction condition. The analysis of the electrophoregrams of modified β-lactoglobulins shows the disappearence of the band corresponding to native β-lactoglobulin. This indicates that all the molecules of β-lactoglobulin were involved in the alkylation procedure, but probably at various degrees. For the glycosylated proteins, the presence of a unique large band seems to be in favour of a rather homogeneous modification. The mal-β-lg had a lower mobility, probably due to the steric

Table I. Extent of modifications of β-lactoglobulins, measured by TNBS method and amino acid analysis.
The experimental values obtained were approximated to the nearest whole number, indicated in parentheses.
Tableau I. *Taux de modification de la β–lactoglobuline, mesuré par la méthode au TNBS et par l'analyse des acides aminés.*

Samples	*TNBS method*		*amino acid analysis*	
	mol NH_2 modified per molecule	*modification degree %*	*lysine modified per molecule*	*modification degree %*
glc-β-lg	13.34(13)	81	11.92(12)	80
mal-β-lg	11.49(11)	69	9.96(10)	67
van-β-lg	5.35(5)	31	3.70(4)	27
bald-β-lg	6.91(7)	44	6.85(7)	47

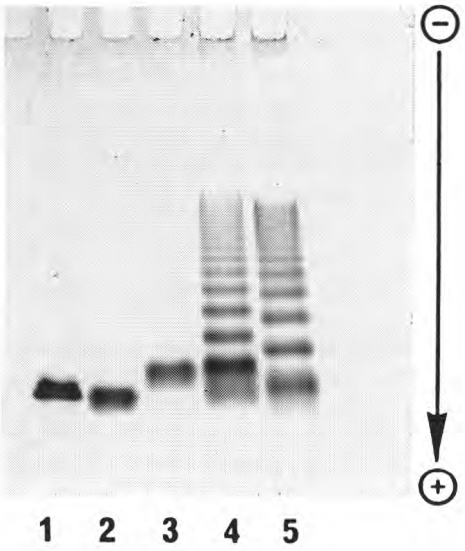

Fig 1. Non-dissociating gel electrophoresis of native and modified β-lactoglobulin. See Methods for details. 1: native β-lactoglobulin; 2: glucose-modified β-lactoglobulin; 3: maltose-modified β-lactoglobulin; 4: vanillin-modified β-lactoglobulin; 5: benzaldehyde-modified β-lactoglobulin.
Electrophorèse non dénaturante de la β-lactoglobuline native et modifiée. Voir les Méthodes pour les détails. 1) β-lactoglobuline native; 2) β-lactoglobuline modifiée par le glucose; 3) β-lactoglobuline modifiée pour le maltose; 4) β-lactoglobuline modifiée par la vanilline; 5) β-lactoglobuline modifiée par le benzaldéhyde.

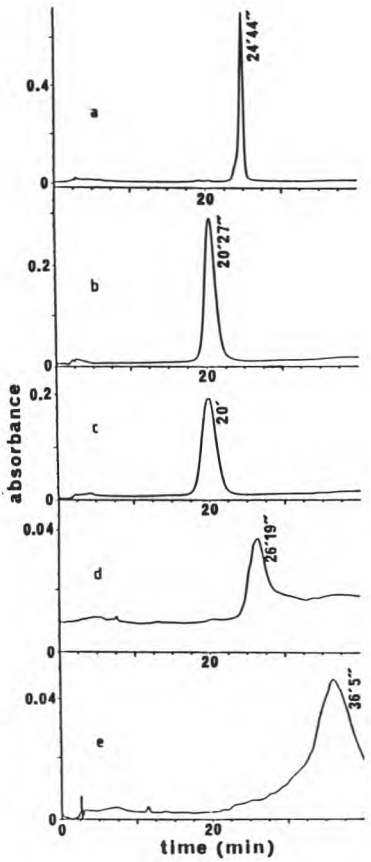

Fig 2. Reversed-phase HPLC profiles of native and modified β-lactoglobulin (gradient from eluant A [0.15 mol.l^{-1} NaCl, pH 2.5] to eluant B [acetonitrile] in 40 min). a: native β-lactoglobulin; β: glucose-modified β-lactoglobulin; c: maltose-modified β-lactoglobulin; d: vanillin-modified β-lactoglobulin; e: benzaldehyde-modified β-lactoglobulin.
Profils en CLHP en phase inverse de la β-lactoglobuline native et modifiée (gradient de l'éluant A [NaCl 0,15 mol.l–1/pH 2,5] vers l'éluant B [acétonitrile] en 40 min]. a) β-lactoglobuline native; b) β-lactoglobuline modifiée par le glucose; c) β-lactoglobuline modifiée par le maltose; d) β-lactoglobuline modifiée par la vanilline; e) β-lactoglobuline modifiée par le benzaldéhyde

hindrance caused by the ligand. Electrophoregrams of van-β-lg and bald-β-lg show several rather slim bands, indicating the presence of heterogeneous classes of modified proteins.

The HPLC analysis confirms these results (fig 2). In the case of glycosylated derivatives, the peaks observed on the chromatograms were slightly larger than that of native β-lactoglobulin. Glc-β-lg and mal-β-

lg were eluted in 20 min 27 s and 20 min, respectively. Their lower retention time as compared to native β-lactoglobulin (24 min 44 s) suggests the decrease in protein hydrophobicity. β-lactoglobulin modified by the aromatic ligands exhibited a broad peak, thus confirming the heterogeneity of the ligand grafting. Van-β-lg and bald-β-lg were eluted in 26 min 19 s and 36 min 5 s, respectively. This extension of retention time is related to the increasing of hydrophobicity of these proteins. In all chromatograms of modified β-lactoglobulins, one should note the disappearance of the peak of native β-lactoglobulin, which is in agreement with the results of electrophoresis.

Solubility

Native β-lactoglobulin displayed a quasi-total solubility on the whole pH range in applied experimental conditions (fig 3). Its solubility decreased a little (minimum 85%) in the isoelectric point region (pI = 5.3 as reported by Tanford and Nozaki, 1959).

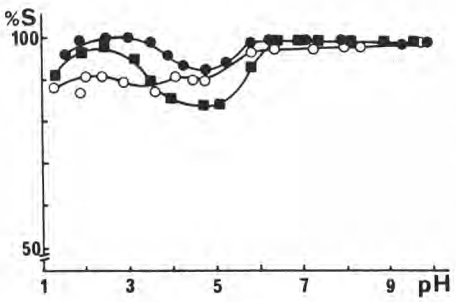

Fig 3. Solubilities of 0.1% w/w suspensions of native β-lactoglobulin (■—■), glucose-modified β-lactoglobulin (O—O) and maltose-modified β-lactoglobulin (●—●) at various pH values; reported as mean of 4 determinations.
Solubilités de suspensions à 0,1% (p/p) de β-lactoglobuline native (■—■), β-lacto-globuline modifiée par le glucose (O—O) et de β-lactoglobuline modifiée par le maltose (●—●) à différentes valeurs de pH; moyenne de 4 déterminations.

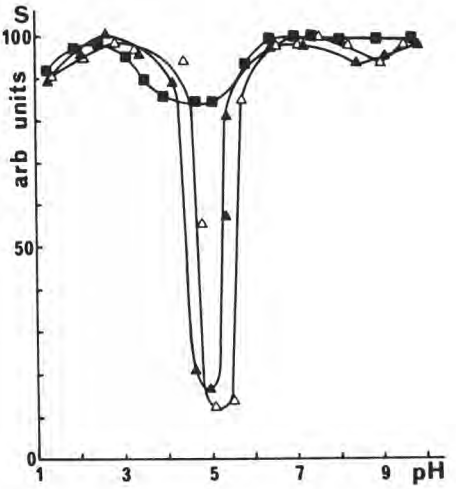

Fig 4. Solubilities of 0.1% suspensions of native β-lactoglobulin (■—■), vanillin-modified β-lactoglobulin (Δ—Δ) and benzaldehyde-modified β-lactoglobulin (▲—▲) at various pH values; reported as mean of 4 determinations.
Solubilités de suspensions à 0,1% (p/p) de β-lactoglobuline native (■—■), β-lactoglo-buline modifiée par la vanilline (Δ—Δ) et β-lactoglobuline modifiée par le benzaldéhyde (▲—▲) à différentes valeurs de pH; moyenne de 4 déterminations.

The patterns of solubility of glc-β-lg and mal-β-lg are shown in fig 3. Both of these modified proteins exhibited an improved solubility in the isoelectric point range, as compared to native β-lactoglobulin. Below pH 3.5, glc-β-lg was slightly less soluble than native β-lactoglobulin. Alkylation does not change the number of positive charges in the protein, and only a small decrease (≈ 0.4-0.6 unit) in the pK'_R of the produced secondary amino groups can be observed (Means and Feeney, 1968). Globally, glycosylation does not drastically change the water solubility properties of β-lactoglobulin.

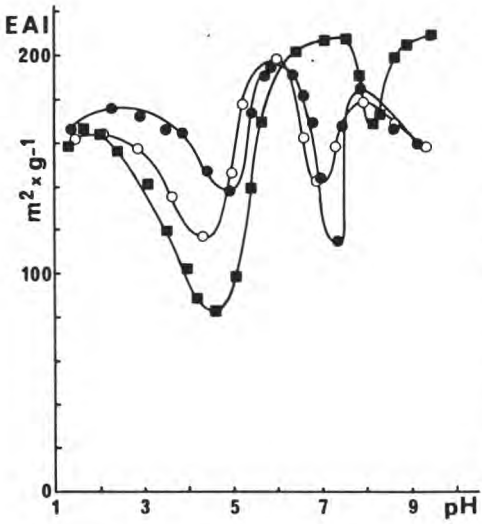

Fig 5. Emulsifying activity index of native and glycosylated β-lactoglobulin as a function of pH. Symbols as in fig 3. Reported as mean of 4 determinations.
Indices d'activité émulsifiante de la β-lactoglobuline native et glycosylée en fonction du pH. Symboles identiques à la fig 3; moyenne de 4 déterminations.

The solubility profiles of van-β-lg and bald-β-lg are shown in fig 4. The solubility values are expressed in arbitrary units because of spectral interferences of aromatic ligand used in the protein assays. Both van-β-lg and bald-β-lg were poorly soluble in the isoelectric point region (13 AU and 16 AU, respectively). In this range of pH, the repulsive forces diminish significantly and consequently the hydrophobic attracting forces predominate between protein molecules. This phenomenon is even more amplified by the coupling of aromatic ligands, increasing the pool of the hydrophobic moieties. For the other pH values, solubilities of van-β-lg and bald-β-lg were identical to that of native β-lactoglobulin.

Emulsifying activity

The emulsifying activity index (EAI) of native β-lactoglobulin was low between pH 3.5 and 5.0 (fig 5). For pH values below 3.5 and above 5.0, the EAI was higher but presented a little drop around pH 8.0. Changes in physicochemical properties of this protein in this pH region have already been described (Tanford and Nozaki, 1959; Timasheff *et al*, 1966; Townend *et al*, 1969). Around pH 7.5, a reversible conformational change occurs which exposes and ionizes 1 abnormal carboxyl group per monomer. This transition is also accompanied by a general molecular expansion as indicated by the decrease in the sedimentation coefficient (Zimmerman *et al*, 1970). These physicochemical changes of the β-lactoglobulin molecule at this pH may explain the decrease of emulsifying activity.

After glycosylation, the emulsifying activity increased in the acidic region (fig 5) as compared to native β-lactoglobulin. For pH values above 6, the drop in EAI value was higher than for native β-lactoglobulin and occurred in a less alkaline pH region (EAI minima at pH 6.8 and 7.2 for glc-β-lg and mal-β-lg, respectively, instead of pH 8.0 for native β-lactoglobulin). This modification is perhaps due to a shift of the pH value, induced by the coupling of sugars to the protein, at which the conformational change occurs.

The binding of aromatic ligands to β-lactoglobulin led to an increase of EAI in the isoelectric point range (pI = 5.3), particularly for bald-β-lg (fig 6). For van-β-lg, the increase of EAI was especially significant at pH 6.5. On the contrary, at this pH value, bald-β-lg exhibited a lower EAI than native β-lactoglobulin.

Whatever the ligand bound to the protein, the increase of EAI at the isoelelectric

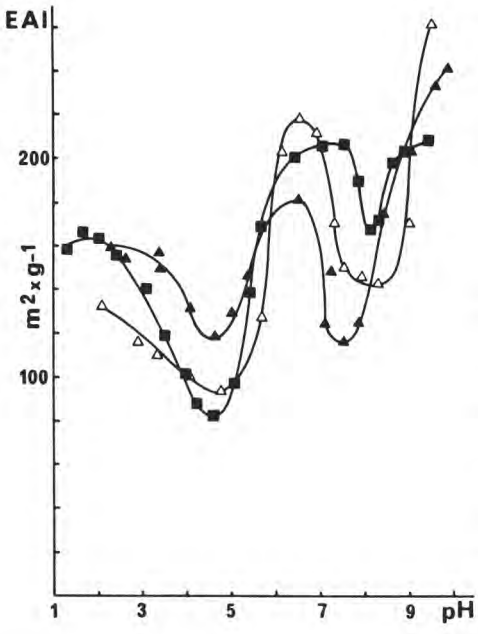

Fig 6. Emulsifying activity index of native β-lactoglobulin and β-lactoglobulin modified with aromatic ligands as a function of pH. Symbols as in fig 4. Reported as mean of 4 determinations.
Indices d'activité émulsifiante de la β-lactoglobuline native et modifiée par les ligands aromatiques en fonction du pH. Symboles identiques à la fig 4; moyenne de 4 déterminations.

point is perhaps due to a partial unfolding of the molecule. Moreover, the fixation of ligands may favour the conformational change occurring at pH 7.5-8.0 and consequently the decrease of EAI in this pH range.

Emulsion stability

After 24 h storage and heating of the emulsion, a pH-dependent decrease of EAI was observed (table II). The emulsions formed with native β-lactoglobulin were unstable below pH 5.0. Above this pH value, their stability increased significantly. All the modified proteins and especially the aromatic derivatives of β-lactoglobulin led to more stable emulsions in the acidic pH range; this was probably due to their lower solubility which stabilized the interface. Above pH 7.0, the opposite was observed: the emulsion stability of modified β-lactoglobulins was lower than that of native β-lactoglobulin, except in the case of glucose binding. For some reason, we still do not understand why glc-β-lg apparently forms the most stable emulsions on the whole pH range, among the β-lactoglobulin derivatives.

Tableau II. Emulsion stability [a] (Δ EAI%) of β-lactoglobulin and β-lactoglobulin derivatives. [a] mean and standard deviation.
Tableau II. *Stabilité des émulsions (Δ EAI%) de la β-lactoglobuline native et de ses dérivés.*

pH	*β-lg*	*glc-β-lg*	*mal-β-lg*	*van-β-lg*	*bald-β-lg*
2	31.1 ± 2.2	25.6 ± 1.5	26.9 ± 2.3	17.3 ± 2.5	9.4 ± 0.4
3	45.7 ± 4.6	27.2 ± 0.4	29.7 ± 1.5	16.7 ± 1.6	4.7 ± 0.5
4	52.6 ± 9.9	23.5 ± 1.7	31.3 ± 4.2	18.0 ± 1.5	13.2 ± 1.5
5	38.3 ± 2.4	33.3 ± 4.2	23.6 ± 1.6	22.5 ± 3.5	10.4 ± 0.1
6	14.7 ± 0.6	4.5 ± 0.4	13.1 ± 0.4	44.2 ± 9.9	11.4 ± 0.5
7	14.4 ± 0.5	18.2 ± 1.3	33.8 ± 2.3	32.4 ± 5.1	27.7 ± 2.5
8	18.4 ± 3.1	18.2 ± 3.5	27.8 ± 3.1	19.3 ± 8.5	32.8 ± 2.6
9	3.4 ± 0.5	17.0 ± 1.3	35.2 ± 4.3	38.2 ± 3.4	29.9 ± 3.0

Since it is now well accepted that hydrophobicity and hydrophobic interactions are important controlling factors for the functional properties of food proteins (Kinsella, 1979; Kato *et al*, 1981; Chobert *et al*, 1987), it was interesting to compare the effect of binding of ligands differing in their hydrophobic characteristics on the lysyl residues of β-lactoglobulin. From the presented results, it appears that the binding of glucose to β-lactoglobulin, by modifying the hydrophile-hydrophobe balance of the molecule, allows both solubility and emulsifying properties of this protein to be improved. To our surprise, the same effect could not be observed for maltosylated β-lactoglobulin. Grafting of vanillin and benzaldehyde to β-lactoglobulin, by increasing the hydrophobicity of the molecule (as shown by RP-HPLC), improved its emulsifying activity. However, these alkylated derivatives have a poor solubility near the isoelectric point.

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