Role of the Polysaccharide Content and Net Charge on the Emulsifying Properties of β-Lactoglobulin−Carboxymethyldextran Conjugates

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β-Lactoglobulin (β-LG)−carboxymethyldextran (CMD) conjugates were prepared by using water-soluble carbodiimide. Three kinds of CMD differing in molecular mass (40, 70, and 162 kDa) were used to investigate the effects of different CMD contents and net charge on the functional changes in β-LG. The emulsifying properties of these β-LG−CMD conjugates were investigated under various conditions by evaluating the stability of oil/water emulsions prepared with oleic acid and the β-LG−CMD conjugates. The emulsifying ability of β-LG was greatly improved by conjugating with CMD in the acidic pH range in the presence of less than 0.5 M NaCl. After heating at 80 °C for 10 min, the emulsifying stability of the β-LG−CMD conjugates was higher than that of β-LG. It is thought that increasing the polysaccharide content and shifting the isoelectric point of β-LG to the acidic side by conjugating with CMD of a high molecular weight would be effective in improving the emulsifying properties of β-LG under unfavorable conditions.

Keywords: *β-Lactoglobulin; neoglycoconjugate; functional improvement; acidic polysaccharide; protein conjugation; emulsification; lipocalin*

INTRODUCTION

Many attempts have been carried out to improve the emulsifying properties of protein by conjugation with nonproteinous materials such as monosaccharides (Waniska and Kinsella, 1988; Bertrand-Harb et al., 1990; Cayot et al., 1991), lipids (Akita and Nakai, 1990), and polysaccharides (Kato et al., 1988, 1992, 1993; Nakamura et al., 1992; Hattori et al., 1994; Nagasawa et al., 1996; Dickinson et al., 1991). Among these conjugates, modification with polysaccharide in particular has remarkably enhanced the emulsifying properties of protein.

Kato et al. (1992, 1993) have reported that protein−polysaccharide conjugates had much higher emulsifying properties than protein alone. We have reported that the emulsifying ability of β-lactoglobulin (β-LG) was improved by conjugation with carboxymethyldextran (CMD) under unfavorable conditions such as in the acidic pH range, in the presence of NaCl, and after heating (Nagasawa et al., 1996). The reason a protein−polysaccharide conjugate show excellent emulsifying properties is not clear. When an oil/water (O/W) emulsion is prepared with a protein−polysaccharide conjugate, assuming that the hydrophobic regions of the protein are adsorbed to the surface of the oil droplets and that the polysaccharide chains are oriented to the aqueous phase, the polysaccharide chains may stabilize the emulsion by covering the oil droplets to inhibit their coalescence (Kato et al., 1993; Nakamura et al., 1993) or hydration with the surrounding water. Furthermore, in the case of a protein-charged polysaccharide conjugate, the addition of an electrical charge is also thought to contribute to improved emulsifying properties of protein (Nagasawa et al., 1996).

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However, there have been very few reports describing the effects of varying the polysaccharide content on improving the emulsifying properties of protein.

Nakamura et al. (1993) have investigated the emulsifying properties of polymannosyl lysozyme and oligomannosyl lysozyme constructed by genetic modification and found that polymannosylation was more effective than oligomannosylation for improving the emulsifying properties of lysozyme.

Moreover, from a comparison of the emulsifying properties of ovalbumin−dextran conjugates prepared with dextran (20, 75, and 250 kDa) at neutral pH, ovalbumin−dextran (75 kDa) had the best emulsifying property, while ovalbumin−dextran (20 kDa), with poor covalent bonding between protein and dextran, had the lowest emulsifying property (Kato et al., 1989). These results mean that the most suitable molecular weight for the modifier or its content brought about the greatest improvement in emulsifying property.

In the case of our previous paper (Nagasawa et al., 1996), the β-LG−CMD conjugates with higher CMD content showed higher emulsifying properties than those with low CMD content under almost all unfavorable conditions. This result suggests that increasing the CMD content would be more effective for improving the emulsifying properties of β-LG. Accordingly, we planned to prepare β-LG−CMD conjugates containing more CMD than the previous conjugates. Since the binding sites for β-LG to CMD are limited, we attempted to conjugate β-LG with CMD of higher molecular masses (about 40, 70, and 162 kDa) than the previously used material (about 10 kDa).

We describe the effects of different CMD contents and net charge on improving the emulsifying properties of β-LG.

MATERIALS AND METHODS

Materials. Dextran T40 and T70 (40 and 70 kDa) were purchased from Pharmacia LKB (Uppsala, Sweden), and dextran (162 kDa) was purchased from Sigma Chemical Co.

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(St. Louis, MO). Water-soluble carbodiimide, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDC), was purchased from Dojindo (Kumamoto, Japan).

Carboxymethylation of Dextran. Three kinds of carboxymethyldextran (CMD40, CMD70, and CMD162) differing in molecular mass (about 40, 70, and 162 kDa) were prepared by carboxymethylation with monochloroacetic acid according to the method described previously (Hattori et al., 1994; Nagasawa et al., 1996). The degree of modification (DM) was determined by hydrochloride−methanol titration (Smith, 1967). DM values for CMD40, CMD70, and CMD162 were about 55, 47, and 128 carboxyl group residues per dextran molecule, corresponding to 23, 11, and 13 residues/100 glucose residues), respectively.

Preparation of β-LG. Crude β-LG (genotype AA) was prepared according to the method of Armstrong et al. (1967) and then purified by ion-exchange chromatography, using a DEAE-Sephacel column (2.5 i.d. × 50 cm; Pharmacia LKB, Uppsala, Sweden) as described previously (Hattori et al., 1994; Nagasawa et al., 1996). The purity of β-LG was confirmed by polyacrylamide gel electrophoresis (PAGE) according to the method of Davis (1964).

Preparation of β-LG−CMD Conjugates. The β-LG−CMD conjugates were prepared as described previously (Hattori et al., 1994; Nagasawa et al., 1996). β-LG (240 mg) and 1050 mg of CMD were dissolved in 30 mL of distilled water, and the solution was adjusted to pH 4.75 with 1 N HCl. An EDC solution (413 mg/mL of distilled water) was gradually added over 30 min while the pH was maintained at 4.75. The reaction mixture was then incubated at room temperature for 3 h. The reaction was stopped by adding 2 mL of a 2 M acetate buffer (pH 5.5), and the solution was dialyzed against distilled water. The crude β-LG−CMD conjugates (Conj. 40, Conj. 70, and Conj. 162) were recovered by lyophilization. Purification of the β-LG−CMD conjugates was carried out as follows. Free CMD in crude Conj. 40 and Conj. 70 was removed by salting out with ammonium sulfate (Hattori et al., 1994). Free CMD in Conj. 162 was removed by hydrophobic chromatography, using a butyl-Toyopearl 650M (Tosoh, 2.5 i.d. × 40 cm) since it could not be salted out. The conjugate (10 mg/mL) was applied to the column and eluted stepwise with 2−0 M ammonium sulfate in a 0.067 M phosphate buffer at pH 7.0.

After salting out or hydrophobic chromatography, free β-LG and polymerized β-LG were removed by ion-exchange chromatography, using a DEAE-Sepharose Fast Flow column (2.5 i.d. × 40 cm) as described previously (Hattori et al., 1994).

Isoelectric Focusing. The isoelectric point of each β-LG−CMD conjugate was determined by isoelectric focusing with the Pharmacia Phast System (Kramlova et al., 1986). The electrophoresed protein bands were detected by staining with Coomassie Brilliant Blue.

CD Spectra. The CD spectrum for each β-LG−CMD conjugate was measured with a Jasco J-720 spectropolarimeter (Tokyo, Japan), using a cell with a 1.0 mm path length. Samples were dissolved in phosphate-buffered saline (PBS; a 0.11 M phosphate buffer at pH 7.1 containing 0.04 M NaCl and 0.02% NaN_3) at a protein concentration of 0.02% (w/v).

Enzyme-Linked Immunosorbent Assay (ELISA). Competitive ELISA was performed as described previously (Hattori et al., 1994; Nagasawa et al., 1996), using two monoclonal antibodies (mAb 21B3 and mAb 61B4). The equilibrium constant for interaction between the monoclonal antibody and the tested β-LG sample was calculated according to the method of Hogg et al. (1987), based on the competitive and noncompetitive ELISA results.

Differential Scanning Calorimetry (DSC). Each sample was dissolved in PBS (pH 7.0) at a protein concentration of 5% (w/v), and 50 μL of the sample solution was sealed in a silver DSC cell. Distilled water was used as a reference. DSC was performed by using a Seiko SSC-5020 DSC 100 instrument (Tokyo, Japan) at a heating rate of 2 K/min.

Evaluation of the Emulsifying Ability of the β-LG−CMD Conjugates. The emulsifying ability of each β-LG−CMD conjugate was evaluated according to the method described previously (Hattori et al., 1994; Nagasawa et al., 1996). A β-LG−CMD conjugate solution (2 mL, 0.1% in protein concentration) and 0.5 mL of oleic acid were mixed and homogenized by Polytron PCU-2 equipment (Kinematica, Switzerland) at 24 000 rpm for 1 min at 25 °C to prepare an O/W emulsion. The emulsifying ability was evaluated by the absorbance at 500 nm of the emulsion 100- or 200-fold diluted with a 0.1% sodium dodecyl sulfate (SDS) solution.

The effect of ionic strength on the emulsifying ability was investigated by measuring the absorbance at 500 nm 30 min after emulsification, using a 0.067 M phosphate buffer (pH 7.0) containing different concentrations of NaCl (0−0.5 M) as the aqueous phase.

The effect of pH on the emulsifying ability was investigated by measuring the absorbance at 500 nm after 30 min, using a 0.05 M citric acid−disodium phosphate buffer as the aqueous phase at different pH values in the range of 3−8.

The effect of heating on the emulsifying ability was investigated by measuring the absorbance at 500 nm of a β-LG−CMD conjugate solution (0.067 M phosphate buffer at pH 7.0) that had been preheated at 80 °C for 10 min before emulsification.

The emulsifying activity of the β-LG−CMD conjugates was evaluated by spectroturbidity according to the method of Pearce and Kinsella (1978). The emulsifying activity is expressed as emulsifying activity index (EAI), calculated by the formula $EAI = 2T/c$, where T (turbidity) $= 2.3\,A/l$ (A being the absorbance at 500 nm and l (light path) $= 10^{-2}$ m), oil phase volume $= 0.2$, and c is the concentration of protein (10^3 g/m^3).

Table 1. Chemical Properties of β-LG−CMD Conjugates

	conjugate			
	40	70	162	β-LG
β-LG:CMD (mol)	2.2:1	2.5:1	4.4:1	
β-LG:CMD (wt)	1:1	1:1.5	1:2	
isoelectric point (pI)	4.55	4.45	4.2	5.2
denaturation terp (T_p,[a] °C)	80.6	83.1	95.5	72.6

[a] T_p, peak temperature of the thermal denaturation evaluated by DSC.

RESULTS

Chemical Properties of β-LG−CMD Conjugates. The conjugation of β-LG and CMD was confirmed by the coincidence of the protein and saccharide stained bands by SDS−PAGE (data not shown) and by the shift of the isoelectric point (pI) of β-LG to the acidic side (Table 1). The chemical properties of the β-LG−CMD conjugates are shown in Table 1. The weight ratios (β-LG:CMD) determined by absorbance at 280 nm and by phenol−sulfuric acid method were about 1:1, 1:1.5, and 1:2, respectively, for Conj. 40, Conj. 70, and Conj. 162. The conjugates containing greater CMD content were obtained by reaction with CMD of higher molecular weight. The pI values for Conj. 40, Conj. 70, and Conj. 162 were 4.54, 4.45, and 4.20, respectively, the degree of the shift of pI increasing with increasing CMD content of the conjugates. The respective thermal denaturation temperatures (T_p) determined by DSC were 80.6, 83.1, and 95.5 °C for Conj. 40, Conj. 70, and Conj. 162, respectively, indicating the enhanced thermal stability of β-LG by its conjugation with CMD.

Conformational Changes of β-LG by Conjugation with CMD. The CD spectra of the β-LG−CMD conjugates are shown in Figure 1. Native β-LG had a negative maximum at 216 nm, since β-LG was rich in β-sheet. In the case of the β-LG−CMD conjugates, the broad negative maximum and the blue shift show that the β-sheet regions had been changed by conjugation with CMD. Such a change was increased by conjugation with CMD of higher molecular weight.

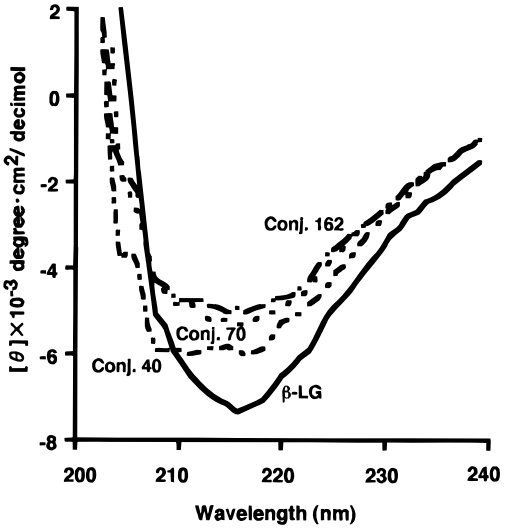

Figure 1. CD spectra of the β-LG−CMD conjugates: (—) β-LG; (— · —) Conj. 40; (- - -) Conj. 70; (— — —) Conj. 162.

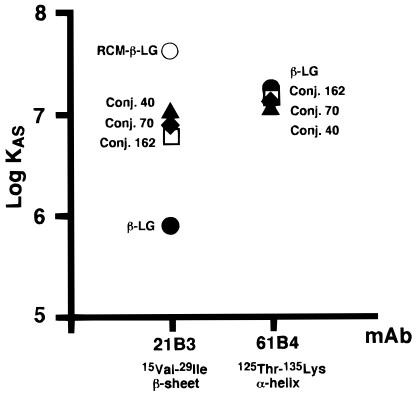

Figure 2. Binding constants of monoclonal antibodies to the β-LG−CMD conjugates: (●) β-LG; (▲) Conj. 40; (◆) Conj. 70; (□) Conj. 162; (○) RCM-β-LG.

The conformational difference between β-LG−CMD conjugates and native β-LG was evaluated by using anti-β-LG mAbs as probes. We have previously shown that mAb can detect the subtle conformational change in local areas within a protein molecule during unfolding and refolding by determining the affinity change (Kaminogawa et al., 1989; Hattori et al., 1993). The epitope regions for mAbs 21B3 and 61B4 are 15Val−29Ile (β-sheet region) and 125Thr−135Lys (α-helix region), respectively. MAb 61B4 reacts preferentially to native β-LG, while mAb 21B3 reacts more strongly to RCM-β-LG (the denatured form of β-LG).

The results of competitive ELISA using two anti-β-LG mAbs (mAbs 21B3 and 61B4) indicate that the binding constants (K_{AS}) of mAb 21B3 to the β-LG−CMD conjugates were larger than that to native β-LG and that K_{AS} values of mAb 61B4 to the β-LG−CMD conjugates were similar to that to native β-LG (Figure 2). Consequently, the conformation around ^{15}Val−^{29}-Ile (the β-sheet region), which is buried in the native state (Papiz et al., 1986), is thought to have been exposed, while ^{125}Thr−^{135}Lys (the α-helix region) almost completely maintained its native form through conjugation with CMD.

Emulsifying Ability of β-LG−CMD Conjugates. The absorbance of the emulsions formed with the β-LG−CMD conjugates at pH 7.0 is shown in Figure 3. Each emulsion with a β-LG−CMD conjugate showed much higher absorbance than that with β-LG alone. Conj. 40, Conj. 70, and Conj. 162 showed high emulsifying stability in comparison with the conjugates with CMD of 10 kDa (Conj. 10A and Conj. 10B) described in the previous paper (Nagasawa et al., 1996). In particular, Conj. 162 displayed a remarkably high emulsifying property.

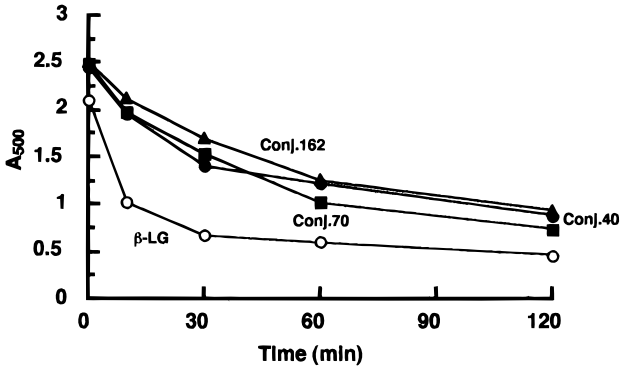

Figure 3. Stability of the emulsions prepared with β-LG−CMD conjugates at pH 7.0: (○) β-LG; (●) Conj. 40; (■) Conj. 70; (▲) Conj. 162. An O/W emulsion [oleic acid/0.1% conjugate solution (based on β-LG), 20/80 (v/v)] was held at 25 °C. The emulsion was 100-fold diluted with a 0.1% SDS solution, and the absorbance at 500 nm was measured.

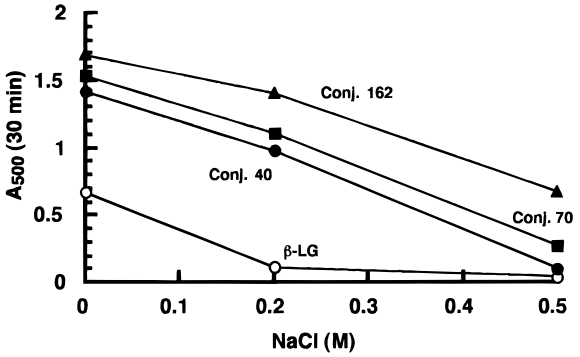

Figure 4. Effect of salt concentration on the emulsifying ability of the β-LG−CMD conjugates: (○) β-LG; (●) Conj. 40; (■) Conj. 70; (▲) Conj. 162. An O/W emulsion [oleic acid/0.1% conjugate solution (based on β-LG), 20/80 (v/v)] was held at 25 °C. A 0.067 M phosphate buffer at pH 7.0 containing 0−0.5 M NaCl was employed as the aqueous phase. The emulsion was 100-fold diluted with a 0.1% SDS solution, and the emulsifying ability was evaluated by the turbidity of the diluted emulsion 30 min after emulsification.

Effect of Salt on the Emulsifying Ability of β-LG−CMD Conjugates. The effect of NaCl on the emulsifying ability of the β-LG−CMD conjugates was evaluated by measuring the absorbance 30 min after emulsification (Figure 4). To avoid an error in the absorbance at 500 nm caused by a slight time lag when emulsion was taken (50 μL), we selected the absorbance 30 min after emulsification, because the decrease in absorbance is relatively gentle. β-LG almost lost its emulsifying ability with 0.2 M NaCl. However, the β-LG−CMD conjugates maintained their emulsifying ability at a high level with 0.2 M NaCl. In particular, Conj. 162 maintained high emulsifying ability with 0.5 M NaCl.

Effect of pH on the Emulsifying Ability of β-LG−CMD Conjugates. The effect of pH on the emulsifying ability of the β-LG−CMD conjugates was also evaluated by measuring the absorbance 30 min after emulsification (Figure 5). β-LG showed good emulsifying ability in the neutral pH range but completely lost its emulsifying ability at pH values of 4.0 and 5.0. On the other hand, the β-LG−CMD conjugates showed much higher emulsifying ability even at these pH values, especially

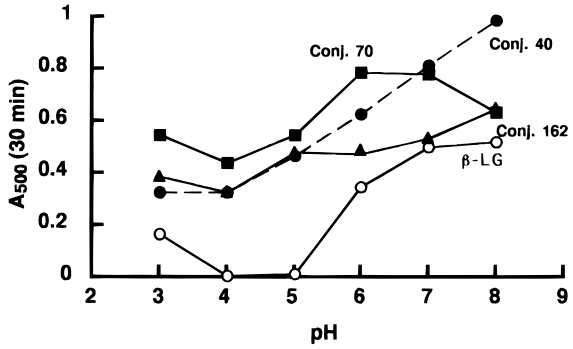

Figure 5. Effect of pH value on the emulsifying ability of the β-LG−CMD conjugates: (○) β-LG; (●) Conj. 40; (■) Conj. 70; (▲) Conj. 162. An O/W emulsion [oleic acid/0.1% conjugate solution (based on β-LG), 20/80 (v/v)] was held at 25 °C. A 0.05 M citric acid−disodium hydrogen phosphate buffer at pH 3.0−8.0 was employed as the aqueous phase. The emulsion was 200-fold diluted with a 0.1% SDS solution, and the emulsifying ability was evaluated by the turbidity of the diluted emulsion 30 min after emulsification.

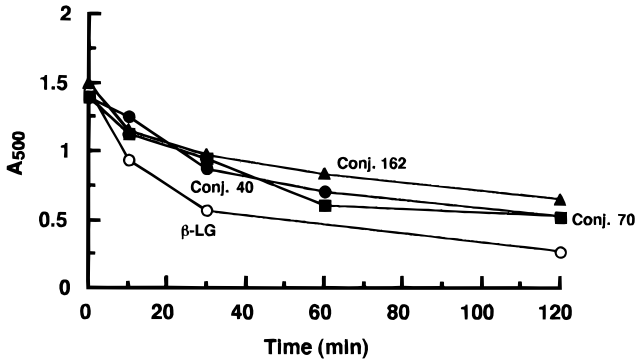

Figure 6. Effect of heating on the emulsifying ability of the β-LG−CMD conjugates: (○) β-LG; (●) Conj. 40; (■) Conj. 70; (▲) Conj. 162. A 0.067 M phosphate buffer at pH 7.0 was employed as the aqueous phase. The emulsion was prepared after heating at 80 °C for 10 min and was then 200-fold diluted with a 0.1% SDS solution.

at pH 5.0, at which they maintained an emulsifying ability similar to that of β-LG at pH 7.0. In comparison with the conjugates with CMD of 10 kDa (Conj. 10A and Conj. 10B), described in the previous report (Nagasawa et al., 1996) at pH 3.0, Conj. 40, Conj. 70, and Conj. 162 maintained high emulsifying ability.

Effect of Heating on the Emulsifying Stability of β-LG−CMD Conjugates. The effect of heating on the emulsifying properties of the β-LG−CMD conjugates was investigated by using samples heated at 80 °C for 10 min (Figure 6), since the emulsifying ability of β-LG increased with increasing temperature up to 60 °C but decreased a bit at 80 °C (Nagasawa et al., 1996). The emulsifying stability of the β-LG−CMD conjugates was higher than that of β-LG. This high emulsifying ability of the β-LG−CMD conjugates after heating at 80 °C could be attributable to amphipathicity being maintained due to the improved thermal stability of β-LG by its conjugation with CMD.

DISCUSSION

It was recognized that the emulsifying ability of β-LG under unfavorable conditions, especially in the acidic pH region, in the presence of salt, and after heating, could be markedly improved by conjugation with CMD of different molecular weights.

In this paper, to analyze the emulsifying properties of the β-LG−CMD conjugates in detail, the results of

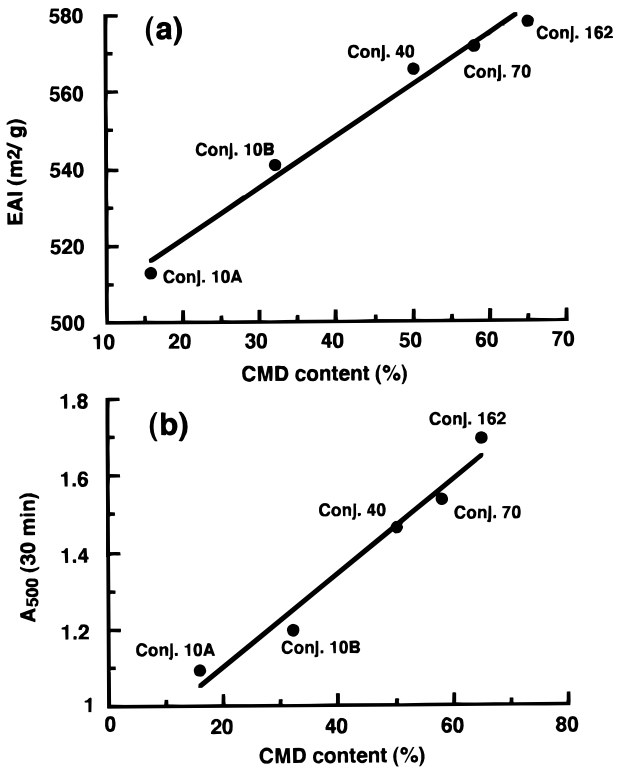

Figure 7. Relationships between the CMD content and emulsifying properties at pH 7.0, and p*I* values of the β-LG−CMD conjugates. The emulsifying properties of the β-LG−CMD conjugates were evaluated by EAI as the emulsifying activity (a) and by the absorbance 30 min after emulsification as the emulsifying stability (b). Conj. 10A and Conj. 10B are the β-LG−CMD conjugates prepared with CMD of 10 kDa, as described in our previous paper (Nagasawa et al., 1996).

the β-LG−CMD conjugates prepared with CMD of 10 kDa (Conj. 10A and Conj. 10B) (Nagasawa et al., 1996) were compared with those of other conjugates. The molar ratios (β-LG:CMD) of Conj. 10A and Conj. 10B were 7:2 and 1:1, respectively. The p*I* of Conj. 10B was 4.8, while the p*I* of Conj. 10A could not be determined.

The addition of hydrophilicity, the negative charge, and the conformational change of β-LG by conjugation with CMD are thought to be the reasons the β-LG−CMD conjugates showed much higher emulsifying properties than β-LG did under unfavorable conditions.

The relationship between the emulsifying properties at pH 7.0 and CMD contents of the β-LG−CMD conjugates is shown in Figure 7. The CMD content determined by absorbance at 280 nm and by phenol−sulfuric acid method were 15.6%, 32%, 50%, 58%, and 65% for Conj. 10A, Conj. 10B, Conj. 40, Conj. 70, and Conj. 162, respectively. The emulsifying properties of the conjugates were evaluated by the EAI value (Figure 7a) and the absorbance 30 min after emulsification (Figure 7b), which could characterize the emulsifying activity and the emulsifying stability. From these plots, it was found that the CMD content had a high positive correlation with both indices ($r = 0.991$, Figure 7a; $r = 0.994$, Figure 7b). Moreover, Conj. 10A and Conj. 10B also approximated to these straight lines. Accordingly, it was strongly suggested that the addition of the physical properties of CMD (hydrophilicity, negative charge, viscosity, and so on) greatly contributed to the improved emulsifying properties of β-LG. In particular, it is thought that the addition of hydrophilicity was one of the most important factors to enhance the emulsifying ability of β-LG. When an emulsion was prepared with

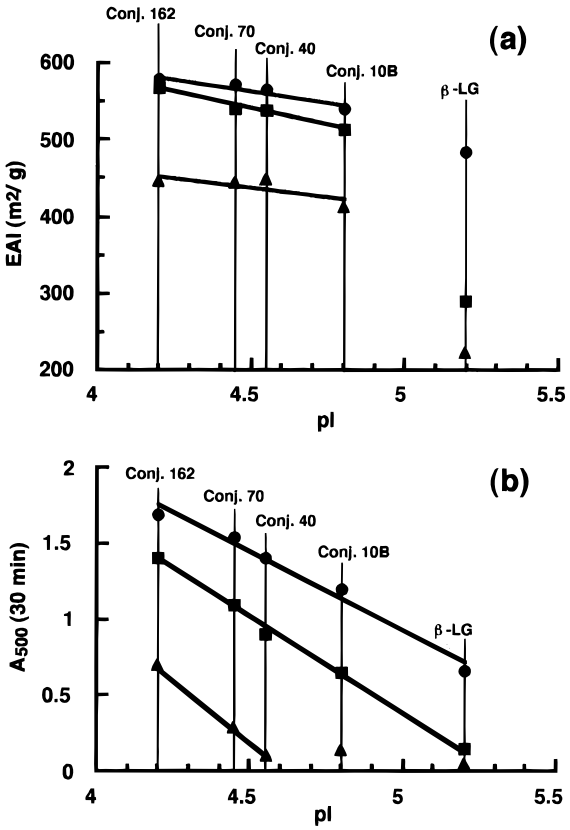

Figure 8. Relationship between the emulsifying properties in the presence of NaCl at pH 7.0 and p*I* of the β-LG−CMD conjugates: (●) 0 M NaCl; (■) 0.2 M NaCl; (▲) 0.5 M NaCl. The emulsifying properties of the β-LG−CMD conjugates were evaluated by EAI as the emulsifying activity (a) and by the absorbance 30 min after emulsification as the emulsifying stability (b).

β-LG−CMD conjugate, the hydrophobic regions of the β-LG molecule may be adsorbed to the surface of oil droplets, while the CMD chains may be oriented to the aqueous phase and stabilize the oil droplets by hydration with the surrounding water. This assumption is suggested by the views of the exterior surface of human milk fat globules, using an electromicroscope (Buchheim, 1986). According to the photomicrograph, polysaccharide chains of glycoprotein adsorbed to the surface of the milk fat globules appear to orient to the aqueous phase.

Although the addition of a negative charge from CMD was related to the CMD content, the three kinds of carboxymethyldextran (CMD40, CMD70, and CMD162) differed in the number of carboxyl group residues per glucose residue, judging from the result of the determination of DM. The shift in p*I* to the acidic side by conjugation was not proportional to the CMD content in the β-LG−CMD conjugate, theoretically. Accordingly, it is thought that p*I* values of the β-LG−CMD conjugates are desirable for index to discuss the effect of charge.

Figure 8 shows the relationship between the p*I* value for each emulsifier and the emulsifying properties in the presence of NaCl at pH 7.0 as monitored by EAI (a) and the absorbance 30 min after emulsification (b). In relation to EAI, it was found that p*I* had high correlation with the emulsifying activity among the β-LG−CMD conjugates [$r = 0.944$ (0 M NaCl), $r = 0.981$ (0.2 M NaCl), and $r = 0.745$ (0.5 M NaCl)]. However, some plots for β-LG were off the straight lines in the presence of 0.2 and 0.5 M NaCl, since the emulsion prepared with β-LG broke down quickly (Figure 8a). On the other hand, it was found that p*I* was highly correlated with the emulsion stability (Figure 8b): $r = 0.990$ (0 M NaCl), $r = 0.998$ (0.2 M NaCl), and $r = 0.999$ (0.5 M NaCl). Since the emulsion was prepared at pH 7.0, the net charge of the emulsifier would increase with decreasing p*I* value. Accordingly, it is suggested that the net charge of the emulsifier played an important role. It is thought that the increase in net charge of protein by conjugation with CMD inhibited the flocculation of oil droplets by the electrostatic repulsion. These results strongly suggest that an increase in the net charge of the emulsifier would be effective for displaying higher emulsifying properties.

The relationship between the difference in molar ellipticity at 216 nm for each conjugate and β-LG and the EAI is plotted to examine the effect of the conformational change. The difference in molar ellipticity at 216 nm is thought to reflect the degree of the conformational change in the β-sheet region. However, high correlation between the conformational change in the β-sheet region and the emulsifying properties was not found (data not shown).

It is thought that the addition of the physical properties of CMD polysaccharide contributed to the improved emulsifying properties of β-LG, rather than the effect of the conformational change. Therefore, increases in the CMD content and net charge are thought to have been the major factors in the improved emulsifying properties of β-LG by its conjugation with CMD.

In this study, three kinds of conjugates (Conj. 40, Conj. 70, and Conj. 162) were prepared to investigate the effect of physical properties of CMD on the improvement of emulsifying properties of β-LG. These β-LG−CMD conjugates showed higher emulsifying properties than did β-LG at acidic pH values and in the presence of NaCl. Since the CMD content and net charge showed high correlation with the emulsifying properties of the β-LG−CMD conjugates, it is suggested that the increase in polysaccharide content and net charge by conjugation with CMD of high molecular weight would be very effective to improve the emulsifying properties of β-LG under unfavorable conditions.

ABBREVIATIONS USED

β-LG, β-lactoglobulin; CMD, carboxymethyldextran; EDC, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide; DM, degree of modification; p*I*, isoelectric point; PBS, phosphate-buffered saline; RCM-β-LG, reduced carboxymethyl β-lactoglobulin; DSC, differential scanning calorimetry.

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