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Binding of Cationic Lipids to Milk β-Lactoglobulin

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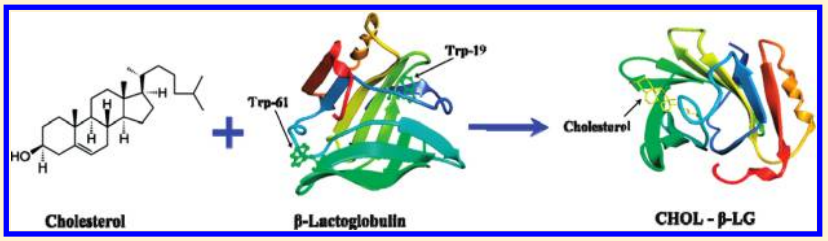
ABSTRACT:



We determined the bindings of several lipids such as cholesterol (CHOL), 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), dioctadecyldimethyl-ammoniumbromide (DDAB), and dioleoylphosphatidylethanolamine (DOPE) to β-lactoglobulin (β-LG) at physiological conditions. FTIR, CD, and fluorescence spectroscopic methods as well as molecular modeling were used to determine the binding of lipid−protein complexes. Structural analysis showed that lipids bind β-LG via both hydrophilic and hydrophobic interactions with overall binding constants of $K_{CHOL-\beta\text{-LG}}$ = 6.0 (±0.6) × 10^3 M^{-1}, $K_{DOPE-\beta\text{-LG}}$ = 6.5 (±0.7) × 10^3 M^{-1}, $K_{DDAB-\beta\text{-LG}}$ = 1.6 (±0.3) × 10^4 M^{-1}, and $K_{DOTAP-\beta\text{-LG}}$ = 2.2 (±0.67) × 10^4 M^{-1}. The number of lipid bound per protein molecule (n) was 0.8 (CHOL), 0.7 (DOPE), 1.0 (DDAB), and 1.3 (DOTAP). Molecular modeling showed the participation of several amino acid residues in lipid−protein complexation with the order of binding DOTAP > DDAB > DOPE > CHOL. Alterations of the protein conformation were observed in the presence of lipids with a minor decrease in β-sheet and an increase in turn structure.

INTRODUCTION

There are several high affinity binding sites for fatty acids, lipids, and aromatic compounds on β-lactoglobulin.[1−4] β-Lactoglobulin (Scheme 1) is the most abundant protein of milk whey (1 g/L) and of a major interest in the food industry because of its nutritional and functional properties. The structure of this protein is well-known.[5] At neutral pH, β-LG exists as a mixture of monomers and dimers of which the equilibrium ratio depends on the association constant of the dimer and on the protein concentration. Each monomer consists of 162 amino acid residues and has a molecular mass of 18 kDa.[6,7] As a member of the lipocalycin family, β-LG is a small globular protein folded into a calyx formed by eight antiparallel β-strands and an α-helix located at the outer surface of the β-barrel.[6] One of the remarkable properties of β-LG is its ability to bind in vitro several hydrophobic compounds such as retinoids, fatty acids, vitamin D, and lipids.[8] Several studies suggest that there are at least two hydrophobic binding sites in the β-LG monomer, one in the internal cavity and the other on the outer surface located between the β-barrel and the α-helix.[8] The ligand binding sites on the β-LG were analyzed,[3,9] and the effect of ligand complexation on protein structure has been reported.[2] However, there has been no report on the interaction of cationic lipids with β-lactoglobulin. Therefore, determination of the lipid binding sites on β-LG and the structural characterization lipid−protein complexes as well as the effect of lipid complexation on protein stability and secondary structure are of major chemical and biochemical importance.

Fluorescence quenching is considered as a technique for measuring binding affinities.[10] Fluorescence quenching is the decrease of the quantum yield of fluorescence from a fluorophore induced by a variety of molecular interactions with quencher molecule.[10,11] Therefore, it is possible to use quenching of the intrinsic tryptophan fluorescence of Trp-61 and Trp-19 in β-LG[1] as a tool to study the interaction of lipids with β-LG in an attempt to characterize the nature of lipid−protein complexation.

We now present spectroscopic analysis and docking studies of the interaction of β-LG with several lipids cholesterol, DDAB, DOPE, and DOTAP (Scheme 1) in aqueous solution at physiological conditions, using constant protein concentration and various lipid contents. Structural analysis regarding lipid binding sites and the effects of lipid−protein complexation on the β-LG stability and conformation is reported here.

MATERIALS AND METHODS

Materials. β-Lactoglobulin (A variant, purity >90%) was purchased from Sigma-Aldrich Chemical Co. (St-Louis, MO) and used as supplied. Cholestrol, DOPE, DDAB, and DOTAP were from Avanti Polar Lipid Inc. and were used as supplied. Other chemicals were of reagent grade and used without further purification.

Preparation of Stock Solutions. β-Lactoglobulin was dissolved in aqueous solution (8 mg/mL to obtain 0.5 mM protein content) containing 10 mM Tris-HCl buffer (pH 7.4). Lipid solution (1 mM) was prepared in ethanol/water 50/50% and

Received: January 3, 2011
Revised: April 18, 2011
Published: May 04, 2011

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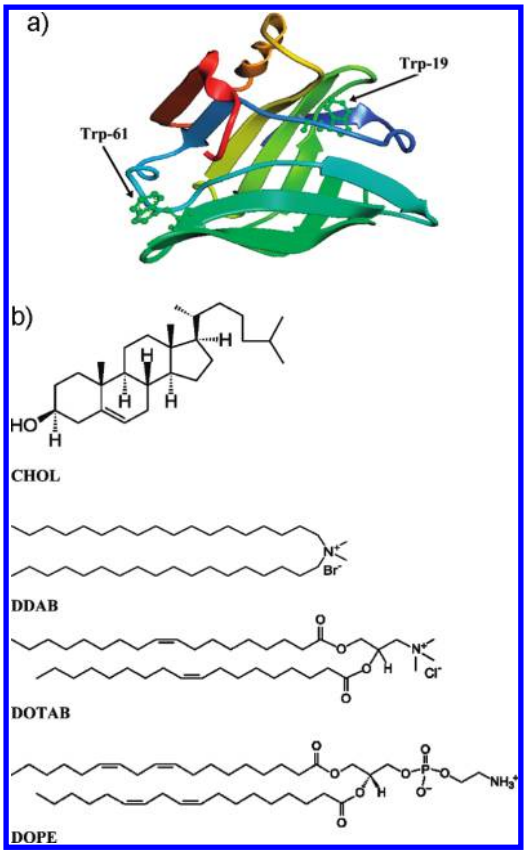
Scheme 1. (a) 3D Structure of β-Lactoglobulin; and (b) Chemical Structures of Lipids



diluted in Tris-HCl to various concentrations in Tris-HCl with the final ethanol concentration of 25%. The presence of 25% ethanol induces no major protein structural changes, according to a recent publication.[12]

The protein concentration was determined spectrophotometrically using the extinction coefficients of 17 600 M^{-1} cm^{-1} (MW = 18 kD) at 280 nm.[1,13]

FTIR Spectroscopic Measurements. Infrared spectra were recorded on a FTIR spectrometer (Impact 420 model, Digilab), equipped with deuterated triglycine sulfate (DTGS) detector and KBr beam splitter, using AgBr windows. Solution of lipid was added dropwise to the protein solution with constant stirring to ensure the formation of homogeneous solution and to reach the final lipid concentrations of 0.125, 0.25, and 0.5 mM with a final protein concentration of 0.25 mM. Spectra were collected after 2 h incubation of β-lactoglobulin with lipid solution at room temperature, using hydrated films. Interferograms were accumulated over the spectral range 4000−600 cm^{-1} with a nominal resolution of 2 cm^{-1} and 100 scans. The difference spectra [(protein solution + lipid solution) − (protein solution)] were generated using water combination mode around 2300 cm^{-1} as standard.[14] When producing difference spectra, this band was adjusted to the baseline level, to normalize difference spectra.

Analysis of Protein Conformation. Analysis of the secondary structure of β-lactoglobulin and its lipid complexes was carried out on the basis of the procedure already reported.[15] The protein secondary structure is determined from the shape of the amide I band, located at 1660−1650 cm^{-1}. The FTIR spectra were smoothed, and

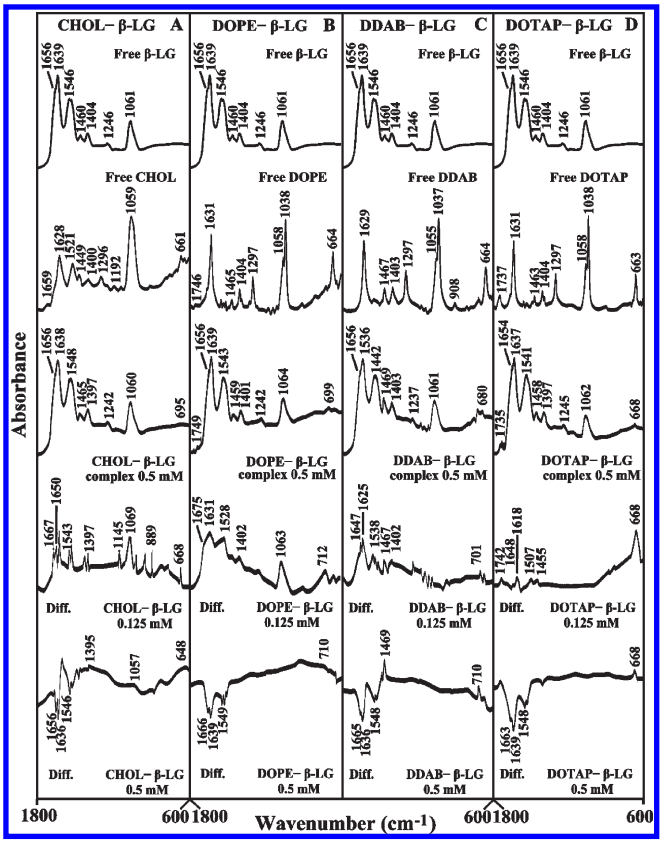


Figure 1. FTIR spectra in the region of 1800−600 cm^{-1} of hydrated films (pH 7.4) for free β-lactoglobulin (0.25 mM), free CHOL (A) (0.5 mM), free DOPE (B) (0.5 mM), free DDAB (C) (0.5 mM), and free DOTAP (D) (0.5 mM) with difference spectra (diff.) of β-LG−lipid complexes (bottom two curves) obtained at different lipid concentrations (indicated on the figure).

their baselines were corrected automatically using the built-in software of the spectrophotometer (OMNIC ver. 7.3). Thus, the root-mean square (rms) noise of every spectrum was calculated. By means of the second derivative in the spectral region 1700−1600 cm^{-1}, five major peaks for β-lactoglobulin and the complexes were resolved. The above spectral region was deconvoluted by the curve-fitting method with the Levenberg−Marquadt algorithm, and the peaks corresponding to α-helix (1660−1650 cm^{-1}), β-sheet (1640−1610 cm^{-1}), turn (1680−1660 cm^{-1}), and β-antiparallel (1692−1680 cm^{-1}) were adjusted and the area was measured with the Gaussian function. The areas of all of the component bands assigned to a given conformation were then summed and divided by the total area. The curve fitting analysis was performed using the GRAMS/AI Version 7.01 software of the Galactic Industries Corp.

Circular Dichroism. CD spectra of β-lactoglobulin and its lipid complexes were recorded with a Jasco J-720 spectropolarimeter. For measurements in the far-UV region (178−260 nm), a quartz cell with a path length of 0.01 cm was used in nitrogen atmosphere. Protein concentration was kept constant (12.5 μM), while varying each lipid concentration (0.125, 0.25, and 0.5 mM). An accumulation of five scans with a scan speed of 50 nm per minute was performed, and data were collected for each nanometer from 260 to 180 nm. Sample temperature was maintained at 25 °C using a Neslab RTE-111 circulating water bath connected to the water-jacketed quartz cuvettes. Spectra were corrected for buffer signal, and conversion to the Mol CD ($\Delta\varepsilon$) was performed

with the Jasco Standard Analysis software. The protein secondary structure was calculated using CDSSTR, which calculates the different assignments of secondary structures by comparison with CD spectra, measured from different proteins for which high-quality X-ray diffraction data are available.[16,17] The program CDSSTR is provided in the CDPro software package, which is available at the Website: http://lamar.colostate.edu/~sreeram/CDPro.

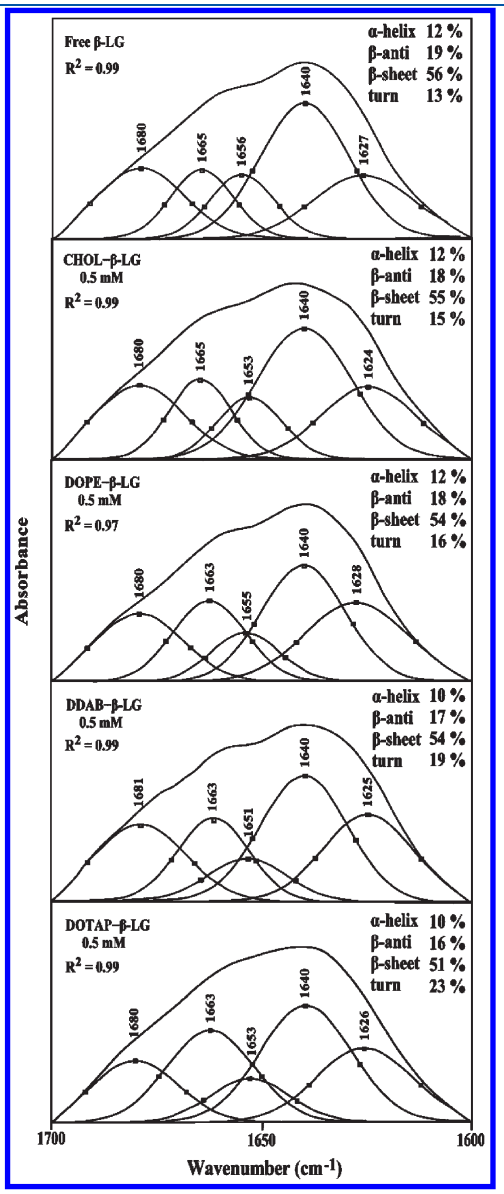


Figure 2. Second derivative resolution enhancement and curve-fitted amide I region (1700−1600 cm^{-1}) for free β-lactoglobulin (A) with their lipid complexes (0.5 mM lipid and 0.25 mM protein concentrations at pH 7.4).

Fluorescence Spectroscopy. Fluorometric experiments were carried out on a Perkin-Elmer LS55 spectrometer. Stock solution of lipid 1 mM was prepared at room temperature (24 ± 1 °C). Various solutions of lipid (5−800 μM) were prepared from the above stock solutions by successive dilutions also at 24 ± 1 °C. A solution of β-lactoglobulin (100 μM) in 10 mM Tris-HCl (pH. 7.4) was also prepared at 24 ± 1 °C. Samples containing 0.4 mL of the above protein solution and various lipid solutions were mixed to obtain a final lipid concentration of 5−800 μM with constant β-LG content 100 μM. The fluorescence spectra were recorded at λ_{exc} = 280 nm and λ_{emi} from 290 to 500 nm. The intensity at 340 nm (tryptophan) was used to calculate the binding constant (K) according to previous literature reports.[18−21]

Molecular Modeling. The docking studies were performed with ArgusLab 4.0.1 software (Mark A. Thompson, Planaria Software LLC, Seattle, WA, http://www.arguslab.com). The β-LG structures were obtained from a literature report,[6] and the lipid three-dimensional structures were generated from PM3 semiempirical calculations using Chem3D Ultra 6.0. The whole protein was selected as a potential binding site because no prior knowledge of such site was available. The docking runs were performed on the ArgusDock docking engine using regular precision with a maximum of 1000 candidate poses. The conformations were ranked using the Ascore scoring function, which estimates the free binding energy. Upon location of the potential binding sites, the docked complex conformations were optimized using a steepest decent algorithm until convergence, with a maximum of 20 iterations. Amino acid residues within a distance of 3.5 Å relative to the lipid were involved in the complexation.

RESULTS AND DISCUSSION

FTIR Spectra of Lipid−β-LG Complexes. The lipid−β-lactoglobulin complexation was characterized by infrared spectroscopy and its derivative methods. Because there was no major spectral shifting for the protein amide I band at 1656 cm^{-1} (mainly C═O stretch) and amide II band at 1546 cm^{-1} (C−N stretching coupled with N−H bending modes)[22−24] upon lipid interaction,

Table 2. Secondary Structure of β-Lactoglobulin Complexes (CD Spectra) with Cationic Lipids at pH 7.4, Calculated with CDSSTR Software

lipid concentration	α-helix (±1%)	β-sheet (±3%)	turn (±1%)	random coil (±2%)
free β-LG	12	50	10	28
CHOL−β-LG (0.5 mM)	10	48	16	26
DOPE−β-LG (0.5 mM)	11	46	18	25
DDAB−β-LG (0.5 mM)	10	45	20	25
DOTAP−β-LG (0.5 mM)	10	45	20	25

Table 1. Secondary Structure Analysis (Infrared Spectra) for the Free β-Lactoglobulin and Its Lipid Complexes in Hydrated Film at pH 7.4

amide I components (cm^{-1})	free β-LG (%) 0.25 mM	CHOL−β-LG (%) 0.5 mM	DOPE−β-LG (%) 0.5 mM	DDAB−β-LG (%) 0.5 mM	DOTAP−β-LG (%) 0.5 mM
1692−1680 β-anti (±1%)	19	18	18	17	16
1680−1660 turn (±1%)	13	15	16	19	23
1660−1650 α-helix (±1%)	12	12	12	10	10
1640−1610 β-sheet (±0.5%)	56	55	54	54	51

dx.doi.org/10.1021/jp200045h |J. Phys. Chem. B 2011, 115, 6683–6690

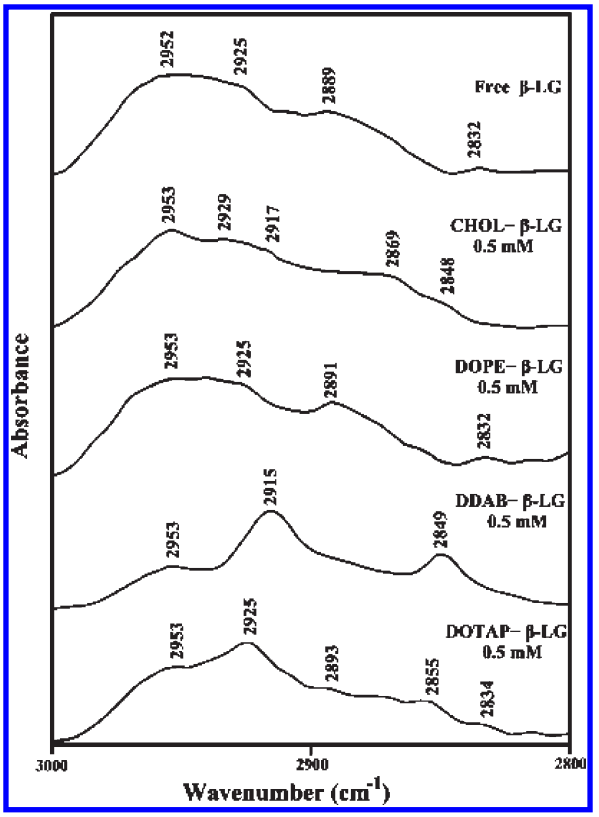


Figure 3. Spectral changes for β-lactoglobulin CH_2 symmetric and antisymmetric stretching vibrations upon lipid complexation (the contribution from free lipid vibrations has been subtracted in this region).

the difference spectra [(protein solution + lipid solution) − (protein solution)] were obtained, to monitor the intensity variations of these vibrations, and the results are shown in Figure 1. Similarly, the infrared self-deconvolution with second derivative resolution enhancement and curve-fitting procedures[15] were used to determine the protein secondary structures in the presence of lipid (Figure 2 and Table 1).

At low lipid concentration (0.125 mM), an increase in intensity was observed for the protein amide I at 1656 and amide II at 1546 cm^{-1}, in the difference spectra of the lipid−β-LG complexes (Figure 1, diff., 0.125 mM). The positive features are located in the difference spectra for amide I and II bands at 1650, 1543 (CHOL−β-LG), 1675, 1631, 1528 (DOPE−β-LG), 1647, 1625, 1538 (DDAB−β-LG), and 1648, 1618, 1507 cm^{-1} (DOTAP−β-LG) in the lipid−β-LG complexes (Figure 1, diff., 0.125 mM). These positive features are related to an increase of the intensity of the amide I and amide II bands upon lipid−protein complexation. The increase in intensity of the amide I and amide II bands is due to lipid binding to protein C═O, C−N, and N−H groups (hydrophilic interaction). Additional evidence to support the lipid interaction with C−N and N−H groups comes from the shifting of the protein amide A band at 3300 cm^{-1} (N−H stretching mode) in the free β-LG to 3290−3280 cm^{-1}, upon lipid complexation (spectra not shown).

As lipid concentration increased to 0.5 mM, major decreases in the intensity of the protein amide I and amide II bands were observed with negative features in the difference spectra for amide I and II bands at 1656, 1636, 1546 (CHOL−β-LG), 1666, 1639, 1549 (DOPE−β-LG), 1665, 1636, 1548 (DDAB−β-LG), and 1663, 1639, 1548 cm^{-1} (DOTAP−β-LG) upon lipid complexation

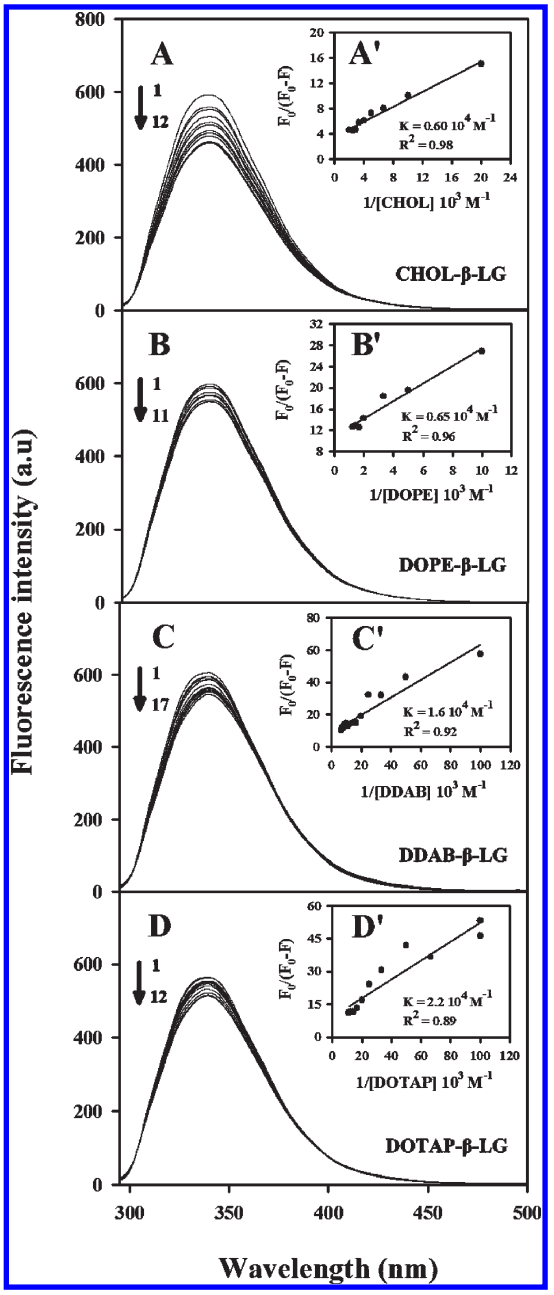


Figure 4. Fluorescence emission spectra of lipid−β-lactoglobulin systems in 10 mM Tris-HCl buffer pH 7.4 at 25 °C for (A) CHOL−β-LG (1) free β-LG 100 μM, (2−12) CHOL at 10, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500 μM; (B) DOPE−β-LG (1) free β-LG 100 μM, (2−11) DOPE at 10, 50, 100, 200, 300, 400, 500, 600, 700, 800 μM; (C) DDAB−β-LG (1) free β-LG 100 μM, (2−17) DDAB at 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150 μM; (D) DOTAP−β-LG (1) free β-LG 100 μM, (2−12) DOTAP at 5, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90 μM. The plot of $F_0/(F_0 - F)$ as a function of 1/lipid concentration. The binding constant K is the ratio of the intercept and the slope for lipid−β-LG complexes.

(Figure 1, diff., 0.5 mM). The decrease in the intensity of the amide I band in the spectra of the lipid−β-LG complexes suggests a minor protein conformational changes at high lipid concentrations.

A quantitative analysis of the protein secondary structure for the free β-LG and its lipid adducts in hydrated films has been carried out, and the results are shown in Figure 2 and Table 1.

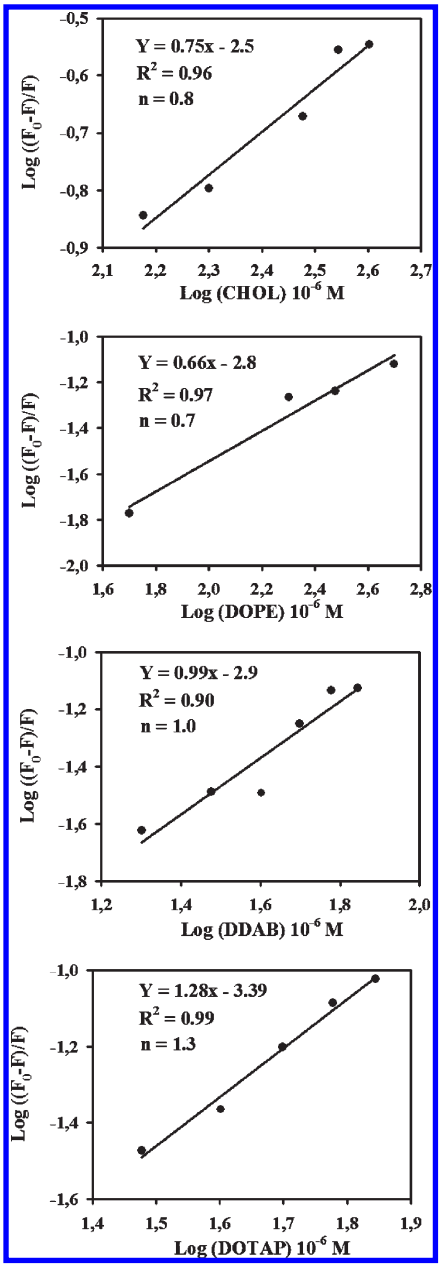


Figure 5. The plot of $\log(F_0 - F)/F$ as a function of log[lipid] for calculation of number of binding (n) in lipid−β-LG complexes.

The free β-LG has major β-sheet content 56% (1640, 1627), α-helix 12% (1656 cm^{-1}), turn 13% (1665 cm^{-1}), and β-antiparallel 19% (1680) (Figure 2 and Table 1). These data are consistent with spectroscopic studies of β-LG previously reported.[24] Upon lipid interaction, a minor decrease in β-sheet and a major increase in turn structure from 13% to 23% were observed, upon lipid−β-LG complexation (Table 1).

CD Spectra. CD spectroscopy was also used to analyze the protein conformation in the lipid−β-LG complexes, and the results are shown in Table 2. The CD results exhibit marked similarities to those of the infrared data (Table 2). The protein conformational analysis based on CD data suggests that free β-LG contains α-helical 10%, β-sheet 50%, turn 12%, and random coil 28% (Table 2), consistent with the literature reports.[4,25] Upon lipid interaction, a decrease in β-sheet structure from 50% (free protein) to 48−45% was observed in the lipid−β-LG complexes (Table 2). The decrease in β-sheet structure was accompanied by an increase in turn structure from 10% to 16−20%, upon lipid complexation (Table 2). The minor decrease in β-sheet and a major increase in turn structure are consistent with the data from infrared results discussed above (Tables 1 and 2).

Table 3. Calculated Binding Constants (K) for the Polyphenol−β-Lactoglobulin Complexes and the Number of Bound Lipids (n) per β-Lactoglobulin

complexes	K-fluorescence (10^4 M^{-1})	n
CHOL−β-LG	0.60 ± 0.06	0.8 ± 0.09
DOPE−β-LG	0.65 ± 0.07	0.7 ± 0.09
DDAB−β-LG	1.6 ± 0.3	1.0 ± 0.16
DOTAP−β-LG	2.2 ± 0.67	1.3 ± 0.09

Hydrophobic Interactions. The spectral changes of the protein CH_2 antisymmetric and symmetric stretching vibrations, in the region of 3000−2800 cm^{-1}, were monitored to locate the presence of hydrophobic contact in the lipid−β-lactoglobulin complexes. The CH_2 bands of the free β-lactoglobulin at 2953, 2925, 2889, and 2832 cm^{-1} shifted (except for 2952 cm^{-1}) to 2929 and 2869 and 2848 cm^{-1} (CHOL−β-LG), to 2891 cm^{-1} (DOPE−β-LG), to 2915 and 2849 cm^{-1} (DDAB−β-LG), and to 2925, 2893, and 2834 cm^{-1} (DOTAP−β-LG), upon lipid complexation (Figure 3). The shifting of the protein antisymmetric and symmetric CH_2 stretching vibrations is due to the presence of hydrophobic interactions via lipid long chains and the hydrophobic pockets in β-LG, which is consistent with fluorescence spectroscopic results discussed below.

Fluorescence Spectra and Stability of Lipid−β-LG Complexes. β-Lactoglobulin has two tryptophan residues, Trp-19 and Trp-61. Trp-19 is in an apolar environment and contributes to 80% of total fluorescence, while Trp-61 is partly exposed to aqueous solvent and has a minor contribution to Trp fluorescence.[4] When other molecules interact with β-LG, tryptophan fluorescence may change depending on the impact of such interaction on the protein conformation.[10,11] On the assumption that there are (n) substantive binding sites for quencher (Q) on protein (B), the quenching reaction can be shown as follows:

$$nQ + B \leftrightarrows Q_nB \quad (1)$$

The binding constant (K_A) can be calculated as:

$$K_A = [Q_nB]/[Q]^n[B] \quad (2)$$

where [Q] and [B] are the quencher and protein concentration, respectively, $[Q_nB]$ is the concentration of non fluorescent fluorophore−quencher complex, and $[B_0]$ gives total protein concentration:

$$[Q_nB] = [B_0] - [B] \quad (3)$$

$$K_A = ([B_0] - [B])/[Q]^n[B] \quad (4)$$

The fluorescence intensity is proportional to the protein concentration as described:

$$[B]/[B_0] \propto F/F_0 \quad (5)$$

dx.doi.org/10.1021/jp200045h |J. Phys. Chem. B 2011, 115, 6683–6690

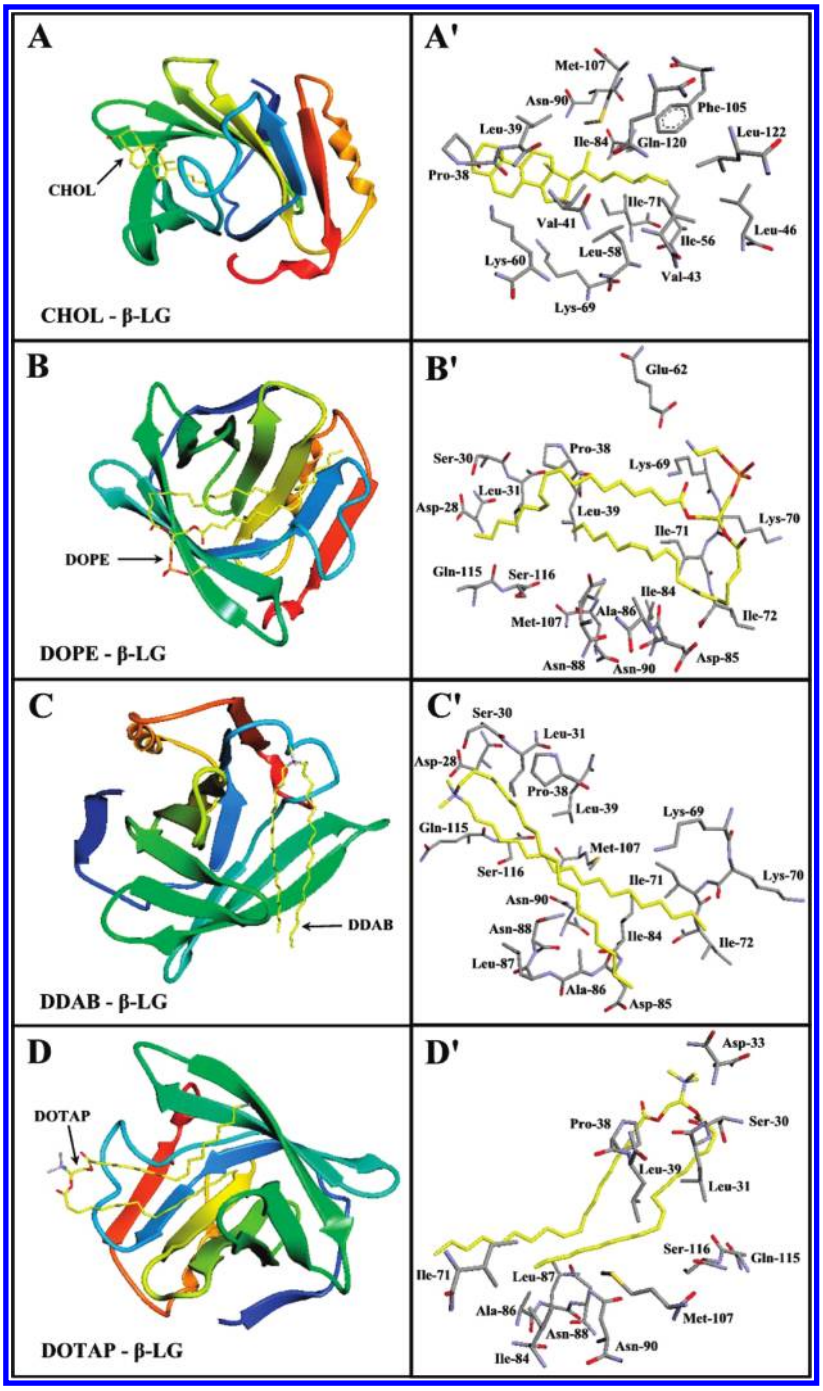


Figure 6. Best docked conformations for lipid−β-LG complexes. (A) For cholesterol complexed to β-LG, (B) for DOPE complexed to β-LG, (C) for DDAB complexed to β-LG, and (D) for DOTAP complexed to β-LG.

Results from fluorescence measurements can be used to estimate the binding constant of lipid−protein complex. From eq 4:

$$\log[(F_0 - F)/F] = \log K_A + n \log[Q] \quad (6)$$

The accessible fluorophore fraction (f) can be calculated by the modified Stern−Volmer equation:

$$F_0/(F_0 - F) = 1/fK[Q] + 1/f \quad (7)$$

where F_0 is the initial fluorescence intensity, and F is the fluorescence intensity in the presence of quenching agent (or interacting molecule). K is the Stern−Volmer quenching constant, [Q] is the molar concentration of quencher, and f is the fraction of accessible fluorophore to a polar quencher, which indicates the fractional fluorescence contribution of the total emission for an interaction with a hydrophobic quencher.[9] The plot of $F_0/(F_0 - F)$ versus $1/[Q]$ yields f^{-1} as the intercept on y axis and $(fK)^{-1}$ as the slope. Thus, the ratio of the ordinate and the slope gives K. The decrease of fluorescence intensity of β-LG is monitored at 340 nm for lipid−β-LG systems (Figure 4A−D shows representative results for each system). Figure 5A′−D′ shows representative plots of $F_0/(F_0 - F)$ versus 1/[lipid]. Assuming that the observed changes in fluorescence

Table 4. Amino Acid Residues Involved in Lipids−β-Lactoglobulin Interaction and Free Binding Energy for the Best Selected Docking Poses

complexes	residues involved in the interaction	$\Delta G_{binding}$ (kcal/mol)
CHOL−β-LG	Asn-90, Gln-120, Ile-56, Ile-71, Ile-84, Leu-39, Leu-46, Leu-58, Leu-122, Lys-60, Lys-69, Met-107, Phe-105, Pro-38, Val-41, Val-43	−12.58
DOPE−β-LG	Ala-86, Asn-88, Asn-90, Asp-28, Asp-85, Gln-115, Glu-62, Ile-71, Ile-72, Ile-84, Leu-31, Leu-39, Lys-69,[a] Lys-70, Met-107, Pro-38, Ser-30, Ser-116	−10.19
DDAB−β-LG	Ala-86, Asn-88, Asn-90, Asp-28, Asp-85, Gln-115, Ile-71, Ile-72, Ile-84, Leu-31, Leu-39, Leu-87, Lys-69, Lys-70, Met-107, Pro-38, Ser-30, Ser-116	−10.06
DOTAP−β-LG	Ala-86, Asn-88, Asn-90, Asp-33, Gln-115, Ile-71, Ile-84, Leu-31, Leu-39, Leu-87, Met-107, Pro-38, Ser-30, Ser-116	−9.57

[a] Hydrogen bonding observed with this residue.

come from the interaction between lipid and protein, the quenching constant can be taken as the binding constant of the complex formation. The K values given here are averages of three-replicate runs for lipid/protein systems, each run involving several different concentrations of lipid (Figure 4): $K_{CHOL-\beta\text{-LG}} = 6.0\ (\pm 0.6) \times 10^3\ M^{-1}$, $K_{DOPE-\beta\text{-LG}} = 6.5\ (\pm 0.7) \times 10^3\ M^{-1}$, $K_{DDAB-\beta\text{-LG}} = 1.6\ (\pm 0.3) \times 10^4\ M^{-1}$, and $K_{DOTAP-\beta\text{-LG}} = 2.2\ (\pm 0.7) \times 10^4\ M^{-1}$ with the order of binding DOTAP > DDAB > DOPE > CHOL (Figure 4 A′−D′ and Table 3). The binding constants calculated for the lipid−β-LG adducts suggest low affinity lipid−β-LG interaction, as compared to the other strong ligand−protein complexes.[25−28] However, similar binding constants (10^3−$10^4\ M^{-1}$) were also reported for several ligand−protein complexes using fluorescence spectroscopic methods.[4,7,29] The binding constants of the cationic lipid−β-LG complexes are bigger than those of helper lipid adducts, which can be due to the presence of the positive charges associated with the cationic lipids that can form ionic interaction with protein polar group (hydrophilic contact) and stabilize lipid−protein complexes (Table 3).

The number of lipid molecules bound per protein (n) is calculated from $\log[(F_0 - F)/F] = \log K_S + n \log[\text{lipid}]$ for the static quenching.[4,30−35] The n values from the slope of the straight line plot in Figure 5 are for lipid−β-LG complexes 0.8 (CHOL), 0.7 (DOPE), 1.0 (DDAB), and 1.3 (DOTAP) (Figure 5 and Table 3).

Docking Study. Our results from FTIR, CD, and fluorescence spectroscopic methods are accompanied by docking experiments in which the CHOL, DOPE, DDAB, and DOTAP molecules were docked to β-lactoglobulin to determine the preferred binding sites on the protein. The stereoview of the dockings of lipids is shown in Figure 6 and Table 4. The docking results show that CHOL is surrounded by Asn-90, Gln-120, Ile-56, Ile-71, Ile-84, Leu-39, Leu-46, Leu-58, Leu-122, Lys-60, Lys-69, Met-107, Phe-105, Pro-38, Val-41, and Val-43 (Figure 6 and Table 4). DOPE is in the vicinity of Ala-86, Asn-88, Asn-90, Asp-28, Asp-85, Gln-115, Glu-62, Ile-71, Ile-72, Ile-84, Leu-31, Leu-39, Lys-69, Lys-70, Met-107, Pro-38, Ser-30, and Ser-116 (Figure 6 and Table 4). DDAB is located in the vicinity of Ala-86, Asn-88, Asn-90, Asp-28, Asp-85, Gln-115, Ile-71, Ile-72, Ile-84, Leu-31, Leu-39, Leu-87, Lys-69, Lys-70, Met-107, Pro-38, Ser-30, and Ser-116 (Figure 6 and Table 4). DOTAP is surrounded by Ala-86, Asn-88, Asn-90, Asp-33, Gln-115, Ile-71, Ile-84, Leu-31, Leu-39, Leu-87, Met-107, Pro-38, Ser-30, and Ser-116 (Figure 6 and Table 4). The binding energy (ΔG) shows more stable lipid−protein complexes formed with DOTAP and DDAB consistent with the spectroscopic results (Tables 3 and 4). This indicates that positively charged cationic lipids interact strongly with negatively charged regions of protein.

CONCLUSIONS

The spectroscopic results and docking studies presented here show that lipids weakly bind to β-lactoglobulin in solution with the order of binding DOTAP > DDAB > DOPE > CHOL. Both hydrophobic and hydrophilic interactions are observed in the lipid−β-lactoglobulin complexation. The participation of several amino acids was observed in the lipid−β-LG complexes. Protein conformational changes were observed upon lipid complexation.

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ACKNOWLEDGMENT

The financial support of the Natural Sciences and Engineering Research Council of Canada (NSERC) to H.-A.T.-R. is highly appreciated.

ABBREVIATIONS

β-LG, beta-lactoglobulin; CHOL, cholesterol; DOTAP, 1,2-dioleoyl-3-trimethylammonium-propane; DDAB, dioctadecyldimethylammonium bromide; DOPE, dioleoylphosphatidylethanolamine; FTIR, Fourier transform infrared spectroscopy; CD, circular dichroism

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