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INTERACTION OF PHOSPHATIDYLCHOLINE WITH β-LACTOGLOBULIN

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Although β-lactoglobulin (β-Lg), the major whey protein of bovine milk, has no known membrane-associated function, it shares certain structural characteristics with membrane proteins. Both average hydrophobicity and the ratio of charged to hydrophobic residues lie between those for intrinsic and extrinsic membrane proteins (1). The circular dichroism (CD) spectrum of native β-Lg shows much less helical structure than predicted by analysis of the amino acid sequence by use of the Chou-Fasman rules (2). Segrest and Feldman (3) identified residues 130–143 of its sequence as a possible amphipathic helix similar to those found in lipoproteins and some membrane proteins. When β-Lg is synthesized in vitro in the presence of microsomes, its signal peptide is cleaved while the protein is passing through the membrane, and β-Lg then assumes its native conformation (4). In a microsome-free system, the signal peptide is not cleaved and is not accessible to enzymatic attack after the protein folds. These observations led us to look for possible interactions of β-Lg with phospholipids. Although no complex formation has been detected between native β-Lg and egg phosphatidylcholine, dimyristoyl phosphatidylcholine (DMPC), or dipalmitoyl phosphatidylcholine (DPPC), solvent-denatured β-Lg readily interacts with each of these lipids.

MATERIALS AND METHODS

Dry lipid was added to β-Lg in a single-phase system formed by diluting 10 mg/ml β-Lg in 0.14M KCl with 2:1 $CHCl_3$:CH_3OH and acidifying with 3% HCl in CH_3OH.[1] Solvents were removed under N_2 and the resulting film was dispersed in buffer. Vesicles formed by the method of Barenholz et al. (5) provided a system suitable for optical and resonance spectroscopy, allowing observation of the effect of lipid on protein and protein on lipid. Measurements at pH 3.7 and pH 7.2 allowed us to observe the effects of changes in the charge distribution on β-Lg. CD spectra were obtained with a J-41C spectropolarimeter (Jasco Inc., Easton, MD)[2], ^{31}P NMR with an FX 60Q spectrometer (JEOL, USA, Cranford, NJ) and electron micrographs with a 10-B electron microscope (Carl Zeiss, New York, NY).

RESULTS AND DISCUSSION

At pH 3.7, DPPC vesicle suspensions were unstable and quickly became turbid. Vesicles formed from the β-Lg-DPPC complex were more stable. The ^{31}P NMR spectrum consisted of a narrow line, indicating rapid motion of the head groups. Electron micrographs of these vesicles showed them to be uniformly dispersed but somewhat flattened, with the short diameter approximately one-third that of the long diameter. These dispersions were suitable for ultraviolet (UV) absorption spectroscopy. The far UV CD spectrum (Fig.1 *a*) shows native β-Lg to have predominately β-structure. Our solvent system is helix forming, but the CD spectrum of solvent-treated, redissolved β-Lg without lipid shows only β- and unordered structures. The double minimum in the spectrum of the β-LG-DPPC vesicle complex represents 200% of the helix in the native protein. The near UV CD spectra (Fig. 1 *b*) of native and solvent-treated β-Lg are similar and distinctly different from the spectrum of the vesicle complex.

At pH 7.2, DPPC vesicle suspensions were stable, but the inclusion of denatured β-Lg led to pelleting of both lipid and protein when the suspension was centrifuged. Electron micrographs of the DPPC vesicles showed a

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[1]Brown, E. M., J. Sampugna, R. J. Carroll, and P. E. Pfeffer. Submitted for publication.

[2]Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

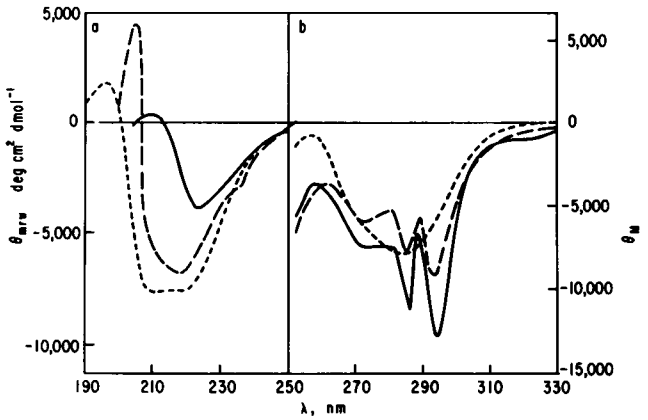

FIGURE 1 The far UV (*a*) and near UV (*b*) CD spectra of (solid line) native β-Lg; (long dashes) denatured, redissolved β-Lg; and (--) β-Lg-DPPC vesicle complex. All samples in 0.02 M acetate, 0.14 M KCl, pH 3.7. θ_M based on a molecular weight of 18,300; θ_{MRW} on a mean residue weight of 113.

uniform dispersion of nearly spherical vesicles with diameters between 200Å and 600Å. The β-Lg-DPPC vesicles were similar in size and shape to those at pH 3.7 but were aggregated to form stacks. The ^{31}P NMR spectrum of the pelleted mixture contained elements of both vesicle and powder pattern spectra but was not simply a sum of these (Fig. 2). When titrated to pH 11, the sample became less turbid and the spectrum of vesicles predominated, confirming vesicle aggregation rather than fusion at pH 7.2.

The pH dependence of the characteristics of the β-Lg-DPPC vesicle complex suggests that electrostatic interactions are involved. In addition, hydrophobic interactions cause an increase in helical structures. We speculate that the required disruption of the native conformation prior to lipid complexation may result in β-Lg secondary structure similar to its state during transportation through the membranes of the endoplasmic reticulum.

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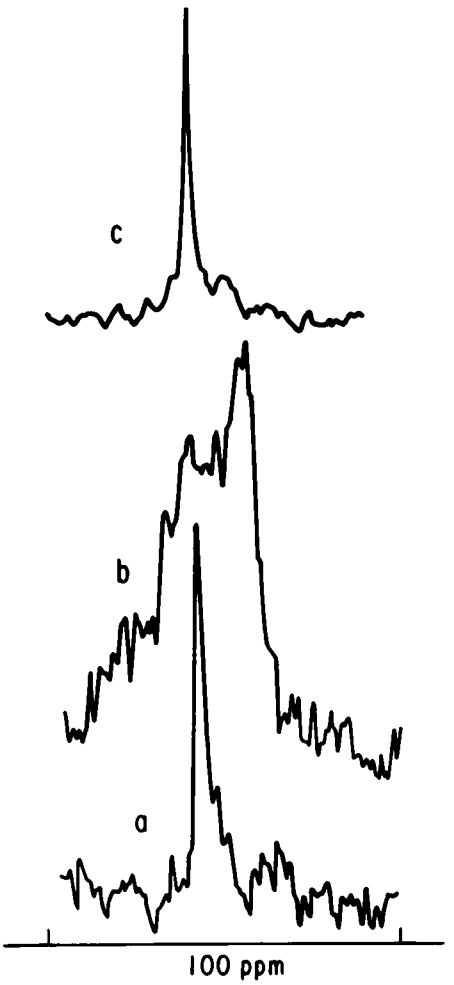

FIGURE 2 24 MHz proton decoupled ^{31}P NMR spectra obtained with sonicated samples of (*a*) DPPC at pH 7.2; (*b*) 1:25 (wt/wt) mixture of β-Lg with DPPC at pH 7.2; and (*c*) after titration of (*b*) to pH 11.