PHOSPHATIDYL CHOLINE INTERACTION WITH BOVINE SERUM ALBUMIN: EFFECT OF THE PHYSICAL STATE OF THE LIPID ON PROTEIN-LIPID COMPLEX FORMATION

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Received April 21,1975

SUMMARY

Radioactively labeled [^{14}C]phosphatidyl choline dispersed on Celite was equilibrated with bovine serum albumin solutions buffered at pH 8.0. Phosphatidyl choline was rapidly solubilized in the presence of serum albumin, and formed stable protein-lipid complexes which were isolated by gel-filtration through a Sepharose 4B column. Under similar conditions, equilibration of the protein with phosphatidyl choline liposome dispersions in buffer did not result in complex formation. The altered physical state of phosphatidyl choline on the weakly adsorbing Celite surface appears to be essential for binding by native bovine serum albumin. This work reports the first observation of phosphatidyl choline binding to native serum albumin in bulk phase and suggests the possibility of exposing monodisperse lipids, under controlled conditions, to proteins having lipid binding properties.

Most of the recent work on protein-lipid interactions has been performed on model systems using either artificial lipid membranes or soluble amphiphiles. In the first case the major limitation is the formation of non-specific complexes of organized lipid phases (e.g., monolayers, bilayers, and bilayer liposomes) with proteins--systems where detailed, quantitative investigations of molecular interactions are not feasible (1). On the other hand, use of soluble amphiphile binding by proteins as a model system of protein-lipid interactions affords detailed binding equilibrium data, and yields information about binding sites and configurational changes in proteins involved in binding (2). However, amphiphile-protein systems cannot be considered representative of true protein-lipid interactions because of the large differences in solubility and behavior in water between soluble amphiphiles, such as sodium dodecyl sulfate, and essentially insoluble lipids, such as phosphatidyl choline (PC)[1] (3).

A different system involving sterol dispersions on Celite, was introduced some time ago by Avigan (4) and Ashworth and Green (5) for the investigation of

[1] Abbreviations: PC, phosphatidyl choline; BSA, bovine serum albumin.

the lipid uptake by serum and serum lipoproteins. The present work describes the successful application of this technique to the binding of PC by bovine serum albumin (BSA)[1], in a system that circumvents the problem of non-specific protein interactions with associated lipid phases, and has the potential of yielding detailed molecular information on the binding process.

MATERIALS AND METHODS

BSA was obtained in crystallized and lyophilized form from Sigma Chemical Co. The free fatty acid content of this preparation was not determined, but was assumed to be less than one fatty acid per BSA, as shown in other similarly prepared crystalline BSA samples (6). Less than 10% dimer was observed on Schlieren patterns obtained in sedimentation velocity experiments on this BSA preparation. Egg PC, purified chromatographically, was purchased from Sigma Chemical Co., and the [^{14}C]PC isolated from algae was obtained from New England Nuclear Co. Purity of both PC preparations was tested by thin layer chromatography on Eastman Kodak Silica Gel-G plates in two solvent systems: chloroform:methanol:water (70:30:1, v/v), and chloroform:methanol:ammonium hydroxide (75:25:4, v/v). Solubility properties were identical for both PC preparations, and purity was found to be better than 97% as detected by I_2 complex formation and autoradiography. Celite 545 was a product of John Manville Co.; it was washed repeatedly with reagent grade chloroform before use. PC liposomes were prepared by the technique of Bangham et al. (7).

Egg PC mixed with appropriate amounts of [^{14}C]PC was added to a chloroform dispersion of Celite in weight ratios of 1 to 4 mg of PC per 100 mg of Celite. The preparation was mixed thoroughly and was dried under nitrogen and subsequently under vacuum, avoiding exposure to light. Equilibration of BSA solutions containing 1 to 5 mg per ml protein in 0.05 M Tris-HCl buffer, pH 8.0, 0.01% EDTA, with Celite-PC dispersions was performed in the dark over 2 to 24 hours at room temperature; 2 ml of protein solution were used per 100 mg of Celite-PC dispersion. Appearance of [^{14}C]PC in solution was followed at timed intervals by removing and filtering aliquots of the dispersion, using 0.45 μ

pore size Millipore filters. Clear samples were then counted in a Beckman LS-100 Scintillation System, using a dioxane-naphthalene based scintillation fluid. Protein was determined either by the technique of Lowry et al. (8) or by using the percent extinction coefficient for BSA at 280 nm, $E^{1\%}_{1cm}$ = 6.6 x $10^2 g^{-1} cm^2$. Stable protein-lipid complexes were isolated by passing 1.5 to 2.0 ml of the solutions containing BSA and [^{14}C]PC through a Sepharose 4B column (2 x 35 cm) equilibrated with the same Tris buffer. Elution patterns of cpm and absorbance at 280 nm were obtained by analyzing alternating column fractions. At the conclusion of the experiments, lipid was extracted from various fractions with ethanol:ether (1:1, v/v) and was chromatographed on thin layer plates in the same solvent systems as described above; at least 90% of the radioactive material was recovered as PC.

RESULTS AND DISCUSSION

Solutions of BSA, containing 2.5 mg of protein per ml, exposed to [^{14}C]PC dispersions on Celite, extracted PC into solution at initial rates at least 10-fold higher than a control buffer (Fig. 1). For the protein containing dis-

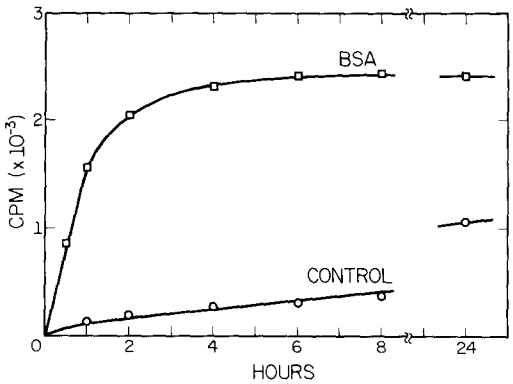

Fig. 1. Rate of uptake of [^{14}C]phosphatidyl choline by a bovine serum albumin solution from a solid dispersion of the lipid on Celite. Protein concentration was 2.5 mg per ml in 0.05 M Tris-HCl buffer, pH 8.0, 0.01% EDTA. Uptake of lipid by the protein solution (—□—□—); uptake by a control buffer solution (—○—○—).

persion, equilibrium was reached in 2 to 6 hours, whereas the control continued to extract PC at the initial rate at the end of 24 hours.

Routinely, solutions of BSA and [^{14}C]PC were filtered after 2 hours of equilibration and were stored at 4°C until the gel-filtration step, in order to prevent PC oxidation. Results of the Sepharose 4B experiments are illustrated in Fig. 2, where cpm correspond to PC elution and the absorbance at 280 nm is contributed almost exclusively by the protein. In this experiment, protein and PC elution coincides at the total volume of the column and corresponds to a molar ratio of 7.2 PC per BSA.

PC liposomes having the same content of lipid as the solution in Fig. 2 were incubated with the same BSA concentration under identical conditions of time of equilibration, buffer, and temperature, as the preceding Celite dispersion experiment. Gel-filtration of this liposome-BSA preparation on the Sepharose 4B column gave the elution patterns shown in Fig. 3, indicating complete separation of protein and lipid. There is no evidence of complex

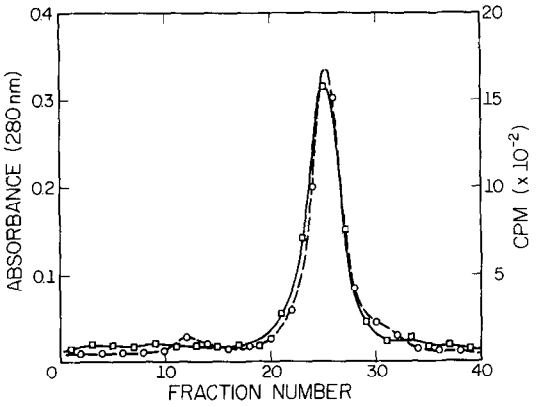

Fig. 2. Elution patterns from a Sepharose 4B column (2 x 35 cm) of bovine serum albumin after equilibration with a Celite-[^{14}C]phosphatidyl choline dispersion. Protein elution (—□—□—) is given in terms of absorbance at 280 nm; lipid elution (—○—○—) is given in cpm. The column was equilibrated with 0.05 M Tris-HCl buffer, pH 8.0, 0.01% EDTA and was operated at 8°. Fractions were 2.5 ml each.

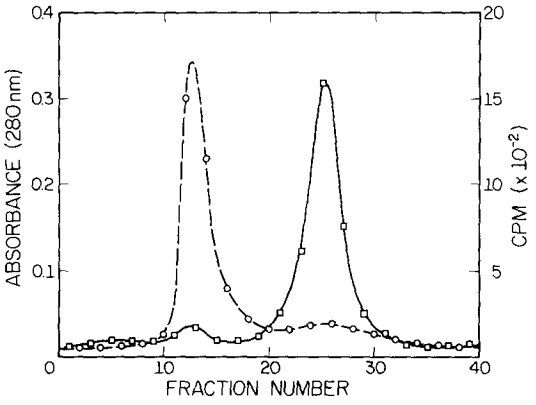

Fig. 3. Elution patterns from a Sepharose 4B column of bovine serum albumin after equilibration with [^{14}C]phosphatidyl choline liposomes. Protein elution (—□—□—) is given as absorbance at 280 nm; lipid elution (—○— ○—) is given in cpm. The same column and the same experimental conditions were used as in Fig. 2.

formation in the liposome fraction excluded from the column, nor in the protein fraction.

It is apparent from these results that PC dispersed on Celite exists in a physical state that allows complex formation with BSA, in contrast to PC liposomes where the lipid molecules are in contact with each other, with only the polar head groups exposed to the aqueous environment. The interaction of BSA with individual molecules of PC from a liposome would depend first, on the ability of the negatively charged protein to approach and interact with the zwitterionic PC liposome surface and second, on the capacity of the protein to overcome the strong lipid-lipid interactions in the PC mesophase. Both of these processes are energetically unfavorable for the formation of BSA complexes with PC molecules from liposomes.

Sweet and Zull (9) have reported the interaction of serum albumin with mixed PC, cholesterol, dicetyl phosphate liposomes at pH's below the isoelectric point of albumin; however, under those conditions the protein is positively charged and expanded, facilitating electrostatic and secondary

hydrophobic interactions with the negatively charged liposomes. Penetration of PC monolayers by albumin was also observed to occur when the protein was denatured by exposure to urea or to low pH; much less penetration took place at neutral pH (10). The serum albumin interaction with PC reported here is a new and very interesting observation in that it occurs at pH 8.0 where BSA is known to exist in its native configuration. Also the interaction takes place under conditions where lipid-lipid interactions are eliminated or minimized, facilitating protein-lipid complex formation.

Additional work is in progress in our laboratory concerning the specificity of the lipid-protein interaction observed in this work and the application of this lipid binding technique to other proteins, particularly to purified proteins isolated from serum lipoproteins.

ACKNOWLEDGMENT

This work was supported by USPHS Grant HL-16059. The author is an Established Investigator of the American Heart Association.

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