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A SUBCLASS OF ALBUMIN RECOGNIZED BY INHIBITION OF PHOSPHATIDYLETHANOLAMINE-MEDIATED AGGLUTINATION OF MOUSE ERYTHROCYTES

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Agglutination of mouse erythrocytes by non-choline phospholipids is inhibited by a factor in mammalian sera. The inhibitor cochromatographed with albumin on dye-agarose conjugates, was retained by an anti-albumin affinity column, was neutralized by anti-albumin antibody and found in a serum fraction in which only albumin could be detected. A variety of commercial preparations of albumin (fraction V, crystalline) did not inhibit. However, they acquired potent inhibitory activity when treated with low molecular weight thiols. The inhibitory activity of serum was increased 8-fold by treatment with dithiothreitol. Other proteins were not activated in this way. Inhibitory activity increased with average free sulphydryl content of treated albumin, up to six thiol groups per molecule. Alkylation of these sulphydryl groups did not diminish inhibitory activity. Thiols also induced polymerization of albumin. Inhibitory albumin in serum was largely monomeric. We propose that the inhibitor is a type of serum albumin which is lost or inactivated during preparation of commercial albumin, and which shares a structural feature, necessary for inhibition, with thiol-reduced albumin and the ligand on mouse erythrocytes.

Introduction

During investigation of the receptor for mouse erythrocytes on leukaemic B lymphocytes, it was found that phosphatidylethanolamine (PE) and other non-choline phospholipids agglutinated erythrocytes from mice but not from other species [1], exactly as the receptor fragment released from B lymphocytes by treatment with trypsin [2]. Haemagglutination by PE was inhibited by a 70 kDa protein in the mouse erythrocyte membrane, and by serum from several species.

We now report studies of the inhibitor in human serum which indicate that it is a type of albumin. This has implications for the understanding of the heterogeneity of albumin and of albumin-phospholipid interactions.

Methods and Materials

Materials. Materials and suppliers thereof were as follows: PE from soybean and egg yolk, fetuin types III and IV, reduced and oxidized glutathione, bovine albumin (Cohn fraction V), human albumin (Cohn fraction V), delipidated human albumin (Cohn fraction V), crystalline human albumin, α_1-acid glycoprotein, Coomassie blue and iodoacetamide (Sigma Chemicals), 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) and dithiothreitol (Calbiochem); chymotrypsinogen, ribonuclease, Blue Sepharose CL-6B, cyanogen bromide-activated Sepharose 4B, rabbit anti-human al-

Abbreviations: PE, phosphatidylethanolamine; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid).

bumin (IgG fraction) and Sephadex G-25 (Pharmacia Fine Chemicals); dye matrix kit (Amicon Corporation); silver stain (Bio-Rad); sodium dithionite, potassium bromide and 2-mercaptoethanol (Merck); sodium metabisulphite (Ajax); butanol (British Drug Houses); Ultrogel AcA-34 (LKB); rabbit anti-mouse albumin (IgG fraction) (Cappel Labs); foetal calf serum (Flow Laboratories). Human adult sera were obtained from healthy subjects. Mouse serum was prepared from Swiss albino mice. Sera were heat-inactivated for 30 min at 56°C. For absorption of natural haemagglutinins equal volumes of serum and fresh-packed mouse erythrocytes were incubated for 30 min at 37°C. Red cells were then removed by centrifugation.

Haemagglutination and haemagglutination inhibition assays. These were performed as in Ref. 1. One haemagglutination unit is defined as the least amount of agglutinin which agglutinates $5 \cdot 10^6$ mouse erythrocytes in a 75 μl volume. One inhibitory unit is defined as the least amount of inhibitor which neutralizes one haemagglutination unit.

Treatment with low molecular weight thiols. Protein or glutathione (5 mg/ml) were treated with dithiothreitol, 2-mercaptoethanol, sodium dithionite or potassium metabisulphite at 1 mg/ml for 90 min at 37°C, unless otherwise indicated. Controls contained phosphate-buffered saline incubated with thiols. The pH value of the reaction was 7.4. After incubation, 2.5 ml solution were applied to disposable P10 (G-25) columns and washed with phosphate-buffered saline. The first 3.5 ml of wash were discarded and the next 2.5 ml collected. In some cases, a second cycle of chromatography was performed to remove any contaminating thiol.

Alkylation. Reduced albumin was reacted with iodoacetamide by a published method [3]. After 60 min at 37°C, the reduced carboxymethylated albumin was dialysed against phosphate-buffered saline.

Dye-ligand chromatography. 1 ml human serum or serum fraction was applied to a column of Blue Sepharose CL-6B (1 × 15 cm), equilibrating and washing with phosphate-buffered saline. Bound material was eluted sequentially with 0.05 M Tris-HCl, pH 7.0, containing 0.5 M NaCl, 1.5 M NaCl, 5 M NaCl, and finally with 6 M urea. Wash and eluates comprised 25 fractions of 1 ml each at a flow rate of 30 ml/h.

500 μl of human serum were applied to each type of dye agarose in the dye matrix kit. Columns were equilibrated with 20 mM Tris-HCl, pH 7.5, at room temperature. After application of serum, columns were washed with 10 ml of equilibration buffer and eluted with 10 ml of 20 mM Tris-HCl, pH 7.5, containing 1.5 M potassium chloride.

Preparation of mouse erythrocyte ligand. This was prepared from mouse red cell stroma by aqueous/phenol extraction [1].

Ultracentrifugation of serum. The method of Havel et al. [4] was modified as follows: human serum, 220 ml containing ethylenediaminetetraacetic acid at 1 mg/ml and potassium bromide (71.5 g) so that the final density was 1.21 g/ml, was centrifuged at 100000 $\times g$ for 48 h in a Beckman model L5-65 preparative ultracentrifuge. Lipoprotein-rich and -free fractions were dialyzed against phosphate-buffered saline and stored at −20°C.

Isolation of serum albumin. 5 ml of human serum, heat-inactivated, were diluted to 25 ml in phosphate-buffered saline and mixed with 100 ml of 40% ethanol at room temperature. After centrifugation, precipitate was taken up into 5 ml of phosphate-buffered saline and mixed with 5 ml of *n*-butanol at room temperature. After centrifugation, the lower aqueous phase was removed, dialyzed against phosphate-buffered saline and concentrated to 1 ml by ultrafiltration (YM-10 membrane). This was applied to a Blue Sepharose column in phosphate-buffered saline and eluted with 0.05 M Tris-HCl, pH 7.0, containing 0.5 M NaCl.

Chromatography with anti-albumin Sepharose. Anti-albumin Sepharose was prepared by conjugating 1 g of cyanogen bromide-activated Sepharose 4B with 1 ml of anti-albumin according to Pharmacia specifications. Samples were incubated on the column for 30 min at room temperature, washed with 20 ml 0.2 M Tris-HCl, pH 8.0, containing 0.5 M NaCl, and then eluted with 20 ml 0.2 M glycine in 0.5 M NaCl, pH 2.8.

SDS-polyacrylamide gel electrophoresis. SDS-polyacrylamide gel electrophoresis was performed by the discontinuous method of Laemmli [5] under reducing conditions, unless otherwise indicated.

Proteins were stained with Coomassie blue or silver.

Determination of albumin and total protein. Albumin content was determined by rocket immunoelectrophoresis [6] or radial immunodiffusion [6] and total protein by absorbance by the method of Lowry et al. [7].

Estimation of sulphydryl content. This was determined using DTNB as described [8].

Ammonium sulphate precipitation. 1 ml human serum was treated with 42.5% saturated ammonium sulphate solution for 15 min at room temperature. Precipitate was collected by centrifugation at 1000 × *g* and both precipitate and supernatant were dialysed extensively.

Ultrogel AcA-34 chromatography. Human serum (4 ml) was applied to a column of Ultrogel (3 × 50 cm) and eluted with phosphate-buffered saline. 1-ml fractions were collected at a flow rate of 40 ml/h. Void volume was measured with blue dextran, and elution volume of monomeric albumin with bovine albumin.

Alkali treatment of albumin. Human serum, absorbed with mouse erythrocytes, was delipidated [9] and then equilibrated with phosphate-buffered saline, pH 7.4, or 0.01 M potassium chloride, pH 9.1, by Sephadex G-25 chromatography. Final concentration of serum was 4% (v/v). After incubation overnight at room temperature, control (pH 7.4) and alkali (pH 9.1) -treated samples were tested for inhibitory activity.

Chromatofocussing. 4 ml human serum dialyzed against starting buffer (0.025 M imidazole-HCl, pH 7.4), were loaded on a Polybuffer exchange 94 column (Pharmacia) and eluted with Polybuffer (Pharmacia) in the pH range 4–7. 5-ml fractions were collected.

Results

1. Inhibition of PE-mediated haemagglutination by serum

Serum (human, mouse or foetal calf) absorbed with mouse erythrocytes to remove natural agglutinins, inhibited the agglutination of mouse erythrocytes by PE (Table I).

2. Characterization of the serum inhibitor

(a) Dye-ligand chromatography. Human serum inhibitor bound to Blue Sepharose conjugate and was eluted by high salt concentration. Of serum proteins, only albumin and some lipoproteins bind to Blue Sepharose under these conditions [11]. Human serum was chromatographed on five dye-agarose columns with different binding specificities. All of the inhibitor was found in the 1.5 M KCl eluates of Blue A (Cibracron Blue)-, Red A- and Green A-agarose and in the washes of Orange A-, Blue B- or control (unconjugated) agarose. This profile has been reported for serum albumin [12], and was confirmed here (data not shown).

TABLE I

INHIBITION OF PE-MEDIATED AGGLUTINATION OF MOUSE ERYTHROCYTES BY SERUM AND SERUM FRACTIONS

Inhibitor	Units of inhibitor	
	(per ml [b])	(Per mg albumin [b]) Albumin
Human serum No. 1.	10240	365
Human serum No. 2.	5120	170
Mouse serum	10240	n.d.
Foetal calf serum	20480	n.d.
Human serum fractions		
Albumin [a]	320	214
42.5% ammonium sulphate precipitate	640	213
42.5% supernatant	640	107
Lipoprotein	2560	191
Non-lipoprotein	10240	277

[a] Made by ethanol precipitation, butanol extraction and Blue Sepharose chromatography (see Methods).

[b] Inhibitory units are expressed per volume or per mg of albumin for each sample of serum or serum fraction.

(b) Lipoprotein isolation. The total lipoprotein and nonlipoprotein fractions obtained by ultracentrifugation were dialyzed and tested without adjusting their volumes, which were 20 and 200 ml, respectively. Each fraction inhibited agglutination of mouse erythrocytes by 16 haemagglutination units of PE. However, the lipoprotein fraction inhibited only to a dilution of 1 : 8, while the nonlipoprotein fraction inhibited at 1 : 32 dilution. After taking into account the respective volumes, it could be concluded that only about 2% of serum inhibitor resided in the lipoprotein fraction (Table I). This correlates well with the content of albumin (as determined by radial immunodiffusion), being 3.5% of that in serum.

(c) Chromatofocussing. Serum inhibitor eluted with proteins in the isoelectric point range 4.7–5.4.

(d) Albumin isolation. Human serum (diluted 5-fold in phosphate-buffered saline) was exposed to varying concentrations of ethanol (16–56%). Maximal inhibitory activity was found in the 32% precipitate which contained a substantial amount of albumin. No activity was found in the 56% precipitate which contained the bulk of albumin (Fig. 1).

The 32% ethanol precipitate was extracted with butanol. Most of the protein in the aqueous phase bound to Blue Sepharose. Inhibitor was found in the 0.5 M NaCl eluate of this column. All of the protein and inhibitor in the 0.5 M NaCl eluate bound to an anti-albumin Sepharose column (Fig. 2). On SDS-polyacrylamide gel electrophoresis, under reducing conditions, there was a major band in the region of albumin (M_r 70000) and several minor bands which may be breakdown products, since they were present in the fraction retained by an anti-albumin column. The major band had a markedly reduced apparent molecular weight on electrophoresis under non-reducing conditions, typical of albumin [13]. Per weight of albumin, inhibitory activity was comparable with that in whole serum (Table I).

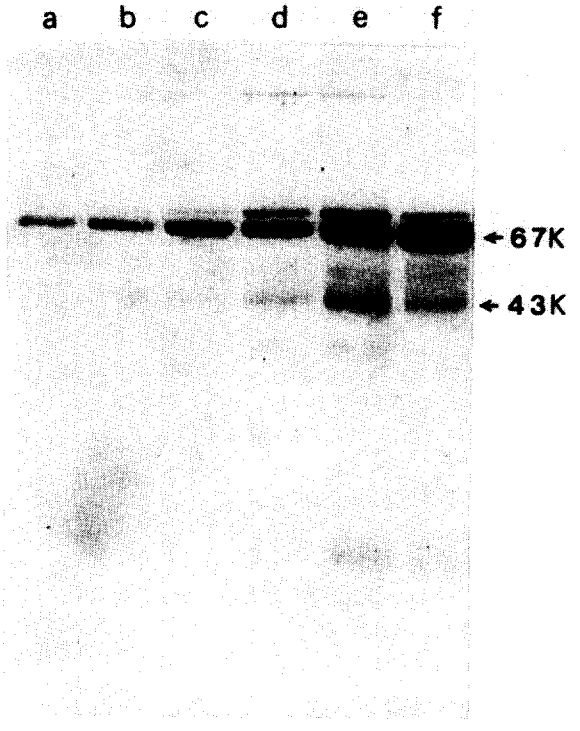

Fig. 1. Ethanol precipitation of serum inhibitor. Ethanol precipitates of 1 ml human serum were redissolved in 1 ml of phosphate buffered saline, tested for inhibitory activity, and analyzed by 10% SDS-polyacrylamide gel electrophoresis. Final concentrations (v/v) of ethanol were (a) 16%, (b) 24%, (c) 32%, (d) 40%, (e) 48% and (f) 56%, respectively. To quantify inhibitor, aliquots of each were incubated with serial dilutions of PE (first well containing 256 haemagglutination units) before addition of red cells. Inhibitory units per ml were (a) 80, (b) 80, (c) greater than 10240, (d) 640 and (f) 0, respectively. (f) contained the maximum amount of albumin (67 K band).

(e) Reaction with soluble anti-albumin. When this albumin was incubated for 30 min with an equal volume of the IgG fraction of a rabbit anti-human albumin antiserum, the solution lost capacity to inhibit.

(f) Commercial serum fractions. No commercial preparation of albumin inhibited haemagglutination. These included crystalline human albumin, human Cohn Fraction V, human delipidated Cohn Fraction V, bovine Cohn Fraction V and mouse Cohn Fraction V. The chromatography profiles of fresh serum albumin and crystalline albumin on Blue Sepharose were significantly different. When human serum (250 μl) was applied to Blue Sepharose CL-6B and albumin was quantified by radial immunodiffusion, the wash, 0.5 M NaCl eluate, 1.5 M NaCl eluate, 5 M NaCl eluate and 6 M urea eluate contained 0.1%, 3%, 49%, 25% and 23% of the total albumin applied, respectively. All of a human crystalline albumin preparation (2 mg) was in the 6 M urea eluate. Commercial preparations of fetuin (types III and IV), derived from foetal calf serum, inhibited haemagglutination. The inhibitor in fetuin was in a 1.5 M KCl eluate of Blue Sepharose CL-6B, which constituted about 10% of the original protein by weight. When human serum was exposed to 42.5% saturated ammonium sulphate at room temperature (method of preparation of fetuin from foetal calf serum [15]), approximately equal amounts of inhibitor were found in the precipitate and supernatant (Table I). These contained 33 and 67% of the serum albumin, respectively.

(g) Mouse erythrocyte ligand. The putative ligand for PE has been solubilized from mouse erythrocytes by extraction with hot aqueous phenol [1]. This ligand has properties similar to those reported here for the serum albumin inhibitor. It is

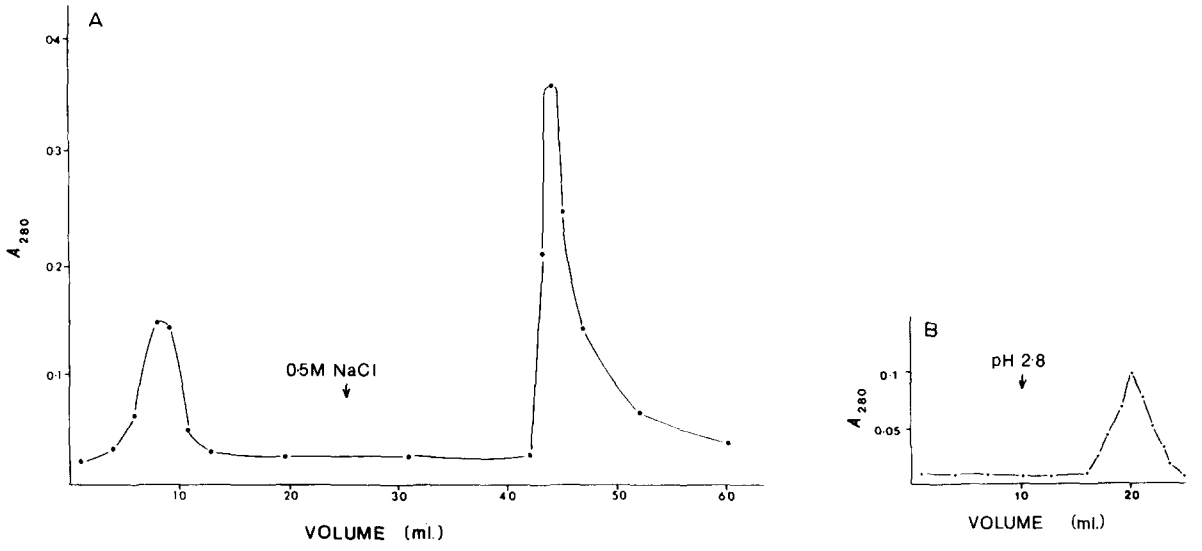

Fig. 2. Chromatography of serum inhibitor. The aqueous phase of a butanol extraction of a 32% ethanol precipitate of human serum (containing inhibitor) was chromatographed on Blue Sepharose. (A) All of the inhibitory activity was found in the 0.5 M NaCl eluate. (B) This was pooled, dialyzed and chromatographed on an anti-albumin Sepharose 4B column. All of the protein and inhibitor bound to this column and was eluted by a 0.2 M glycine (pH 2.8) buffer. Protein concentrations were determined by absorbance at 280 nm (●——●).

not yet possible to identify positively the mouse erythrocyte-derived inhibitor as albumin, because of the small quantity of material available. An analysis of the crude extract by radial immunodiffusion indicated that about 7% of it was mouse albumin. This is equivalent to the proportion of protein in this extract which bound to Blue Sepharose.

3. Thiol-activated albumin

When non-inhibitory commercial albumin preparations (crystalline human, delipidated fraction V human, fraction V human and bovine) were treated with dithiothreitol, they contained at least 512 inhibitory units per mg. Similar treatment of control proteins (chymotrypsinogen, α_1 acid glycoprotein and ribonuclease) and the tripeptide glutathione did not induce inhibitory activity. Treatment of human serum with dithiothreitol for 45 min at 37°C induced an 8-fold increase in inhibitory activity (from 215 to 1720 units per mg albumin). Activity in albumin treated for 60 min with dithiothreitol (1 mg/ml) was comparable to that in whole human serum. Thus, dithiothreitol-treated albumin contained 215 inhibitory units per mg albumin, while human serum contained 170–365 units per mg.

Treatment of crystalline albumin (5 mg/ml) with sodium dithionite and potassium metabisulphite induced 8 units, 2-mercaptoethanol induced 16 units and dithiothreitol induced 512 units of inhibitory activity per mg albumin.

Mechanism of activation

(i). Reduction of disulphides. Crystalline albumin was treated with dithiothreitol for varying periods, before chromatography on Sephadex G-25. Initially, there were approx. 0.5 sulphydryl groups per molecule of albumin and no inhibition of haemagglutination. Control treatment for 120 min had no effect. After 30 min with dithiothreitol, there were 2.5 sulphydryl groups per molecule of albumin and 50 units of inhibitor per mg albumin. Inhibitory activity peaked at 60 min (200 units) when 6 thiol groups on average were exposed. No more or less activity was seen at 120 min when about 12 thiols were available. No inhibitory activity was lost when dithiothreitol-reduced albumin was alkylated with iodoacetamide.

(ii). Alkali treatment. Incubation of delipidated

serum at pH 9.1 and low ionic strength did not affect inhibitory activity.

(iii). Polymerization. Increasing turbidity occurred with increasing time of exposure to dithiothreitol. A sample treated for 90 min was completely excluded from an Ultrogel AcA-34 column, indicating that all of the protein had a molecular weight greater than 130000. When human serum was fractionated on AcA-34, most of the inhibitor eluted in fractions having an approximate molecular weight of 65000–70000, suggesting that it was monomeric albumin. Some inhibitor and albumin eluted in the void volume.

Discussion

The agglutination of mouse erythrocytes by non-choline phospholipids [1] is a unique model of phospholipid-protein interaction with special relevance to the phenomenon of human B cell rosette formation with mouse erythrocytes [2]. The isolated receptor from human B cells is a protein-PE complex, in which PE forms the binding site for a 70 kDa mouse erythrocyte ligand [22]. The haemagglutination-inhibiting substance in human serum may be a natural ligand for this B cell receptor.

Efforts to isolate the serum inhibitor failed to separate the active material from albumin. The chromatographic profile using a series of dye-agarose columns was similar to that of albumin. On chromatofocussing, it behaves like albumin. A potent preparation of inhibitor obtained by ethanol precipitation, butanol extraction and Blue Sepharose chromatography was entirely retained by an anti-albumin affinity column. Analysis by SDS-polyacrylamide gel electrophoresis under reducing and non-reducing conditions was consistent with it being albumin [13]. The small amounts of inhibitory material in a lipoprotein fraction of human serum could be accounted for by the presence of albumin either as a contaminant or complexed with lipoprotein [14]. The inhibitor in commercial preparations of fetuin had the properties of albumin. Its presence in fetuin, in contrast to commercial preparations of human albumin, may be ascribed to the use of ammonium sulphate precipitation in preparing fetuin, but not human albumin. Albumin is known to be the major contaminant of fetuin [15]. A fraction of human serum obtained by an analogous method (precipitation with 40–45% ammonium sulphate) contained albumin and inhibitory activity. Finally, inhibitor reacted with soluble as well as Sepharose-bound anti-albumin.

Absence of inhibitory activity in a variety of commerical albumin preparations may be due to a loss or inactivation during Cohn fractionation. High concentrations of ethanol (at neutral pH and room temperature) inactivated inhibitor. It has been reported that commercial preparation alters some properties of albumin [16], and we observed a difference between the Blue Sepharose chromatography profile of fresh serum albumin and crystalline albumin.

Human crystalline albumin, after treatment with a variety of thiol reagents, completely inhibited the agglutination of mouse erythrocytes by PE. Inhibitory activity increased in thiol-treated albumin with increasing sulphydryl content, but blocking of the sulphydryl groups with iodoacetamide did not affect inhibitory activity, and other proteins were not similarly activated. The ability of different thiols to induce inhibitory activity increased with an increase in their negative redox potential [17], suggesting that their mechanism of action is by reduction of disulphides. 'Alkaline ageing' induces a disulphide bond interchange in monosulphydryl albumin, causing a different arrangement of intrachain S-S links and slightly different physicochemical properties [10]. This is catalyzed by thiols. 'Alkaline ageing' of serum albumin did not affect inhibitory activity. The possibility cannot be excluded that the thiols are acting on a low molecular weight substance tightly bound to albumin. Glutathione forms mixed disulphides with albumin [18]. However, neither reduced nor oxidized glutathione inhibited haemagglutination.

Thiols induced polymerization of albumin. It has been reported that liver cells have receptors for polymerized albumin and that some abnormal sera contain antibodies to these polymers [19]. The observation that naturally occurring serum inhibitor is both monomeric and polymeric argues against polymerization as the activating mechanism.

It remains to be determined whether all serum albumin molecules have some inhibitory capacity.

Augmentation of inhibitory activity by dithiothreitol treatment of serum may be due to conversion of noninhibitory to inhibitory albumin or to enhancement of the activity of inhibitory albumin.

That the putative mouse erythrocyte ligand also binds to Blue Sepharose and has a molecular weight similar to that of albumin [1] suggests that PE binds to albumin in mouse erythrocyte membranes. Thus, the mechanism of inhibition of PE-mediated haemagglutination by serum albumin is probably the binding to PE. Albumin is known to bind free fatty acids and lysophosphatidylcholine [20] and to interact with celite-bound phosphatidylcholine through its fatty acids [21]. However, to our knowledge, there has been no previous report of interaction of albumin with non-choline phospholipids, which must involve the head group, phosphate and fatty acids [1].

It is essential to determine whether PE actually binds to inhibitory albumin. Preliminary experiments partitioning free and protein-complexed PE in an aqueous/phenol system have been inconclusive.

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References

1 Forbes, I.J., Zalewski, P.D. and Valente, L. (1983) Biochim. Biophys. Acta 732, 179–185
2 Zalewski, P.D., Forbes, I.J., Gee, D. and Valente, L. (1982) Clin. Exp. Imm. 47, 405–413
3 Fuller-Noel, J.K. and Hunter, M.J. (1972) J. Biol. Chem. 247, 7391–7406
4 Havel, R.J., Eder, H.A. and Bragdon, J.H. (1955) J. Clin. Inv. 34, 1345–1353
5 Laemmli, U.K. (1970) Nature (London) 227, 680–685
6 Ouchterlony, O. and Nilsson, L.A., (1973) in Handbook of Experimental Immunology. Weir, D.M., ed., 2nd Edn, Blackwell Scientific Publications Oxford 19.1–19.39
7 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
8 Habeeb, A.F.S.A. (1972) Methods Enzymol. 25, 457–464
9 Chen, R.F. (1967) J. Biol. Chem. 242, 173–181
10 Stroupe, S.D. and Foster, J.F. (1973) Biochem. 12, 3824–3830
11 Travis, J. and Pannell, R., (1973) Clin. Chim. Acta 49, 49–52
12 Fulton, S., (1980) in Dye-Ligand Chromatography (Marois, M., ed.), Amicon Corporation, Picton Printing, Inc. MA, p. 10
13 Owen, M.J., Barber, B.H., Faulkes, R.A. and Crumpton, M.J. (1978) Biochem. Soc. Trans. 6, 920–922
14 Wille, L.E. (1977) Scand. J. Clin. Lab. Invest. 37, 175–181
15 Puck, T.T., Waldren, C.A. and Jones, C. (1968) Proc. Natl. Acad. Sci. U.S.A. 59, 192–199
16 Foster, J.F. (1977) in Albumin Structure, Function and Uses, (Rosenoer, U.M., Oratz, M. and Rothschild, M.A., eds.), pp. 53–84, Pergamon Press, New York
17 Liu, T.-Y., (1973) in The Proteins (Neurath. H. and Hill, R.L., eds.), 3rd Edn, pp. 240–402
18 Janatova, J., Fuller, J.K. and Hunter, M.J. (1968) J. Biol. Chem. 243, 3612–3622
19 Lenkei, R., Onica, D. and Ghetie, V. (1977) Experientia 33, 1046–1047
20 Switzer, S. and Eder, H.A. (1965) J. Lipid Res. 6, 506–511
21 Jonas, A. (1975) Biochim. Biophys. Res. Commun. 64, 1003–1008
22 Zalewski, P.D., Forbes, I.J. and Valente, L. (1984) J. Immunol., in the press