Renal plasma membrane receptors for certain modified serum albumins

Evidence for participation of a heparin receptor

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Binding of formaldehyde-treated (f-alb), reduced-carboxymethylated (ac-alb) or reduced-acetamidated (am-alb) bovine serum albumins to purified rat renal plasma membranes was studied. Radioiodinated f-alb or ac-alb bound to kidney membranes while am-alb neither bound significantly nor competed with f-alb binding to kidney membranes. The binding was specific, saturable and heat- and proteinase-sensitive. Competition studies showed that f-alb and ac-alb sites may be the same on these membranes. To determine the role played by charge in binding, competition experiments with polyanions were performed. Polyanions such as nucleic acid or glycosaminoglycans were effective competitors of f-alb binding to cell membranes. Heparin was especially inhibitory, being several-fold more so than chondroitin sulphate. Completely reduced and carboxymethylated albumin was a better competitor than its partially modified counterpart. Furthermore, f-alb was a significant competitor of [³⁵S]heparin binding to kidney membranes. Also, partially purified heparin receptor demonstrated specific binding of ¹²⁵I-f-alb. These data suggest that a heparin receptor is responsible for binding and internalization of intravenously injected f-alb. A Scatchard plot revealed two classes of receptors with dissociation constants of 3.2×10^{-6} M and 4.7×10^{-5} M.

INTRODUCTION

Serum albumin modified by treatment with formaldehyde (Mego & McQueen, 1965a,b) or by reduction and acetylation of disulphide bonds (Mego, 1984) injected intravenously into mice is rapidly cleared from the blood stream and localized in the liver and kidney. Endocytosis of denatured albumin followed by intralysosomal proteolysis has been demonstrated in mouse liver and kidneys (Mego *et al.*, 1967; Mego, 1973). F-alb is exclusively taken up and degraded by the sinusoidal liver cells (Nilsson & Berg, 1977). The endothelial compartment of liver is mainly responsible for the uptake of acetyl-LDL (Nagelkerke *et al.*, 1983; Blomhoff *et al.*, 1984a,b). Receptors on macrophages for acetylated or maleylated LDL (Goldstein *et al.*, 1979) and normal VLDL (Kramer *et al.*, 1983) have been demonstrated. A hepatic scavenger receptor (Blomhoff *et al.*, 1984b) that can also recognize denatured proteins such as f-alb has been hypothesized. Such receptors apparently bind proteins with increased negative charge as well as certain polyanions such as poly(vinyl sulphate) (Brown *et al.*, 1980). Degradation of cationized LDL by macrophages from homozygous familial hypercholesterolaemia patients has been shown (Basu *et al.*, 1976). A common hepatic receptor for both f-alb and acetyl-LDL was envisaged (Blomhoff *et al.*, 1984b) but demonstrated otherwise (Horiuchi *et al.*, 1985a). A hepatic receptor for f-alb has been purified and characterized (Horiuchi *et al.*, 1985b,c).

Uptake of f-alb is equal to or greater in the kidney than in liver, per g wet wt. of tissue, after intravenous injection into mice (Mego, 1971). Hepatic receptors for

native albumin do not exist (Stremmel *et al.*, 1983) while vasopressin receptors exist in mouse kidney but not in liver (Assimakopoulos-Jeannot *et al.*, 1983). Acetyl-LDL receptors are present in mouse macrophages but absent from non-macrophage cells such as mouse Y-1 adrenal cells (Brown & Goldstein, 1983). For these reasons, we investigated the possibility of a specific receptor for f-alb in kidney. In this paper we present data indicating the presence on kidney membranes of receptors specific for albumins modified in some ways but not others. Two classes of such receptors with different affinities appear to exist for which negative charge is required but not sufficient for recognition and/or binding. This receptor, which apparently scavenges xenobiotics such as f-alb, may be a heparin receptor.

MATERIALS AND METHODS

Materials

Iodoacetate, iodoacetamide, sucrose, citrate, Triton X-100, *Streptomyces griseus* proteinase type VI ('Pronase') and certain other chemicals were purchased from Sigma Chemical Co. Carrier-free Na¹²⁵I and [³⁵S]heparin were obtained from ICN. Spectropor dialysis tubings were from Fisher Scientific, and nitrocellulose filters (0.45 µm) were from Schleicher & Schüll. All other chemicals were reagent grade or the purest available from laboratory chemical distributors.

Animals

Sprague-Dawley rats, obtained from Charles River Breeding Laboratories, Wilmington, MA, U.S.A., were maintained in this institution's animal facilities until use.

Abbreviations used: alb, albumin; f-, ac-, am-, formaldehyde-treated, reduced-carboxymethylated, reduced-acetamidated; BSA, bovine serum albumin; LDL, VLDL, low and very low density lipoproteins.

Proteins

Recrystallized fat-free BSA (Sigma Chemical Co.) was labelled with carrier-free Na^{125}I by the chloramine-T method to a specific radioactivity of 10^7 c.p.m./mg and treated with formaldehyde as previously described (Mego, 1971, 1973) to yield ^{125}I -f-alb. Disulphides of undenatured ^{125}I -labelled albumin were reduced and alkylated by treatment with a 10-fold molar excess (with respect to disulphides) of dithiothreitol in 8 M-urea and 50 mM-Tris/acetate buffer, pH 8.0, for 4 h. Ac₁-alb was prepared by adding stoichiometric amounts of dithiothreitol just sufficient to reduce at most eight disulphide bonds. A 2-fold molar excess (with respect to dithiothreitol) of iodoacetate or iodoacetamide was then added and left at 23 °C for 30 min. The proteins were extensively dialysed against 0.1 M-NaCl at 4 °C with several changes.

Plasma membrane preparations

Renal plasma membranes were prepared as previously described (Alkhawajah, 1984). Briefly, male albino Sprague-Dawley (200–300 g) rats were killed after stunning, and kidneys were quickly removed and decapsulated. Ice-cold buffer (0.25 M-sucrose, 10 mM-Tris, 1 mM-EDTA, pH 7.4) was added to the tissues in a ratio of 1:3 (w/v) and homogenized with 10 strokes in a Dounce homogenizer. The homogenate was filtered through two layers of cheesecloth and centrifuged at 1475 g for 10 min at 4 °C. The sediment, containing nuclei, cell debris, mitochondria and membrane fragments, was resuspended in cold 2 M-sucrose (1 ml/g of original tissue), homogenized with three strokes and centrifuged at 13300 g for 10 min at 4 °C. The supernatant, containing membranes and mitochondria, was diluted with 7 vol. of ice-cold distilled water and centrifuged at 35000 g for 15 min, resulting in a two-layered pellet. The upper layer, containing membranes, was removed. The lower pellet was washed with the buffer, washings were mixed with the upper layer, mixed by two strokes in the homogenizer and centrifuged at 35000 g for 15 min. This pellet was resuspended in the buffer and recentrifuged. The final pellet was divided into portions in buffer and stored at –70 °C until use. The plasma membrane preparations used in this research are purified approx. 5-fold with respect to adenylate cyclase and 14-fold with respect to ($\text{Na}^+ + \text{K}^+$)-ATPase (Alkhawajah, 1984). Specific activities of marker enzymes for contaminating membranes are decreased approximately as follows: β -glucuronidase (lysosomes) from 8.8 to 0.9; succinic dehydrogenase (mitochondria), 1.3 to 0.3; and glucose-6-phosphatase (endoplasmic reticulum), 2.3 to 0.58 (Alkhawajah, 1984).

Receptor purification

All steps were performed at 4 °C. Frozen portions (2 ml) of renal plasma membrane preparations were thawed and centrifuged at 16000 g for 10 min. The pellet was sonicated three times for 10 s each with 20 s intervals in 1 ml of solubilization buffer (50 mM-Tris/HCl, 0.15 M-NaCl, 1 mM- CaCl_2 , 1% Triton X-100, pH 7.5) and incubated for 30 min, followed by centrifugation at 105000 g for 1 h. The supernatant was mixed with 5.5 ml (packed gel volume) of heparin-agarose gel pre-equilibrated with solubilization buffer and incubated on a rocking platform for 4 h. This gel slurry was poured into a 10 ml pipette and washed with 15 vol. of

solubilization buffer. Elution was performed with buffer E (50 mM-glycine/HCl, 0.15 M-NaCl, 1 mM- CaCl_2 and 0.25% Triton X-100, pH 3.0), collecting 14 fractions (each 1 ml) and immediately adding 7 μl of 1 M-NaOH to each fraction to raise the pH to the vicinity of 7.5. Based on protein content the first seven fractions were pooled and concentrated using an Amicon Diaflo apparatus fitted with a UM-10 membrane. This receptor preparation was divided into portions for protein estimation and future use.

Protein assay

Protein was determined according to Lowry *et al.* (1951), with BSA as standard. Membrane proteins were assayed in the presence of 0.8% sodium deoxycholate, pH 11.0.

Receptor binding assay

Renal plasma membranes were washed with 10 mM- NaHCO_3 /HCl, pH 7.5, before the assay. The indicated amounts of membrane fraction were incubated in a final volume of 0.12 ml of buffer A (50 mM-Tris/HCl, 0.15 M-NaCl, 1 mM- CaCl_2 , pH 7.5, with 18 mg of BSA/ml) and the indicated amounts of ^{125}I -ac-alb or ^{125}I -f-alb were added in the presence or absence of varying amounts of unlabelled f-alb or other competing ligands. Reactions were initiated by the addition of ^{125}I -f-alb or ^{125}I -ac-alb and continued for 1 h at 22 °C with four 10 s periods of vortex-mixing. Membrane-bound ^{125}I -f-alb was obtained by pelleting the membranes at 35000 g for 1 h at 4 °C followed by washing the pellet with 0.5 ml of ice-cold buffer A and recentrifuging at 35000 g for 30 min at 4 °C. After removing the supernatant, the attached cap of the tube was cut off and the radioactivity in the pellet was determined using a Beckman Gamma Counter 4000 (40% efficiency with ^{125}I) with automatic sample changer.

^{35}S Heparin binding to kidney plasma membranes experiments were as described above, except that buffer A contained 1.8 mg of BSA/ml. Membrane-bound radioactivity was measured by solubilizing the final pellet in 5 ml of toluene-based liquid scintillation fluid (Triton X-100/ethanol/Spectrofluor/toluene, 33:20:5.5:41.5, by vol.) (Spectrofluor is 25 g of PPO and 0.31 g of POPOP in 250 ml of toluene) and counting in the computerized Beckman liquid-scintillation counter model LS 3801.

Equilibrium dialysis of ^{35}S heparin against BSA or f-alb

^{35}S Heparin (25 μg) in a total volume of 1 ml was dialysed at 4 °C for 24 h against 150 ml of distilled water in Spectropor 3 (M_r cut-off 3500) dialysis tubing in order to remove any dialysable material. This was followed by an identical dialysis but in dialysis tubing with a cut-off of M_r 12000–14000. Buffer (50 mM-Tris, 0.15 M-NaCl and 1 mM- CaCl_2 , pH 7.5) alone or with added 1.8% BSA in the presence or absence of 1.88 mg of f-alb, each in a total volume of 1.00 or 1.06 ml, was further dialysed at 4 °C for 24 h against the second diffusate. Radioactivity trapped by each of the four dialysis residues was measured, as mentioned in the previous paragraph.

Dot-blot assay

Membrane preparation or purified receptor (15 μl each containing 8 μg of protein) was spotted onto a nitrocellulose filter and air-dried. Exposed areas on the

Fig. 1. ^{125}I -f-alb binding to kidney membrane as a function of ligand concentration

The indicated amounts of ^{125}I -f-alb were incubated with $45 \mu\text{g}$ of kidney membrane protein as described under 'Materials and methods', in the presence (○) or absence (▲) of $300 \mu\text{g}$ of unlabelled f-alb. Specific binding (●) equals total (▲) minus non-specific (○) binding. Each value is the mean of three experiments.

Fig. 2. ^{125}I -f-alb binding to kidney membranes as a function of membrane quantity

The indicated amounts of plasma membranes were incubated with $4 \mu\text{g}$ of ^{125}I -f-alb as described under 'Materials and methods', in the presence (○) or absence (▲) of a 100-fold molar excess of unlabelled f-alb. Specific binding (●) equals total (▲) minus non-specific (○) binding. Each value is the mean of two experiments.

filter were blocked with 1% non-fat dried milk in phosphate-buffered saline, dried and incubated with $2 \mu\text{g}$ of ^{125}I -f-alb (2.5×10^6 c.p.m.)/spot in 5 ml of buffer A on a rocking platform for 1 h at 22°C . The filter was then washed in 5 ml of half-strength buffer A each for 30, 30 and 60 min intervals, air-dried overnight and autoradiographed using Kodak X-Omat AR film exposed for 48 h at -70°C .

RESULTS

Binding of f-alb to rat kidney membranes was saturable both with respect to ligand (Fig. 1) and membrane (Fig. 2) concentrations. In order to determine if this binding was due to a scavenger receptor responsible for binding polyanions, as proposed by Brown *et al.* (1980), several experiments were performed to determine if negatively charged macromolecules would compete with f-alb for binding sites on kidney plasma membranes. Fig. 3 shows that heparin was an effective competitor but the closely related glycosaminoglycan, chondroitin sulphate, was not effective even at 10 times molar excess quantities. The only polyanions tested that were as effective as heparin were dextran sulphate and poly-L-aspartate (Fig. 3). Heparin, in fact, was almost as effective as f-alb itself in 2 h incubations (Fig. 4). Table 1 shows that fully reduced and carboxymethylated albumin was an effective competitor of f-alb binding. Partially reduced and carboxymethylated albumin was less effective, and untreated albumin had no effect. Am-alb neither bound by itself (results not shown) nor was an effective competitor of f-alb. Reciprocal binding experiments (Table 2) using ^{125}I -ac-alb as a probe not only shows that ac-alb bound to the membrane but was inhibited by excess f-alb or heparin.

Fig. 3. Effect of pretreatment with different concentrations of glycosaminoglycans and polyanions on ^{125}I -f-alb binding to kidney membranes

Membrane protein ($40 \mu\text{g}$) was incubated with $7 \mu\text{g}$ of ^{125}I -f-alb in the absence (control) or presence of the indicated molar excess of heparin (Δ) or chondroitin sulphate ($\blacktriangle$) as described under 'Materials and methods'. Pretreatment was for 15 min. Each value is the mean of two experiments. In experiments comparing the effect of dextran sulphate (○) or poly-L-aspartate (●), $20 \mu\text{g}$ of membrane protein was incubated with $2.5 \mu\text{g}$ of ^{125}I -f-alb and each value is the mean of two to four experiments. All other conditions remain the same.

Fig. 4. Comparison of inhibition of ^{125}I -f-alb binding to kidney membranes by heparin and f-alb

Membrane protein ($48\text{ }\mu\text{g}$) was incubated with $8\text{ }\mu\text{g}$ of ^{125}I -f-alb in the absence (control) or presence of the indicated molar excess of f-alb (○) or heparin (△) as described under 'Materials and methods', except that incubation was for 2 h. Pretreatment was for 15 min. Each value is the mean of two experiments.

Table 2. ^{125}I -ac-alb binding to kidney plasma membranes and competition by heparin or f-alb

Indicated amounts of ^{125}I -ac-alb were incubated with $43\text{ }\mu\text{g}$ of plasma membrane protein and assayed as described under 'Materials and methods'. Where applicable, a 10-fold molar excess of f-alb or heparin was added 10 min prior to the addition of ^{125}I -ac-alb. Each value is the mean of two to four experiments.

^{125}I -ac-alb (μg)	Competing compound (molar excess)	^{125}I -ac-alb bound	
		(ng/ μg of protein)	(% of control)
10	None (control)	11.9	100
20	None	15.0	—
30	None	21.2	—
10	$10\times$ f-alb	7.7	65
10	$10\times$ heparin	7.5	63

These results suggest charge to be at least one of the factors involved in the binding. Heating to 80°C or treatment with 'Pronase' almost completely abolished the binding of f-alb, suggesting that the receptor was a protein.

Treatment of kidney membranes with the lectin concanavalin A also effectively inhibited the binding of f-alb, suggesting that the receptor was a glycoprotein

Table 1. Effect of various compounds on ^{125}I -f-alb binding to kidney membranes

^{125}I -f-alb ($4\text{ }\mu\text{g}$) was incubated with $40\text{ }\mu\text{g}$ of plasma membrane protein and assayed as described under 'Materials and methods'. Indicated molar excess of competing compounds were added 10 min prior to the addition of ^{125}I -f-alb. In the case of heparin, it was added either simultaneously or after the incubation with ^{125}I -f-alb for 20 min. Treatment at 80°C was for 12 min. Each value is the mean of two to four experiments.

Expt.	Competition (molar excess)	^{125}I -f-alb bound	
		(ng/ μg of protein)	(% of control)
1	None	6.60	100
	$100\times$ f-alb	2.70	41
	$100\times$ BSA	6.65	101
	$100\times$ ac ₁ -alb	4.90	75
	$100\times$ ac-alb	2.60	39
2	None	8.10	100
	$50\times$ f-alb	3.90	48
	$70\times$ chondroitin sulphate	7.10	90
	$150\times$ poly-L-aspartate	5.20	64
	DNA ($300\text{ }\mu\text{g}$)	5.10	63
3	None	2.14	100
	$300\times$ f-alb	0.27	13
	$300\times$ heparin (simultaneous)	0.27	13
	$1\times$ heparin (simultaneous)	0.42	20
	$1\times$ heparin (post-treatment)	1.56	73
4	None	2.8	100
	$100\times$ f-alb	0.0	0
	$50\times$ am-alb	2.7	96
5	None	3.50	100
	80°C	0.10	3
	Pronase (0.1%)	0.06	2

Table 3. Effect of concanavalin A on ^{125}I -f-alb binding to kidney membranes

^{125}I -f-alb ($5\text{ }\mu\text{g}$) was incubated with $32\text{ }\mu\text{g}$ of plasma membrane protein in the absence of any competing compound (control) or in the presence of the indicated molar amounts of f-alb or concanavalin A and assayed as described under 'Materials and methods'. In the case of concanavalin A plus α -methyl D-mannoside, concanavalin A was added first followed by α -methyl D-mannoside, and vice-versa.

Incubation conditions	^{125}I -f-alb bound	
	(ng/ μg of protein)	(% of control)
Control	2.2	100
$200\times$ f-alb	0.9	41
Concanavalin A (72 pmol)	1.3	59
α -Methyl D-mannoside (2 nmol)	1.8	82
and then concanavalin A (72 pmol)		
Concanavalin A (72 pmol) and then α -methyl D-mannoside (2 nmol)	1.8	82

(Table 3). This inhibitory effect of concanavalin A was noticeably reduced by either pre-treatment or post-treatment with α -methyl D-mannoside, a known ligand for concanavalin A (Goldstein *et al.*, 1965).

Fig. 5 shows that ^{35}S heparin became bound to kidney plasma membranes in a concentration-dependent manner. This binding was almost completely abolished by a 10-fold excess of f-alb and significantly reduced by a 4-fold excess of ac-alb.

The inhibition of f-alb binding by heparin could have

Fig. 5. ^{35}S heparin binding to kidney membranes as a function of ^{35}S heparin concentration and competition by heparin, f-alb and ac-alb

Plasma membrane protein ($44\text{ }\mu\text{g}$) was incubated with the indicated amounts of ^{35}S heparin in the absence (concentration dependency, $\bullet$) or presence (competition) of heparin (500-fold molar excess, $\circ$), f-alb (10-fold molar excess, $\blacktriangle$) and ac-alb (4-fold molar excess, $\triangle$) and assayed as described under 'Materials and methods'.

Table 4. Equilibrium dialysis of ^{35}S heparin against BSA or f-alb

BSA and/or f-alb was dialysed to equilibrium against ^{35}S heparin, with the proper control, as described under 'Materials and methods'. Total radioactivity trapped by each dialysis residue was measured.

Expt. no.	Solution in dialysis tubing	^{35}S heparin trapped by dialysis residue	
		(c.p.m.)	(ng)
1	Buffer (control)	298741	98.9
2	Buffer + 1.88 mg of f-alb	272265	90.1
3	Buffer containing 1.8% BSA	702405	232.5
4	Buffer containing 1.8% BSA + 1.88 mg of f-alb	748083	247.6

been due either to heparin binding f-alb directly and thus preventing it from binding to the receptor, or to the competition being for the receptor itself. In order to distinguish between these two possibilities an equilibrium dialysis of ^{35}S heparin against f-alb was performed. Table 4 clearly shows that the inhibition of binding was not due to inter-molecular affinity between heparin and f-alb and hence could have been only for the receptor itself.

Fig. 6(a) shows the saturability of the receptors by increasing amounts of unlabelled f-alb. A Scatchard

Fig. 6. ^{125}I -f-alb binding to kidney membranes as a function of total f-alb concentration

(a) Membrane protein ($20\text{ }\mu\text{g}$) was incubated with $0.3\text{ }\mu\text{g}$ of ^{125}I -f-alb in the presence of the indicated amounts of unlabelled f-alb as described under 'Materials and methods'. Each value is the mean of two experiments. (b) Scatchard plot of the same data.

Fig. 7. ^{125}I -f-alb binding to whole membranes and partially purified heparin receptor

Protein (8 μg /spot) was incubated with 2 μg of ^{125}I -f-alb and assayed as described under 'Materials and methods', 1, Liver membrane; 2, kidney membrane; 3, partially purified heparin receptor; 4, as 3, but pretreated with 400 μg of unlabelled f-alb for 15 min.

(1949) plot (Fig. 6b) of data obtained over the concentration range 2.7–1366 $\mu\text{g}/\text{ml}$ indicated the presence of two classes of receptors with different affinities. The calculated apparent dissociation constants (K_d) for the two classes were: high affinity, $K_d 3.2 \times 10^{-6}$ M; low affinity, $K_d 4.7 \times 10^{-6}$ M.

A heparin receptor was partially purified from detergent-solubilized rat kidney membranes by heparin-agarose affinity chromatography. This partially purified receptor protein was then used in dot-blot experiments for f-alb binding and competition studies. Results of the dot-blot (Fig. 7) showed that unlabelled f-alb competed effectively with ^{125}I -f-alb for the heparin receptor and that an equivalent quantity of intact membrane protein under identical conditions was insufficient to bind detectable ^{125}I -f-alb, suggesting significant purification of the receptor.

DISCUSSION

BSA is a single polypeptide with 61 lysine residues (Putnam, 1965) and 17 disulphides (Brown, 1974, 1975). Using ^{125}I -f-alb as the main probe we have identified a receptor on rat kidney plasma membranes that may mediate the uptake and degradation of certain chemically modified serum albumins in kidney. The data presented in this paper demonstrate that f-alb became bound to the renal plasma membrane in a concentration-dependent manner, both with respect to ligand and membrane protein concentrations. Sensitivity to heat and proteinase (Table 1) and concanavalin A (Table 3) suggested that the binding site was a glycoprotein. Table 1 shows results of five separate experiments. The reason for difference in the extent/degree of inhibition of ^{125}I -f-alb binding to membranes by unlabelled f-alb (Table 1, expt. 1 versus expt. 4, or versus Fig. 4) is not clear. We can only attribute this to variations between experiments and age of membrane and ligand preparations. For this reason, most experiments were performed with their own (internal)

controls and comparisons were made within and not between experiments.

The fact that polyanions such as DNA, poly-L-aspartate, heparin and dextran sulphate effectively competed with f-alb binding strongly suggests a crucial role for the negative charge on the ligand(s). Such results have been reported elsewhere (Basu *et al.*, 1976; Brown & Goldstein, 1983; Horiuchi *et al.*, 1985a). Moreover, am-alb was neither an effective competitor nor showed significant binding by itself, probably because am-alb, though structurally modified, should have no net change in charge. A similar result has been reported by Brown *et al.* (1980) using reductively methylated LDL as the ligand. Furthermore, completely reduced and carboxymethylated albumin was a better competitor for f-alb binding than was the partially modified form. The former, due to the addition of approximately double the number of carboxyl groups than in the latter, would have a stoichiometric increase in the negative charges added. Carboxymethylation of thiol groups adds further carboxyl groups (negative charges) whereas formaldehyde treatment eliminates amino groups (positive charges) of albumin. The albumin used in these studies may be arranged with respect to increasing negative charge in the following order: BSA or am-alb, ac₁-alb, ac-alb and f-alb. This arrangement paralleled increasing competition with f-alb, suggesting a threshold negative charge on the ligand to be a crucial factor for binding to the receptor. BSA and am-alb may fall below this threshold. The nature of the negative charge seems to be immaterial. We emphasize that a more exhaustive study is warranted here, although the above conclusion is a possibility and hence cannot be ignored. Such a threshold effect has been well demonstrated with acetyl-LDL receptor on human monocyte-derived macrophages (Haberland *et al.*, 1982). Because native albumin and glycosaminoglycans such as chondroitin sulphate were not effective competitors of f-alb binding, it is probable that a certain magnitude rather than density of negative charge was necessary but not sufficient for binding.

DNA, poly-L-aspartate and heparin, all of which competed effectively with f-alb for binding, are linear polymers with uniformly distributed negative charges. On the other hand, dextran sulphate, though not a linear polymer, has uniformly distributed negative charges and was an effective competitor. These data (Tables 1 and Fig. 3) raise a question whether a charge localization, as proposed by Brown & Goldstein (1983) is a necessity or only an aid to binding.

It has been shown that even untreated BSA is taken up in the lysosomal fraction, to some extent, when compared with f-alb (Mego, 1971). This, when viewed in conjunction with results obtained with ac₁-alb and ac-alb in the present studies, tempts one to correlate binding directly with the degree of denaturation. Such a correlation has been invoked in uptake studies using liver cells (Mego *et al.*, 1967) and rat yolk-sac epithelium (Moore *et al.*, 1974, 1977).

Reciprocal binding experiments using [^{35}S]heparin as the probe and f-alb as the competitor gave comparable results (Fig. 5). F-alb, unlike BSA, did not bind heparin much more than the control (Table 4). This may have been due to the increased negative charge density of f-alb and its increased compactness, as evidenced by its increased electrophoretic mobility (P. N. Ranganathan & J. L. Mego, unpublished work) compared with BSA. The

possibility of BSA losing its heparin binding site as a result cannot be ignored. This suggested the existence of at least one class of receptors common to heparin and f-alb. Additionally, such a putative heparin receptor, though only partially purified, exhibited binding of ^{125}I -f-alb that was replaceable by unlabelled f-alb. An equal quantity of total membrane protein, under identical conditions, failed to show significant f-alb binding (Fig. 7). More important is the fact that heparin, in low concentrations, is physiological whereas f-alb is xenobiotic.

In lysosomal uptake studies of f-alb in mice, the usual quantity injected was 1 mg (Mego, 1971). Assuming a blood volume of 2 ml, the f-alb concentration is 7×10^{-6} M, which is higher than the K_d of 3.2×10^{-6} M (Fig. 6b) obtained in the present studies. The concentration of heparin in plasma (human) varies from 48 to 138 mg/kg (Serafin, 1957) whereas it is approx. 16 mg/kg in rat serum (Mergenthaler & Paff, 1956). From this standpoint, 14.7–83 mg of heparin/l, the concentration range in which significant binding of heparin to rat kidney membranes occurred, would fall within the physiological concentration range of heparin in rats. In view of these data it is reasonable to suggest that it was a heparin receptor in kidney that bound f-alb. The results obtained in these studies also suggested the presence of two classes of receptors, one with a high affinity and another with a relatively low affinity. The low-affinity kidney receptor requires more than ten times higher concentrations of f-alb and hence is probably of little significance with respect to binding and removal of this protein from the bloodstream after intravenous injections. Table 1 and Figs. 1 and 2 showed that rat kidney plasma membranes bound 2–4 ng of f-alb/ μg of membrane protein, which suggests that about 0.2–0.4% of the proteins in these membranes may be a scavenger (heparin) receptor. In similar studies using purified rat liver sinusoidal membranes, Horiuchi *et al.* (1985c) obtained values of 0.2–0.5 μg of f-alb bound/mg of membrane protein and a K_d of 7.8 $\mu\text{g}/\text{ml}$, which corresponds to 1.1×10^{-7} M. This only suggests that the receptor in the kidney may have a lower affinity for f-alb than does the liver receptor.

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