THE JOURNAL OF BIOLOGICAL CHEMISTRY
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Vol. 261, No. 11, Issue of April 15, pp. 4962–4966, 1986
Printed in U.S.A.

Scavenger Receptor for Aldehyde-modified Proteins*

(Received for publication, December 3, 1985)

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This paper describes an unexpectedly broad ligand specificity of a scavenger receptor of sinusoidal liver cells that is responsible for endocytic uptake of formaldehyde-treated bovine serum albumin (f-Alb). Binding of ^{125}I-f-Alb to the isolated cells was effectively inhibited by bovine serum albumin (BSA) modified with aliphatic aldehydes such as glycolaldehye, DL-glyceraldehyde, and propionaldehyde whereas albumin preparations modified by aromatic aldehydes such as pyridoxal, pyridoxal phosphate, salicylaldehyde, and benzaldehyde did not affect this binding process. Binding of ^{125}I-glycolaldehyde-treated BSA to the cells exhibited a saturation kinetics with an apparent K_d = 3.3 μg of the ligand/ml. This binding process was inhibited by unlabeled f-Alb as well as by the antibody raised against the f-Alb receptor. Indeed, ^{125}I-glycolaldehyde-treated BSA underwent a rapid plasma clearance ($t_{1/2}$ ~ 2 min) which was markedly retarded by unlabeled f-Alb. Upon treatment by these aldehydes, other proteins such as ovalbumin, soybean trypsin inhibitor, and hemoglobin were also converted to active ligands for the f-Alb receptor, while no ligand activity was generated with γ-globulin and RNase A. These results clearly show that the f-Alb receptor, originally described as being specific for f-Alb, exhibits a broad ligand specificity in terms of both aldehydes and proteins and, hence, should be described as a scavenger receptor for aldehyde-modified proteins.

Scavenger function of macrophages or macrophage-derived cells for chemically modified proteins has been known with formaldehyde-treated bovine serum albumin (f-Alb[1]) (1–4), maleylated albumin (5, 6), and malondialdehyde-modified (7) and acetylated low-density lipoprotein (5, 8–10). These chemically modified proteins have a common biological feature of being endocytosed via a receptor-mediated mechanism, when infused intravenously, by sinusoidal liver cells, major scavenger cells *in vivo*.

Earlier studies from this laboratory have demonstrated the presence of a scavenger receptor for f-Alb on the plasma membrane of sinusoidal liver cells (11, 12). Moreover, this receptor was found to be distinct (13) from the scavenger receptor claimed as being specific for the negatively charged proteins (5, 14) such as acetylated, molondialdehyde-modified low-density lipoprotein and maleylated albumin. Thus, at least two distinct scavenger receptors seem to be present on plasma membranes of sinusoidal liver cells (15–17) and peritoneal macrophages (18, 19). However, the physiological role of the f-Alb receptor is poorly understood largely due to the argument that the formation of the ligand is unlikely to occur *in vivo* under physiological conditions. To elucidate its physiological function, knowledge on the molecular basis of the ligand specificity of this receptor appears to be essential.

In the present study we have addressed two questions of whether or not the receptor would recognize albumin modified by aldehydes other than formaldehyde, and whether or not this phenomenon could be extended to any proteins other than bovine serum albumin (BSA). The results indicate that the f-Alb receptor, originally described as specific for albumin modified by formaldehyde, recognizes several proteins modified by aliphatic aldehydes as its ligand. Thus, this unexpectedly broad ligand specificity of the receptor suggests its role as a scavenger receptor for aldehyde-modified proteins.

MATERIALS AND METHODS

Chemicals—BSA (Fraction V) from Sigma was chromatographed on a Sephacryl S-200 column and the monomeric fraction was used as an albumin source for preparation of chemically modified ligands. RNase A, ovalbumin, soybean trypsin inhibitor, human hemoglobin A, and bovine γ-globulin were purchased from Sigma. Formaldehyde, DL-glyceraldehyde, proprionaldehyde, benzaldehyde, dihydroxyacetone, and collagenase were purchased from Wako Chemical Co. (Osaka, Japan). Glycolaldehyde, dihydroxyacetone phosphate, pyridoxal 5′-phosphate, and salicylaldehyde were obtained from Sigma. Na^{125}I (15.8 mCi/μg of iodine) was from Amersham. All reagents used were of the best grade available from commercial sources.

Preparation of Aldehyde-modified Proteins—The f-Alb was prepared by a slight modification of the method reported previously (11). BSA was treated with 0.33 M formaldehyde at 37 °C for 1 h. The modification of BSA by other aldehydes was performed as follows. The reaction mixture contained, in a total volume of 1 ml of 0.1 M sodium carbonate buffer (pH 10.0), 7.2 mg of BSA and either of 50 mM glycolaldehyde, 50 mM glyceraldehyde, 0.1 M acetaldehyde, 0.1 M propionaldehyde, 0.1 M dihydroxyacetone, 20 mM dihydroxyacetone phosphate, 0.1 M pyridoxal, 0.1 M pyridoxal 5′-phosphate, 0.1 M salicylaldehyde, or 0.1 M benzaldehyde. Each reaction mixture was incubated at 37 °C for 5 h in the dark, followed by extensive dialysis against 20 mM sodium phosphate buffer (pH 7.4) containing 0.15 M NaCl at 4 °C. Under the same conditions, soybean trypsin inhibitor (3.6 mg), hemoglobin (6.2 mg), ovalbumin (5.4 mg), γ-globulin (12.5 mg), and RNase A (2.6 mg) were treated with glycolaldehyde, DL-glyceraldehyde, or propionaldehyde. Under the identical conditions, these proteins were treated with glycolaldehyde, DL-glyceraldehyde, or propionaldehyde in the presence of 50 mM $NaBH_4$. After extensive dialysis, all these modified proteins were determined both for the extent of lysine modification and for their ligand activity by the binding assay described below. The extent of lysine modification by these aldehydes was determined according to the method of Habeeb (20) with trinitrobenzenesulfonic acid as the difference in lysyl residues of modified and unmodified protein preparations. The extent of lysine modified was expressed as percentage of modification. ^{125}I-f-Alb and ^{125}I-glycol-Alb was prepared as described previously (11) by iodination with ^{125}I to a specific radioactivity of 5400 and 3200 cpm/

* This work was supported in part by a grant-in-aid for scientific research from the Ministry of Education, Science and Culture of Japan. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] The abbreviations used are: f-Alb, formaldehyde-treated BSA; BSA, bovine serum albumin; glycol-Alb, glycolaldehyde-treated BSA; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

ng, respectively. The protein concentrations were determined by the method of Lowry *et al.* (21).

Binding Assay—Sinusoidal liver cells were prepared from male Wistar rats (200–240 g) as described previously (11) by a modification of the collagenase perfusion method (22). The isolated cells were suspended in Eagle's minimal essential medium containing 3% BSA buffered with 20 mM Hepes to pH 7.4 (buffer A). Binding assay was performed in a 1.6-ml polyethylene centrifuge tube (Eppendorf) as described previously (13). The reaction mixture contained, in a total volume of 0.1 ml of buffer A, 3×10^6 cells and a fixed amount of ^{125}I-labeled ligand in the absence or presence of an unlabeled ligand to be tested. The reaction was initiated by the addition of ^{125}I-f-Alb or ^{125}I-glycol-Alb and incubated on ice for 1 h with several intervals on a Vortex mixer. Upon termination of the reaction, 1 ml of ice-cold buffer A was added to each reaction tube, followed by centrifugation at 12,800 × *g* for 25 s at 4 °C. The supernatant was discarded and the pelleted cells were resuspended in 1.0 ml of ice-cold buffer A. After the cells were washed twice more, the cell-associated radioactivity was measured as described previously (13). Unless otherwise specified, each value in the figures and table represents the mean value of duplicate assays. The Scatchard analysis was performed as described (12).

Plasma Clearance—Effects of unlabeled glycol-Alb and f-Alb on the plasma clearance of ^{125}I-glycol-Alb were performed as described previously (23). The injected solution contained 2 μg of ^{125}I-glycol-Alb (1800 cpm/ng) alone or with either 2.6 mg of unlabeled glycol-Alb or 3.6 mg of unlabeled f-Alb. Each sample dissolved in buffer A (0.1 ml/100 g of body weight) was injected intravenously via the femoral vein of a male Wistar rat (200–220 g). Blood samples (100 μl) were withdrawn at various times from the jugular vein into heparinized tubes, followed by centrifugation at 1000 × *g* for 5 min at 4 °C. Each plasma aliquot (30 μl) was measured for trichloroacetic acid-precipitable radioactivity. The amount of acid-precipitable radioactivity in plasma was expressed as percentage of the injected dose, assuming a plasma volume of 3.13 ml/100 g body weight as described previously (23). Each plasma clearance curve represents a typical pattern obtained from three separate experiments.

Anti-f-Alb Receptor Antibody—The antiserum was raised in rabbits against the f-Alb receptor purified from rat liver as described previously (12). IgG fraction was prepared from both the antiserum and the preimmune serum by chromatography on a DEAE-Sephacel column as described previously (24).

RESULTS

Effect of BSA Modified with Glycolaldehyde and DL-Glyceraldehyde on f-Alb Receptor—To test whether the f-Alb receptor also recognizes BSA modified by aldehydes other than formaldehyde, effects of glycol-Alb and DL-glyceraldehyde-treated BSA on the f-Alb receptor were examined. As Fig. 1 shows, glycol-Alb inhibited effectively the binding of ^{125}I-f-Alb to sinusoidal liver cells: 50% inhibition was achieved at a concentration as low as 10 μg/ml of glycol-Alb (Fig. 1*B*). Comparison with the data for f-Alb (Fig. 1*A*) clearly shows that glycol-Alb serves as an excellent ligand for the f-Alb receptor. Although less effective, BSA treated by DL-glyceraldehyde was also similarly recognized by the f-Alb receptor (Fig. 1*C*). However, BSA treated by these aldehydes in the simultaneous presence of $NaBH_4$, a reagent known to reduce the aldimine bond (Schiff base) formed between aldehyde and lysine residue(s) of the protein, failed to compete with ^{125}I-f-Alb for the f-Alb receptor. Thus, it is likely that the reaction of these aldehydes with BSA via its peptidyl lysine(s) may lead to the generation of the ligand activity, and that the reduced form of aldimine may not be recognized by the f-Alb receptor.

Since previous studies (13, 25) have shown that the f-Alb receptor binds polyanionic compounds such as dextran sulfate and poly(L-glutamic acid), a possible role of increase in negative net charges of BSA for the specific recognition by the receptor was examined. Unlike f-Alb, modification of BSA with glycolaldehyde or glyceraldehyde resulted in only slight increase in negative net charges as judged from their electrophoretic mobility. However, acetylated BSA which was much more negative did not affect the binding of ^{125}I-f-Alb to the cells (data not shown). Thus, the increase in negative net changes *per se* may not play a major role in the receptor recognition.

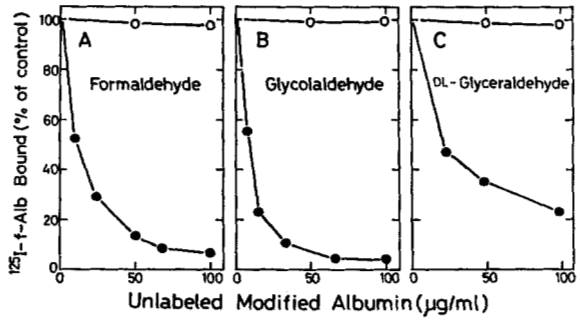

FIG. 1. **Effect of BSA modified by glycolaldehyde and DL-glyceraldehyde on ^{125}I-f-Alb binding to sinusoidal liver cells.** BSA was treated with formaldehyde, glycolaldehyde, or DL-glyceraldehyde in 0.1 M sodium carbonate buffer (pH 10.0) for 3 h at 37 °C in the absence (●) or presence (○) of 50 mM $NaBH_4$ as described under "Materials and Methods." After extensive dialysis, each sample was tested for the ligand activity by the binding assay. Each tube contained, in a total volume of 0.1 ml of buffer A, 3×10^6 cells, 0.37 μg of ^{125}I-f-Alb (4500 cpm/ng), and indicated amounts of unlabeled f-Alb (*A*), glycol-Alb (*B*), or DL-glyceraldehyde-treated BSA (*C*). After incubation on ice for 1 h with several intervals on a Vortex mixer, the cell-associated radioactivity was determined as described under "Materials and Methods." The extent of lysine modification of each competing ligand was as follows: f-Alb, 46.2%; glycol-Alb, 58.3%; and glyceraldehyde-treated BSA, 62.3%.

Effect of BSA Modified by Other Aldehydes on the Binding of ^{125}I-f-Alb to the f-Alb Receptor—To further probe into the ligand specificity of the f-Alb receptor, BSA derivatives prepared by treating with various aldehydes were examined for their effects on the binding of ^{125}I-f-Alb to sinusoidal cells. Treatment with aliphatic aldehydes such as propionaldehyde, acetaldehyde, dihydroxyacetone, and dihydroxyacetone phosphate converted BSA to active ligands for the f-Alb receptor (Table I). In sharp contrast, treatment with aromatic aldehydes such as pyridoxal, pyridoxal phosphate, salicylaldehyde, and benzaldehyde failed to produce active ligands. With all of these aldehyde-treated samples, the extent of modification of lysyl residues was similar to or even higher than that by formaldehyde (Table I). These findings indicate that derivatives of BSA modified by aliphatic aldehydes but not by aromatic aldehydes could be active ligands for the f-Alb receptor.

Binding of ^{125}I-Glycol-Alb to Sinusoidal Liver Cells—The binding of ^{125}I-glycol-Alb to sinusoidal cells reached an equilibrium within 80 min at 0 °C. In the presence of 100-fold excess of unlabeled glycol-Alb, the binding of ^{125}I-glycol-Alb was reduced by more than 90% (data not shown), indicating that the unlabeled glycol-Alb and radioactive glycol-Alb were competing for a limited number of common binding sites. The binding of ^{125}I-glycol-Alb to the cells at 0 °C as a function of the concentration of ^{125}I-glycol-Alb in the incubation medium exhibited a typical saturation curve (Fig. 2). The Scatchard analysis revealed a straight line indicating the involvement of a single binding mode with an apparent K_d = 3.3 μg of protein/ml and B_{max} = 2.3 ng of the ligand/10^6 cells (Fig. 2, *inset*). The binding of ^{125}I-glycol-Alb to the cells was also effectively inhibited in a dose-related manner by unlabeled f-Alb (Fig. 3*A*). This finding, together with the data shown in Fig. 1*B*, provides evidence that both f-Alb and glycol-Alb bind to a single site of the f-Alb receptor.

TABLE I

Effect of BSA preparations treated with various aldehydes on ^{125}I-f-Alb binding to sinusoidal liver cells

Each assay contained, in a total volume of 0.1 ml of buffer A, 3 × 10^6 cells and 0.37 μg of ^{125}I-f-Alb (4500 cpm/ng) in the presence or absence of indicated amounts of albumin preparations treated with various aldehydes. After incubation on ice for 1 h, the amount of cell-associated radioactivity was determined as described under "Materials and Methods." Nonspecific binding was determined by parallel incubations in the presence of 1.0 mg of unlabeled f-Alb. The specific binding was determined by subtracting nonspecific binding from the total binding. Procedures for modification by these aldehydes was described under "Materials and Methods." Each value represents the mean of duplicate assays. Figures in parentheses show the percentage of the control value for the specific binding.

Aldehydes	Extent of lysine modification	Ligand concentration	Total binding	Specific binding
	%	μg/ml	cpm/system	
None			5430	4920 (100)
f-Alb	46.2	50	1020	510 (10.4)
		100	770	260 (5.3)
Propionaldehyde	64.4	50	1130	620 (12.6)
		100	830	320 (6.5)
Acetaldehyde	53.8	50	1420	910 (18.5)
		100	1030	520 (10.6)
Dihydroxyacetone	59.3	50	1280	770 (15.7)
		100	940	430 (8.7)
Dihydroxyacetone phosphate	56.9	50	1630	1120 (22.8)
		100	1220	710 (14.4)
Pyridoxal	48.3	50	5370	4820 (98.0)
		100	5270	4760 (96.7)
Pyridoxal phosphate	68.2	50	5360	4850 (98.6)
		100	5220	4710 (95.7)
Salicylaldehyde	73.4	50	5260	4750 (96.5)
		100	5230	4720 (96.0)
Benzaldehyde	77.2	50	5200	4690 (95.3)
		100	5120	4610 (93.7)

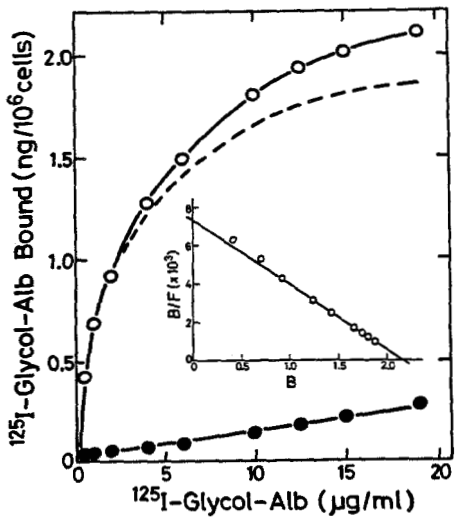

FIG. 2. **Binding of ^{125}I-glycol-Alb to sinusoidal liver cells as a function of its concentration.** Each tube contained, in a final volume of 0.1 ml of buffer A, 3 × 10^6 cells and the graded amounts of ^{125}I-glycol-Alb (3200 cpm/ng). After incubation for 1 h at 0 °C, the total binding (○) was determined as described under "Materials and Methods." Nonspecific binding (●) was determined by parallel incubation in the presence of 200 μg of unlabeled glycol-Alb. Specific binding (– – –) was obtained by subtracting the nonspecific binding from the total binding. The *inset* shows the Scatchard plot for the specific binding: *F*, free ^{125}I-glycol-Alb; *B*, bound ^{125}I-glycol Alb.

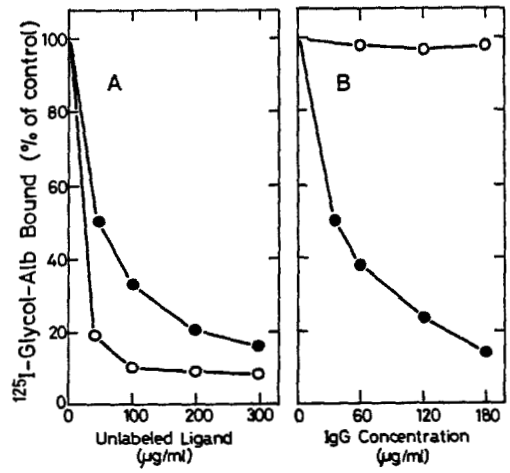

FIG. 3. *A*, effect of unlabeled f-Alb on ^{125}I-glycol-Alb binding to sinusoidal cells. Each assay tube received 0.1 ml of buffer A containing 3 × 10^6 cells, 0.31 μg of ^{125}I-glycol-Alb (3200 cpm/ng), and indicated amounts of unlabeled f-Alb (●) or glycol-Alb (○). The cell-associated radioactivity was determined as described under "Materials and Methods." *B*, effect of anti-f-Alb receptor antibody on ^{125}I-glycol-Alb binding to sinusoidal cells. Each tube received 0.1 ml of buffer A containing 3 × 10^6 cells and increasing amounts of either anti-f-Alb receptor IgG (●) or preimmune rabbit (○). After incubation for 20 min at 0 °C, the binding reaction was initiated by adding 10 μl of ^{125}I-glycol-Alb (3.1 μg/ml, 3200 cpm/ng), followed by incubation for 1 h at 0 °C. The cell-associated radioactivity was determined as described under "Materials and Methods."

Based on the previous finding that the antibody raised against the f-Alb receptor has a capacity to block specifically the binding of ^{125}I-f-Alb to both sinusoidal cells (13) and their plasma membranes (12), we tested for the effect of the anti-receptor antibody on the binding of ^{125}I-glycol-Alb to sinusoidal cells. As Fig. 3*B* shows, the binding of ^{125}I-glycol-Alb was effectively blocked by treatment with the antibody whereas IgG purified from a preimmune serum had no effect on this binding process. This result lends further support to the contention that glycol-Alb serves as an active ligand for the f-Alb receptor.

The conclusion drawn from the *in vitro* experiments was also supported by the following *in vivo* observation. Intravenous injection of a trace amount of ^{125}I-glycol-Alb resulted in a rapid disappearance from the blood stream within a few minutes. The plasma clearance was significantly retarded by simultaneous injection of a loading amount of unlabeled f-Alb as well as unlabeled glycol-Alb (Fig. 4). Reciprocally, the plasma clearance of ^{125}I-f-Alb was also significantly retarded by the simultaneous injection of a loading amount of unlabeled glycol-Alb in a manner similar to that observed above (data not shown).

Ligand Activity of Other Proteins Treated with Aliphatic Aldehydes—Since no ligand activity was generated when the reductively methylated BSA was further treated with aliphatic aldehydes (data not shown), it is likely that BSA becomes an active ligand by reacting with aliphatic aldehydes via its lysyl residue(s). Then, one may ask a question whether or not any protein containing lysyl residues could be converted to an active ligand for the f-Alb receptor when its peptidyl lysine(s) is modified by aliphatic aldehydes. This possibility was investigated by examining the effect of several proteins treated with glycolaldehyde, glyceraldehyde, and propionaldehyde on the binding of ^{125}I-f-Alb to sinusoidal cells. To our surprise, ovalbumin, soybean trypsin inhibitor, and hemoglobin treated with these aldehydes were found to inhibit the binding of ^{125}I-f-Alb to the cells whereas γ-globulin and RNase A similarly

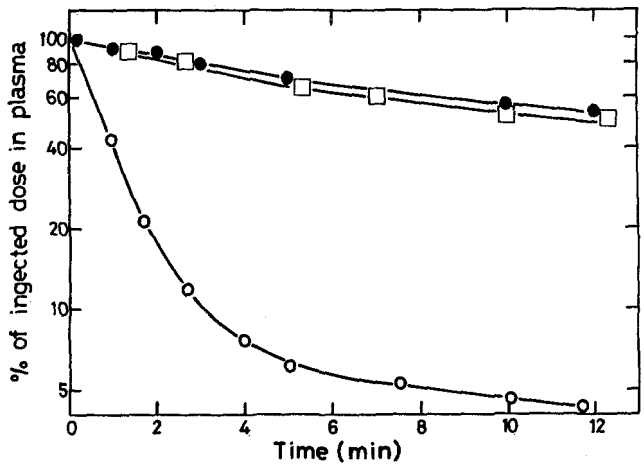

FIG. 4. **Effect of f-Alb on plasma clearance of ^{125}I-glycol-Alb.** The samples containing 2 μg of ^{125}I-glycol-Alb (1800 cpm/ng) (○), 2 μg of ^{125}I-glycol-Alb plus 2.6 mg of unlabeled glycol-Alb (●), or 2 μg of ^{125}I-glycol-Alb plus 3.6 mg of f-Alb (□) were prepared in buffer A. Each sample was injected (0.1 ml/100 g of body weight) intravenously into a rat. Blood samples were taken at indicated times and the plasma was separated by brief centrifugation, followed by determination of the trichloroacetic acid-precipitable radioactivity. The amount of acid-precipitable radioactivity in plasma was expressed as percentage of the injected dose assuming a plasma volume of 3.13 ml/100 g body weight as described under "Materials and Methods."

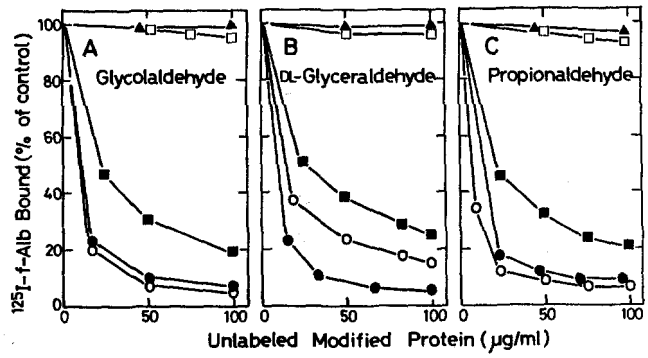

FIG. 5. **Effect of several proteins treated with glycolaldehyde, DL-glyceraldehyde, and propionaldehyde on binding of ^{125}I-f-Alb to sinusoidal liver cells.** Soybean trypsin inhibitor (○), human hemoglobin (●), ovalbumin (■), γ-globulin (□), and RNase A (▲) were modified either by glycolaldehyde (*A*), DL-glyceraldehyde (*B*), or propionaldehyde (*C*) as described under "Materials and Methods." The effects of these modified proteins on the binding of ^{125}I-f-Alb to the cells were examined at the indicated concentrations by the binding assay as described under "Materials and Methods." Each point represents the mean value of the duplicate assays.

treated showed no ligand activity (Fig. 5). Neither of these proteins showed any ligand activity upon modification with aromatic aldehydes.

DISCUSSION

The results obtained in the present study are 2-fold: (i), derivatives of BSA modified with several aliphatic aldehydes other than formaldehyde are also recognized by the f-Alb receptor, and (ii) other proteins such as ovalbumin, soybean trypsin inhibitor, and hemoglobin could also serve as active ligands for the receptor when modified with these aldehydes. Thus, it is proposed that the f-Alb receptor, originally reported as being specific for albumin treated with formaldehyde (1, 15), might play an important role as a general scavenger receptor for aldehyde-modified proteins.

The phenomenon that, when treated *in vitro* with formaldehyde, BSA was rapidly cleared from the circulation was discovered in 1965 (1). Subsequent studies showed that this chemically modified albumin was taken up by sinusoidal liver cells via receptor-mediated endocytosis (15). Since the condition for treatment of BSA employed by these investigators was obviously drastic (the protein was incubated with 6.6 M formaldehyde at 37 °C for 2 days), there has since been a tacit understanding that formaldehyde treatment of the protein may be equivalent to its denaturation. Thus, it was assumed that such modification might unlikely occur to albumin molecules *in vivo*. In our previous studies (11, 12), however, drastic denaturation of BSA *per se* was shown to be unrelated with the receptor recognition. Moreover, it was demonstrated that modification of only 11 lysine residues out of the total 59 lysine residues in BSA led to the formation of a maximally active ligand (26). The modification to this limited extent was achieved by treating BSA with 0.2 M formaldehyde at 37 °C for 1 h, far milder conditions than those used by the original investigators. This finding strongly suggested the presence of some unique lysine-containing domains in the BSA molecules which could potentially provide the receptor recognition site(s) upon modification with formaldehyde. This notion was further supported by the fact that other proteins such as rat and human albumin, ovalbumin, soybean trypsin inhibitor, and human hemoglobin could be converted to active ligands upon treatment with formaldehyde (26). Upon treatment with aliphatic aldehydes such as glycolaldehyde, DL-glyceraldehyde and propionaldehyde, albumin, ovalbumin, soybean trypsin inhibitor, and hemoglobin yielded active ligands for the f-Alb receptor while γ-globulin and RNase A did not (Fig. 5). This finding seems to indicate the presence of a unique domain(s) in common in the former group of proteins and the absence of such domain structure in the latter group of proteins. All these proteins have many lysyl residues modifiable by these aldehydes. However, the lysyl residues whose modification by these aldehydes is critical to the generation of the ligand activity appear to be limited to those occurring in a certain unique sequence of a potential ligand protein. Proteins thus examined for their potentiality to exhibit the ligand activity upon modification with aldehydes are rather limited in number. However, identification of such unique sequence(s) in these proteins would shed light on the molecular mechanism for the apparently broad ligand specificity of the f-Alb receptor.

Acharya and Manning (27) demonstrated that the Schiff base adduct of glycolaldehyde with a protein undergoes Amadori rearrangement to generate a new aldehyde function which is capable of forming Schiff base with another amino group to cross-link proteins. Treatment of BSA with glycolaldehyde indeed gave rise to polymeric molecules in addition to the monomeric species. These monomeric and polymeric species were equally effective in inhibiting the binding of ^{125}I-f-Alb to sinusoidal liver cells.[2] Thus, polymerization *per se* did not contribute to the generation of the ligand activity. The precise structure of protein-aldehyde adduct in the ligand molecules remains to be studied. However, one may derive partially its chemical nature from the following findings so far obtained: (i) BSA derivatives whose lysyl residues were reductively methylated to various extents from 25% to nearly 100% under controlled conditions showed no ligand activity; (ii) further formaldehyde treatment of the reductively methylated derivatives of BSA did not generate any ligand activity; (iii) the ligand activity generated upon treatment with either formaldehyde or glycolaldehyde was not lost by treatment with a

[2] S. Horiuchi, M. Murakami, K. Takata, and Y. Morino, unpublished observation.

reducing reagent such as $NaBH_4$ or $NaCNBH_3$. The findings i and ii strongly suggest that the reaction of lysyl residues with aldehydes is involved in the formation of an active ligand. The third finding favors the contention that the protein-aldehyde adduct might not be of a Schiff base type but of a carbinolamine type. No evidence has been described for the formation of a stable Schiff base in the reaction of formaldehyde with lysines (28–30). Formaldehyde is known to react also with side chains of histidine, asparagine, glutamine, and arginine residues. However, the finding ii is not consistent with the possibility that modification of these residues is responsible for the formation of an active ligand.

Several aldehydes are known to be produced in the intermediary metabolism in animals (31). Modification of proteins by aldehydes *in vivo* is not known except for the following two cases: nonenzymic glucosylation of proteins by glucose particularly in diabetes mellitus (32–35) and modification by malondialdehyde generated via the metabolism of arachidonic acid by platelets (36–38) as well as by lipid peroxidation (39, 40). However, since aldehydes have in general a high reactivity to form covalent adducts with α- and ϵ-amino groups of proteins, it would be possible that the reaction with aldehydes generated *in situ* may transform these proteins into the forms which could be recognized by an endocytic receptor and hence cleared from the circulation. Although it is not clear at present whether or not the f-Alb receptor may be involved in this process, the finding that the binding of ^{125}I-f-Alb to sinusoidal liver cells was inhibited to a significant degree by the presence of rat serum (41) may indicate the occurrence in the serum of a natural ligand structurally similar to f-Alb.

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