Secreted Forms of the Amyloid-β Precursor Protein Are Ligands for the Class A Scavenger Receptor*

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Upon activation, platelets secrete a 120-kDa protein that competes for the binding and internalization of acetyl low density lipoproteins (AcLDL) by macrophages. From the amino-terminal amino acid sequence, amino acid composition, and immunoblot analysis, we identified the active factor in platelet secretion products as sAPP, an α-secretase cleavage product of the β-amyloid precursor protein (APP), that contains a Kunitz-type protease inhibitor (KPI) domain. We showed that both sAPP751 (also called Nexin II) and sAPP695, which does not contain a KPI domain, are ligands for the class A scavenger receptor (SR-A). Chinese hamster ovary cells stably transfected to express the SR-A bound and internalized 4-fold more human platelet-derived sAPP than control cells. The binding and internalization of sAPP were inhibited by the SR-A antagonist fucoidin. In addition, sAPP competed as effectively as fucoidin for SR-A-mediated cell association and degradation of ^{125}I-AcLDL. To determine if the KPI domain is required for the binding of sAPP to the SR-A, APP751 and APP695 were expressed in Chinese hamster ovary cells, and sAPP751 and sAPP695 purified from the medium were tested for their binding to the SR-A. sAPP751 and sAPP695 were equally effective in competing for the cell association of ^{125}I-AcLDL by SR-A-expressing cells, demonstrating that the KPI domain is not essential for binding. We also found that sAPP751 is present in extracts of atherosclerotic lesions and that sAPP competes for the SR-A-mediated cell association of oxidized low density lipoprotein. Deletion mutagenesis indicated that a negatively charged region of APP (residues 191–264) contributes to binding to the SR-A. These results suggest that the SR-A contributes to the clearance of sAPP and that sAPP competes for the cell association of other SR-A ligands.

An early event in atherogenesis is the formation of fatty streak lesions in the arterial wall. These lipid-laden lesions consist primarily of macrophage foam cells containing large amounts of cholesterol ester, much of it derived from plasma lipoproteins. The accumulation of lipids in macrophages is thought to reflect uptake of modified lipoproteins by scavenger receptors. Plasma lipoproteins retained by proteoglycans in the subendothelial space of the arterial wall are thought to be modified, initially through lipid peroxidation and then by interaction of the reactive aldehydes that are formed with lysine residues of the apolipoproteins (for a review, see Ref. 1). Modified low density lipoproteins (LDL),[1] which have an increased negative charge, are recognized by cell-surface receptors expressed by macrophages and are internalized, leading to foam cell formation (1).

Macrophages express several scavenger receptors that bind modified lipoproteins (2–6). The class A scavenger receptor (SR-A) was the first to be cloned (2, 7, 8). The type I and type II SR-As are trimeric integral membrane glycoproteins generated by alternative splicing of a single gene product (2, 8). They bind a wide variety of polyanions, including certain chemically modified proteins and lipoproteins such as acetyl-LDL (AcLDL) and oxidized LDL (OxLDL), polysaccharides such as fucoidin and dextran sulfate, and polyribonucleotides such as poly(I) and poly(G). All ligands for the SR-A are polyanions, but not all polyanions are ligands for the SR-A. Eliminating SR-A expression in mice significantly reduces the uptake of modified LDL by peritoneal macrophages from these animals and inhibits atherogenesis (9–11).

We have shown that secretion products from thrombin-activated human platelets contain a factor that competes for the binding, internalization, and degradation of AcLDL by mouse peritoneal macrophages (12, 13). At physiologic concentrations, secretion products from activated platelets inhibited the binding of AcLDL and the ability of AcLDL to induce cholesterol ester formation in these cells. We subsequently purified this protein to homogeneity and produced an antibody to it that abolished the ability of the platelet secretion products to inhibit the binding of AcLDL to macrophages. The purified secretion product bound directly to macrophages and was named platelet-derived macrophage-binding proteoglycan (PDMBP) (13). The binding of PDMBP was competitively inhibited by unlabeled PDMBP and by fucoidin. PDMBP inhibited the binding of AcLDL to macrophages but not to aortic endothelial cells, sug-

[1] The abbreviations used are: LDL, low density lipoprotein; Aβ, amyloid β peptide; AcLDL, acetyl low density lipoprotein(s); AD, Alzheimer's disease; APP, amyloid precursor protein; CHO, Chinese hamster ovary; KPI, Kunitz-type serine protease inhibitor; OxLDL, oxidized low density lipoproteins; PDMBP, platelet-derived macrophage-binding proteoglycan; sAPP, α-secretase cleavage product of APP; SR-A, class A scavenger receptor: PBS, phosphate-buffered saline; LRP, LDL receptor-related protein.
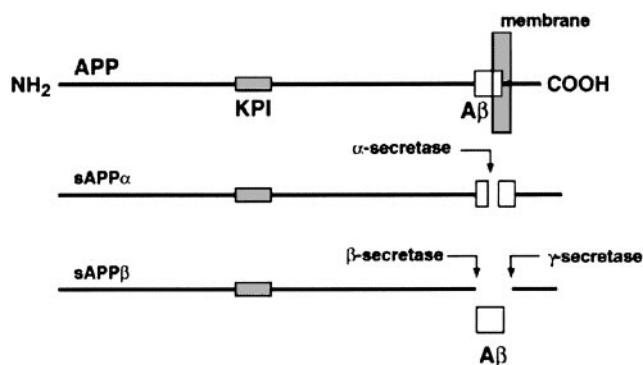
FIG. 1. **Schematic showing the proteolytic processing of APP.** APP751, APP770, and APP695 are processed in a similar manner. α-Secretase cleaves in the middle of the 40–42-amino acid Aβ peptide, precluding its formation and releasing sAPPα. β- and γ-secretase cleavage results in the formation of the Aβ peptide and the release of sAPPβ, which is 16 amino acids shorter than sAPPα. sAPPα is a form of APP that is constitutively released by cells. In this article, sAPP refers to sAPPα unless sAPPβ is explicitly mentioned.

gesting that PDMBP interacts with a specific receptor on macrophages. However, the sequence of PDMBP and the receptor it interacts with were not determined.

Upon platelet activation, α-granules release numerous proteins, including soluble forms of the amyloid precursor protein (sAPP) (14–16). APP is a plasma membrane protein containing a single transmembrane domain and a large extracellular region. Three major APP isoforms are generated by alternative splicing of the mRNA encoded by a single gene (17–19). They are referred to as APP695, APP751, and APP770, reflecting the number of amino acids encoded by their respective mRNAs. APP751 and APP770 contain a 56-amino acid domain that is homologous to the Kunitz-type serine protease inhibitor (KPI) domain; APP695 does not (17–19).

APP is expressed by all cell types. Most tissues, including brain, express APP751, but neurons (and to a lesser extent glia) also express APP695 (20). Normally, APP is constitutively cleaved by an α-secretase, which releases the extracellular domain (sAPP) (20). The α-secretase cuts close to the plasma membrane between residues 668 and 669 (APP751 numbering) (Fig. 1) within the amyloid β (Aβ) peptide region (21), precluding the formation of Aβ peptide, a major component of the amyloid plaques in the brains of people with Alzheimer's disease (20). A minority of APP molecules undergo proteolysis through the β- and γ-secretase pathway, generating the Aβ peptide (20, 22) and sAPPβ, which is 16 residues shorter than sAPP generated by the α-secretase cleavage. (In this article, sAPP refers to the α-secretase product unless stated otherwise.) sAPP751 and sAPP770 are also referred to as Nexin II (23, 24).

Nexin II is a potent inhibitor of certain serine proteases, including coagulation factor XIa, trypsin, chymotrypsin, epidermal growth factor-binding protein, and the γ subunit of nerve growth factor (24–27). Platelet α-granules contain relatively large amounts of Nexin II, which is secreted upon platelet activation (14–16). sAPP751 and sAPP770 are the predominant isoforms secreted by platelets (28).

In the current study, we discovered that PDMBP is sAPP/Nexin II, that both Nexin II and sAPP695 bind to the SR-A, that sAPP competes for the binding of oxidized LDL to the SR-A, and that it is present in atherosclerotic lesions, where it might compete for the binding of atherogenic scavenger receptor ligands.

EXPERIMENTAL PROCEDURES

Materials—Human platelet concentrates were obtained from the Peninsula Blood Bank (Burlingame, CA). Thrombin, hirudin, benzami-

dine, and fucoidin were purchased from Sigma. DEAE-Sephacel, Sephacryl S-300, Q-Sepharose, phenyl-Superose, heparin-Sepharose, and PD-10 columns were obtained from Amersham Pharmacia Biotech (Uppsala, Sweden). Na^{125}I was purchased from Amersham Pharmacia Biotech (Piscataway, NJ). Dulbecco's modified Eagle's medium, F12 medium, trypsin-EDTA solution, LipofectAMINE, geneticin, and penicillin-streptomycin solution were purchased from Life Technologies (Gaithersburg, MD). Fetal calf serum was obtained from Hyclone (Logan, UT). Tissue culture dishes and other plasticware were obtained from Falcon (Franklin Lakes, NJ), Corning (Acton, MA), and Nalge Nunc International (Rochester, NY). Three synthetic peptides ([191]EESDNVDSADAEEDDSDVWWGGADTDYADGSEEDKVV[227], [228]EVAEEEEVAEVEEEEADDDEDDDEDGDEVEEEAEEPYEE[264], and [191]EESDNVDSADAEEDDSDVWWGGADTDYADGSEEDKVVEVA-EEEEVAEVEEEEADDDEDDDEDGDEVEEEAEEPYEE[264]) corresponding to negatively charged regions of APP were obtained from Alpha Diagnostic (San Antonio, TX). The antibody 22C11 was purchased from Roche Molecular Biochemicals (Indianapolis, IN). The antibodies 3D6, R7, 8E5, and 2H3 were kindly provided by Dr. Lennart Mucke (Gladstone Institute of Neurological Disease).

Purification and Radiolabeling of PDMBP and sAPP—PDMBP was purified to homogeneity from platelets as described (13). Briefly, platelets were activated with thrombin, and the secreted products were subjected to ion-exchange chromatography (DEAE-Sephacel) and gelfiltration chromatography (Sephacryl S-300). PDMBP/sAPP was labeled with ^{125}I to specific activities of 250–400 cpm/ng with IODO-BEADS (Pierce, Rockford, IL), as recommended by the manufacturer. To determine if the KPI domain was important for binding to the SR-A, sAPP751 and sAPP695 were expressed in Chinese hamster ovary (CHO) cells. CHO cells were transiently transfected with pcDNA3 containing the cDNA for human APP695 or APP751, using LipofectAMINE as recommended by the manufacturer. sAPP695 and sAPP751 were partially purified from conditioned media of the transfected cells by DEAE-Sephacel chromatography as described for the isolation of PDMBP from platelets (13). Fractions containing sAPP as determined by immunoblotting were pooled, dialyzed against PBS and applied to a heparin-Sepharose column (0.5 × 1 cm). The column was washed with 5 ml of PBS, bound protein eluted with 2 ml of 0.5 M NaCl in PBS, and the eluted material dialyzed against PBS prior to use in competition assays. All steps were performed at 4 °C in the presence of complete protease inhibitors (Roche Molecular Biochemicals).

Deletion Mutagenesis—Three deletion mutants of APP695 were created by removing residues 191–229, 220–264, or 191–264. Deletion mutagenesis was performed by polymerase chain reaction with the cDNA for APP695 cloned in pcDNA3 and the Excite kit (Stratagene, La Jolla, CA), according to the manufacturer's recommendations. Residues 191–229 were deleted with primers 5'-AGGCAGTGGGCAACACA-CAAACTCTACCC-3' (antisense) and 5'-GAGGAGGAAGAAGTGGCT-GAGG TG-3' (sense). Residues 220–264 were deleted with 5'-CCCAT-CTGCATAGTCTGTGTCTGCTCC-3' (antisense) and 5'-GCCACAGAG-AGAACCACCAGC-3' (sense). Residues 191–264 were deleted with primers 5'-AGGCAGTGGGCAACACACAAACTCTACCC-3' (antisense) and 5'-GCCACAGAGAGAACCACCAGC-3' (sense). The deletion mutations were confirmed by DNA sequencing. The cDNAs for APP695 and the deletion mutants were amplified by polymerase chain reaction with previously reported primers (29) and cloned into the *Xma*I site of the vector pHIL-S1 for expression in *Pichia pastoris*. *P. pastoris* cells were transfected by electroporation and were grown and selected according to the recommendations for the *Pichia* expression kit (Invitrogen, Carlsbad, CA). Several colonies for each of the deletion mutants and wildtype APP695 were analyzed for APP expression by immunoblotting with antibody 22C11 (Roche Molecular Biochemicals). APP-expressing colonies were grown in 1-liter cultures, and sAPP was purified from the growth medium by Q-Sepharose and phenyl-Superose chromatography as described (29).

Immunoblot Analysis—Proteins were separated on 7.5% polyacrylamide-sodium dodecyl sulfate gels and transferred to nitrocellulose membranes. The membranes were blocked for 1 h at room temperature in PBS containing 5% nonfat milk and 0.05% Tween 20 and then incubated for 1 h at room temperature in the same solution containing the primary antibody. The membranes were washed and incubated for 1 h at room temperature with the secondary antibody in PBS containing 0.1% Tween 20. The immunoblots were visualized with the chemiluminescent reagent system (Amersham Pharmacia Biotech) and exposed to x-ray film.

Protein Sequence—For amino-terminal sequence analysis, ~150 pmol of PDMBP purified from platelet secretory products was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis as de-

scribed above and transferred to a polyvinylidene difluoride membrane. The membrane was stained with 0.1% Coomassie Blue and destained with 50% methanol and 10% acetic acid, and the band corresponding to PDMBP (~120 kDa) was submitted to the Biomolecular Resource Center (University of California, San Francisco, CA) for amino-terminal sequence analysis. Total amino acid composition was determined at the Beckman Center (Stanford University, Palo Alto, CA), on PDMBP prepared in a similar manner.

Lipoproteins—Human LDL (d = 1.02–1.05 g/ml) were isolated from the plasma of normal fasting donors by sequential density ultracentrifugation at 4 °C (30). LDL were radiolabeled by the iodine monochloride method (31) to a specific activity of 150–350 cpm/ng protein. LDL were acetylated with acetic anhydride (32). LDL were oxidized by incubation for 18 h at 37 °C in PBS containing 10 μM Cu(SO)$_4$. Lipoproteins were dialyzed against 0.15 M NaCl and 0.01% EDTA, pH 7.2, before use.

Cell Culture—Control CHO cells were grown in Dulbecco's modified Eagle's/F-12 (50/50, v/v) medium containing 10% fetal calf serum. CHO cells stably transfected to express the type I SR-A were grown in the same medium containing 400 μg/ml G418. The preparation and properties of these cells have been described (33). To examine the cell association and degradation of sAPP751, the cells were washed twice in serum-free medium and incubated for 5 h at 37 °C in serum-free medium containing ^{125}I-sAPP751 (1 μg/ml). Cell-associated radioactivity (*i.e.* bound and internalized lipoprotein) and trichloroacetic acid-soluble lipoprotein degradation products in the medium were quantitated as described (34, 35). For competition experiments, the cells were washed twice with serum-free medium and incubated for 5 h at 37 °C in serum-free medium containing ^{125}I-AcLDL (2 μg/ml) alone or with the indicated concentrations of competitors.

sAPP in Atherosclerotic Lesions—New Zealand White rabbits were fed a diet containing 0.5% cholesterol for 8 weeks. The rabbits were anesthetized and perfused with saline to clear the tissues of blood, and the aorta was removed. Raised white atherosclerotic lesions were peeled from the luminal surface of the vessel. The media under the lesion and tissue from grossly normal aorta were also collected. The samples were homogenized at 4 °C with a Polytron in buffer containing complete protease inhibitors (Roche Molecular Biochemicals). The samples were centrifuged at 15,000 rpm in a microcentrifuge at 4 °C, and the proteins in the supernatant were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and subjected to immunoblot analysis as described above.

RESULTS

PDMBP Is sAPP—To characterize PDMBP, we isolated it from human platelet secretion products as described (see "Experimental Procedures") and determined that the sequence of the amino-terminal 20 amino acids of the purified PDMBP was LEVPTDGNAGLLAEPQIAMF, which exactly corresponds to the amino-terminal sequence of sAPP after removal of the signal peptide (23, 24). The amino acid composition of PDMBP agrees favorably with the amino acid composition of sAPP751 but differs in some respects from the composition we reported previously (13) (Table I).

Immunoblot analysis with antibodies to epitopes spanning the entire length of sAPP showed that PDMBP reacted with all of the antibodies against the extracellular domain of APP (Fig. 2*A*). Antibody 22C11 reacts with amino acids 60–100 near the amino terminus (22), antibody R7 reacts with the KPI domain (36), antibody 8E5 reacts with amino acids 436–573 (37), and antibody 2H3 reacts with amino acids 652–664 near the carboxyl terminus (38). The amino acid numbers are based on APP751. As expected, the isolated protein was also detected by a polyclonal antibody against PDMBP (13) (Fig. 2*A*), which detects the platelet secretory product that competes for the binding of AcLDL to macrophages (12, 13). To determine if this polyclonal antibody reacts with authentic sAPP, serum-free conditioned medium from CHO cells transiently expressing APP695 or APP751 was subjected to immunoblot analysis. Both sAPP695 and sAPP751 reacted with the polyclonal antibody to PDMBP (Fig. 2*B*). The electrophoretic mobility of the purified PDMBP differed slightly from that of human sAPP751 secreted by transfected CHO cells. The difference in mobility most likely results from differences in glycosylation and from

TABLE I
Comparison of the amino acid composition of purified PDMBP to the amino acid composition calculated for sAPP751

Amino acid residue	PDMBP[a]	sAPP751[b]	A[c]
	mol %		
Aspartic acid/asparagine	12.7	11.4	14.2
Threonine	5.4	6.5	4.1
Serine	6.2	4.6	9.4
Glutamic acid/glutamine	18.6	17.5	15.2
Proline	4.1	4.6	5.9
Glycine	5.1	4.6	9.5
Alanine	8.4	8.0	4.6
Cysteine	ND[d]	2.8	—[e]
Valine	6.9	8.0	3.5
Methionine	2.6	2.8	1.9
Isoleucine	2.9	2.9	2.8
Leucine	6.8	6.3	10.3
Tyrosine	2.9	2.6	2.3
Phenylalanine	2.6	2.6	4.3
Histidine	3.1	4.9	2.1
Lysine	6.2	4.9	3.8
Tryptophan	ND	1.2	ND
Arginine	5.7	5.2	6.1

[a] Amino acid composition of the gel-purified PDMBP (sAPP751/770) used for amino-terminal sequence analysis in the current study.

[b] The composition of sAPP751 calculated from the reported amino acid sequence (17).

[c] Amino acid composition of PDMBP previously reported (13).

[d] ND, not determined.

[e] The cysteine content was 1.6 mol% in the original report but was deleted in the current calculation to facilitate comparison.
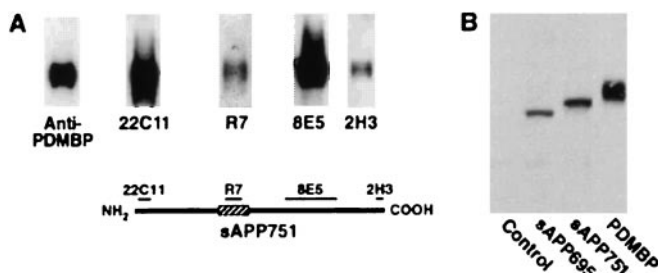

FIG. 2. **Antibodies to epitopes of APP recognize PDMBP.** *A*, immunoblot showing the interaction of PDMBP, isolated from human platelets, with antibodies to epitopes in APP and with anti-PDMBP. The schematic shows the approximate location of epitopes of the antibodies on a diagram of sAPP751. *B*, immunoblot showing that an antibody to PDMBP detects sAPP695 and sAPP751 from conditioned medium (40 μl) of CHO cells transfected to express APP695 or APP751. Medium from nontransfected CHO cells (control) and PDMBP isolated from platelets are also shown as negative and positive controls, respectively.

the fact that the PDMBP contains both sAPP751 and sAPP770. These findings demonstrate that PDMBP is sAPP751/770, which contains a KPI domain.

PDMBP Is a Ligand for the SR-A—Next, we determined whether the SR-A binds PDMBP. Competition studies with CHO cells stably transfected to express the type I SR-A showed that PDMBP isolated from human platelets competed as effectively as AcLDL and fucoidin (a potent inhibitor of SR-A ligand interactions) for the cell association and degradation of ^{125}I-AcLDL (Fig. 3).

To examine the interaction of sAPP with the SR-A more directly, we evaluated the binding of ^{125}I-PDMBP to SR-A-expressing CHO cells. The cell association, which represents bound and internalized ligand, was ~4.3-fold higher in SR-A-expressing cells than in control cells (Fig. 4*A*). The cell association of ^{125}I-AcLDL was also enhanced by SR-A expression in CHO cells. Fucoidin, a well characterized antagonist for the binding of ligands to the SR-A, competed effectively with ^{125}I-PDMBP for binding and internalization by SR-A-expressing CHO cells (Fig. 4*B*), whereas AcLDL did not (Fig. 4*B*). Taken
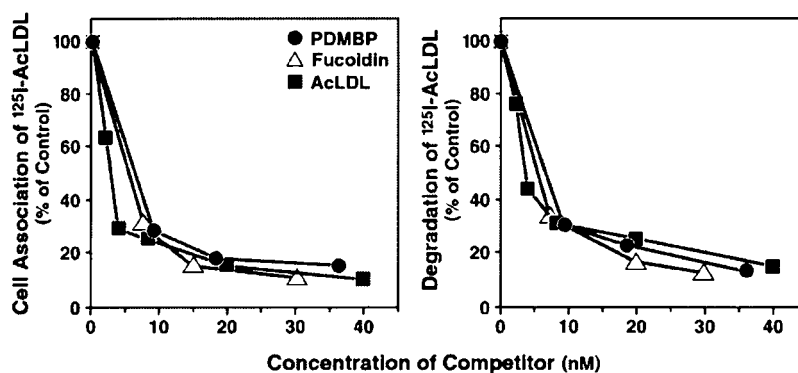
FIG. 3. **PDMBP competes for the SR-A-mediated cell association and degradation of ^{125}I-AcLDL.** CHO cells stably transfected to express the SR-A were incubated with ^{125}I-AcLDL (2 μg/ml) alone or in the presence of increasing concentrations of the nonlabeled competitors PDMBP (sAPP751/770), fucoidin, or AcLDL. After 5 h at 37 °C, the cell association (*left panel*) and degradation (*right panel*) of ^{125}I-AcLDL were determined. In the absence of competitors, the cell association was 192 $\pm$ 9 and the degradation was 216 $\pm$ 13 ng/mg cell protein. Each data point represents the mean ($\pm$ S.D.) of four experiments in duplicate. The experimental error bars are within the data points.

FIG. 4. **PDMBP is a ligand for the SR-A.** *A*, control CHO cells and CHO cells stably expressing the SR-A were incubated with ^{125}I-PDMBP (1 μg/ml) or ^{125}I-AcLDL (2 μg/ml) for 5 h at 37 °C. The cells were then washed to remove nonbound ligand, and the cell association (bound and internalized) was determined. The cell association of ^{125}I-PDMBP was 5.2 $\pm$ 0.7 in control CHO cells and 22.9 $\pm$ 1.6 ng/mg cell protein in CHO cells expressing the SR-A. For ^{125}I-AcLDL, the cell associations were 17.5 $\pm$ 1.6 and 192 $\pm$ 9 ng/mg cell protein, respectively. *B*, competition for the cell association of ^{125}I-PDMBP by a 100-fold molar excess of AcLDL or fucoidin.
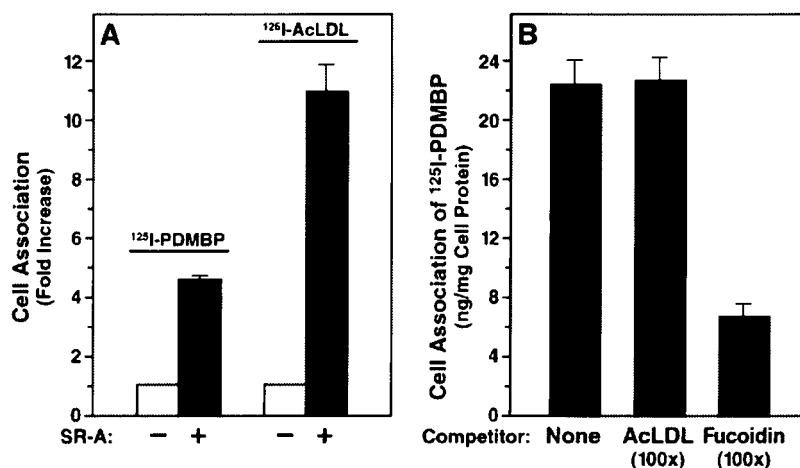
together, these results demonstrate that PDMBP (sAPP751/770) is a ligand for the SR-A.

The KPI Domain of APP Does Not Mediate Binding to the SR-A—The KPI domain is essential for the binding of sAPP751 and sAPP770 to the LRP (39, 40). To determine if the KPI domain plays a role in the interaction of sAPP751 with the SR-A, we expressed APP695, which lacks the KPI domain, and APP751 in CHO cells and isolated sAPP from the cell culture medium. In competitive binding experiments, sAPP695 and sAPP751 were equally effective in competing for the cell association of ^{125}I-AcLDL (0.5 μg/ml) (Fig. 5A). Therefore, both sAPP751 and sAPP695 are ligands for the SR-A, and the KPI domain is not necessary for the binding of APP to the SR-A. sAPP also competed for the cell association of ^{125}I-OxLDL (2 μg/ml) (Fig. 5B).

A Region of APP with a High Concentration of Negatively Charged Amino Acids Contributes to Binding to the SR-A—All known ligands for the SR-A are polyanions. sAPP contains a negatively charged region between amino acids 191 and 264, where 40 of the 74 amino acids are aspartic or glutamic acid; between amino acids 229 and 264, 26 of 35 amino acids are acidic. To test the hypothesis that this region contributes to the binding of sAPP to the SR-A, we generated three deletion mutants of APP695-(Δ191–229, Δ220–264, and Δ191–264) and expressed them, as well as wild-type APP695, in *P. pastoris*. sAPP was purified from the culture medium and tested for its ability to compete for the cell association of ^{125}I-AcLDL. Deletion of the entire negatively charged region of APP (residues 191–264) abolished the ability of sAPP to compete for the cell association of ^{125}I-AcLDL in SR-A-expressing CHO cells (Fig.

6); partial deletion of this region reduced competition. It took 2.5-fold more sAPP-(Δ191–229) and 3-fold more sAPP-(Δ220–264) than wild-type sAPP695 to compete for 50% of the cell association of ^{125}I-AcLDL. Therefore, the entire negatively charged region of APP may be involved in binding to the SR-A.

To determine if the primary sequence of the negatively charged region of APP is the major determinant for binding to the SR-A, we examined the ability of peptides corresponding to amino acids 191–227, 228–264, and 191–264 of sAPP to compete for the interaction of AcLDL with the SR-A. None of these peptides competed effectively for the cell association of ^{125}I-AcLDL in SR-A-expressing CHO cells (data not shown). Thus, the specific conformation of these sequences in the protein, not simply the negative charge, may be important for binding.

sAPP Is Present in Rabbit Atherosclerotic Lesions—Finally, we sought to identify sAPP in atherosclerotic lesions. Immunoblot analysis of proteins extracted from rabbit atherosclerotic lesions or from grossly normal aorta demonstrated the presence of sAPP (Fig. 7). More APP was detected in the lesion than in the media of the aorta under the lesion or in the grossly normal aortic tissue (Fig. 7).

DISCUSSION

This study shows that PDMBP is sAPP751/770 (α-secretase cleavage products of APP), that sAPP binds to the SR-A, and that the KPI domain of sAPP is not required for SR-A binding. These conclusions are based on several findings. The amino-terminal sequence of purified PDMBP was identical to that of APP, and its amino acid composition was similar to that of sAPP751. Antibodies against different epitopes of APP reacted
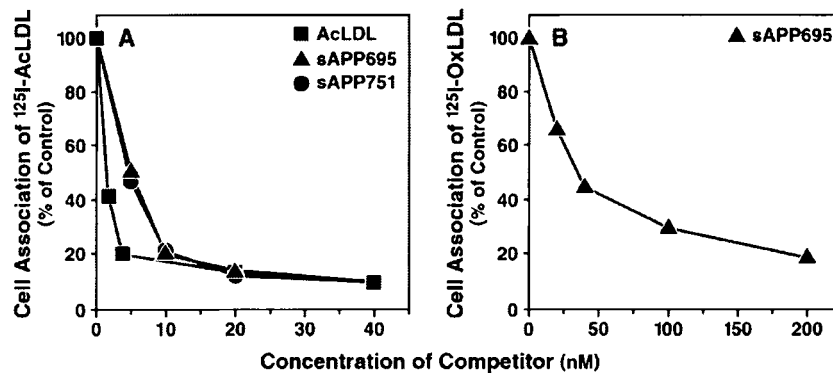
FIG. 5. **sAPP695 and sAPP751 compete for the cell association of ^{125}I-AcLDL and ^{125}I-OxLDL.** *A*, sAPP695 and sAPP751 expressed and purified from CHO cells were tested for their ability to compete for the cell association of ^{125}I-AcLDL in SR-A-expressing CHO cells as indicated in the legend of Fig. 2, except that the concentration of ^{125}I-AcLDL was 0.5 μg/ml. Each data point represents the mean ($\pm$ S.D.) of three experiments in duplicate. The experimental error is within the data points. *B*, CHO cells expressing the SR-A were incubated with 2 μg/ml ^{125}I-OxLDL and the indicated concentrations of sAPP695 isolated from *P. pastoris*. Each data point is the average of duplicates. The experiment was performed twice with similar results.
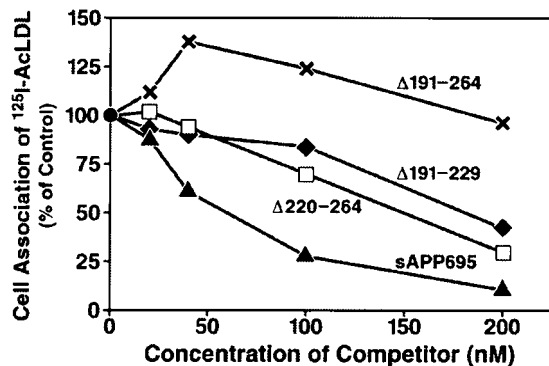

FIG. 6. **Deletion of the negatively charged region of APP abolishes its ability to compete with the SR-A for the cell association of AcLDL.** sAPP695 and sAPP deletion mutants (Δ191–229, Δ220–264, and Δ191–264) expressed and purified from the medium of *P. pastoris* were tested for their ability to compete for the cell association of ^{125}I-AcLDL in SR-A-expressing CHO cells as described in the legend of Fig. 2. Each data point is the average of duplicates. The experiment was performed three times with similar results.
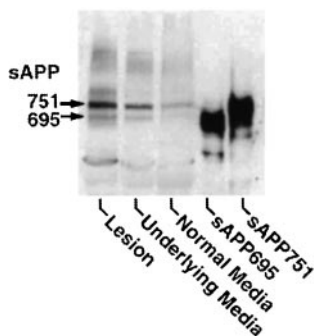

FIG. 7. **sAPP751 is present in rabbit atherosclerotic lesions.** Rabbits were fed a hypercholesterolemic diet for 2 months. Proteins from atherosclerotic lesions, from the underlying media, and from a grossly normal portion of aorta were extracted by homogenization in buffer containing protease inhibitors. The homogenates were centrifuged, and proteins (100 μg) in the supernatant were separated by SDS-PAGE and transferred to a nitrocellulose membrane. sAPP was detected by immunoblot analysis with the antibody 22C11, which detects all isoforms of APP. sAPP695 and sAPP751 isolated from the medium of transfected CHO cells were used as positive controls.

with PDMBP, and a polyclonal antibody against PDMBP detected authentic APP695 and APP751 expressed in CHO cells. The binding and internalization of sAPP751/770 by SR-A-expressing CHO cells were about 4-fold higher than that of control CHO cells and were blocked by fucoidin, an SR-A antago-

nist. Both sAPP751 and sAPP695, which does not contain a KPI domain, competed for the cell association of ^{125}I-AcLDL by SR-A-expressing CHO cells as effectively as fucoidin, a potent inhibitor of SR-A ligand interactions, and sAPP695 competed for the cell association of ^{125}I-OxLDL, another ligand for the SR-A.

Upon activation, the predominant forms of APP released by platelets are sAPP751 and sAPP770 (28). The exact ratio of sAPP751/sAPP770 and whether it differs significantly in different individuals are not known. Both forms of APP contain the KPI domain. Previously, we identified PDMBP as a chondroitin-4-sulfate proteoglycan with a core protein of ~32 kDa (13). Chondroitin sulfate forms of APP, which vary in apparent molecular mass from 140 to 250 kDa, have been reported to occur in the central nervous system (41–43), but have not been identified in platelets. In the current study, treatment of PDMBP with chondroitinase AC or ABC did not alter the electrophoretic mobility of the ~120-kDa band of PDMBP/sAPP (data not shown), and the amino acid analysis of the PDMBP is in better agreement with the composition of sAPP751 than previously reported (13) (Table I). It is difficult to know the reason for these differences; however, our earlier findings might reflect contamination of the chondroitinase with proteases, resulting in a 32-kDa proteolytic cleavage product. Differences in amino acid composition could result from differences in the ratio of sAPP751/sAPP770 in the two preparations of PDMBP or from low level contamination with other proteins, since in the previous study the PDMBP was purified by column chromatography without final gel purification.

sAPP has not previously been reported to bind to scavenger receptors; interestingly, however, both sAPP751 and sAPP770 are ligands for the LDL receptor-related protein (LRP), a member of the LDL receptor gene family. The LRP binds, internalizes, and degrades both sAPP751 and sAPP770 (39, 40). The binding and degradation are inhibited by the receptor-associated protein, an LRP antagonist (39). The KPI domain of sAPP appears to be essential for recognition of sAPP by the LRP, since sAPP695, which lacks a KPI domain, is not a ligand for that receptor (39, 40). Our studies clearly demonstrate that the KPI domain is not necessary for the binding of sAPP to the SR-A, since both sAPP751 and sAPP695 are equally effective in binding to the SR-A.

Interaction between the SR-A and its ligands is complex not only because of the broad ligand specificity of this receptor, but also because it exhibits nonreciprocal cross-competition (*i.e.* one ligand efficiently competes for the binding of a second ligand, while the second ligand does not compete, or competes only partially, for the binding of the first) (33, 44–47). Simi-

Binding of APP to the SR-A

larly, in our study sAPP695 and sAPP751 effectively competed for the cell association of ^{125}I-AcLDL with the SR-A expressed by transfected CHO cells, but AcLDL did not compete for the cell association of ^{125}I-sAPP751. This lack of cross-competition suggests that sAPP and AcLDL may bind to partially overlapping sites on a single receptor molecule, as observed with OxLDL and AcLDL in CHO cells expressing the SR-A (44). In addition, SR-A-expressing CHO cells showed ~4-fold greater cell association of ^{125}I-sAPP751 and 11-fold greater cell association of ^{125}I-AcLDL than control CHO cells. This difference might be due to differences in affinity or, more likely, to the binding of sAPP751 to multiple receptors.

At a concentration of 5–10 nM, sAPP isolated from human platelets or sAPP695 and sAPP751 isolated from transfected CHO cells competed for 50% of the cell association (bound and internalized) of ^{125}I-AcLDL. In contrast, ~60 nM sAPP isolated from *P. pastoris* produced this level of competition. The difference in affinity may have resulted from the different purification procedures used to isolate sAPP from the different sources, which could have affected the conformation of the sAPP and its ability to compete for the binding of ^{125}I-AcLDL to the SR-A. Alternatively, the difference in affinity might result from differences in glycosylation of the sAPP isolated from different sources, raising the possibility that the degree and nature of the carbohydrate side chains might contribute to binding.

All ligands for the SR-A are polyanions that interact with lysines in the binding domain of this receptor (2). Using deletion mutagenesis, we explored the possibility that a negatively charged region of APP is responsible for binding to the SR-A. Deletion of the negatively charged region of sAPP (residues 191–264) abolished the ability of sAPP to compete for the binding of ^{125}I-AcLDL to the SR-A. However, a synthetic peptide corresponding to this negatively charged region of APP did not compete for the binding of ^{125}I-AcLDL. There are at least two possible explanations for these findings. First, the conformation of the negatively charged region in APP may differ from that of the peptide in solution. Second, deletion mutagenesis may cause conformational changes in other regions of APP that are important for binding to the SR-A. It remains to be determined whether the negatively charged region of APP *per se* constitutes the binding domain or whether this region interacts with other regions of the molecule to maintain a conformation necessary to maintain the binding site.

Numerous studies have suggested that the SR-A is critically involved in the internalization of modified lipoproteins and in the deposition of cholesterol in arterial wall macrophages during atherogenesis (48). Recent studies with SR-A knockout mice support these observations (9–11). As shown previously (12), levels of sAPP necessary to inhibit the binding, uptake, and degradation of modified LDL by macrophages are secreted by physiologic concentrations of platelets. Since platelet secretory products inhibit the ability of modified LDL to promote cholesterol ester formation in the cells, the secretion products may modulate the ability of macrophages to accumulate lipids, thereby modulating the formation of foam cells in the artery wall (12). As noted above, we found that 5–10 nM sAPP competed for ~50% of the binding of ^{125}I-AcLDL to the SR-A. Normal citrated human plasma contains ~60 pM sAPP (15). However, it has been estimated that, upon activation of platelets and release of their α-granule contents, plasma concentrations of Nexin II could reach 30 nM (15). Even higher concentrations can be achieved in the immediate microenvironment of the activated platelet surface (15). Thus, sAPP secreted from platelets might be present at a high enough concentration to compete for the binding of modified lipoproteins to the SR-A in atherosclerotic lesions and reduce foam cell formation, one of

the early events in the development of atherosclerosis. Indeed, sAPP751 was present in extracts of atherosclerotic lesions. It is not possible to determine if this sAPP was platelet-derived or was produced within the lesion by resident cells.

The SR-A may also play a role in the central nervous system, where sAPP is also produced and where the Aβ peptide, a product of β- and γ-secretase cleavage of APP, contributes to the development of AD. There is high level expression of the scavenger receptor on activated microglia in the vicinity of amyloid plaques in AD brains (49), and microglia up-regulate SR-A expression in response to central nervous system injury (50). Cleavage of APP by β- and γ-secretase results in production of the amyloidogenic Aβ peptides (Aβ40 and Aβ42) and of sAPPβ. The SR-A binds and internalizes microaggregates of Aβ42 (51). The uptake of Aβ microaggregates was reduced by coincubation with SR-A ligands, such as AcLDL, OxLDL, fucoidin, maleylated bovine serum albumin, and dextran sulfate (51). Although the LRP is expressed on microglia, it is not involved in the uptake of Aβ42 microaggregates (51). The Aβ peptide is a major constituent of senile plaques and cerebrovascular deposits in patients with AD and Down's syndrome (20, 52, 53). The scavenger receptor may therefore play a role in clearance of Aβ, which is produced continuously in normal and AD brains (52, 54–56). Our data suggest that the SR-A is more prominent in APP metabolism than previously appreciated, since the SR-A binds sAPP. We predict that the SR-A will bind sAPPβ as well as sAPPα. Therefore, the SR-A may function in the clearance of sAPP under both normal and pathological conditions.

We have begun to test the importance of the SR-A in the central nervous system by examining the effect of SR-A expression on Aβ deposition in mice. In an initial study, we found that elimination of SR-A expression in transgenic mice expressing an amyloidogenic form of human APP in neurons did not affect amyloid plaque formation or neurodegeneration in this model (57). However, these results need to be extended to include older mice, different APP mutants, and different strains of mice before definitive conclusions can be drawn (58).

In summary, we have shown that sAPP, which is constitutively produced in all tissues, is a ligand for the SR-A. These data raise the intriguing possibility that the SR-A may contribute to the clearance of sAPP from the brain and other tissues and, furthermore, that sAPP could compete for the uptake of other ligands by the SR-A. Competition of sAPP for the SR-A-mediated binding and internalization of modified lipoproteins by macrophages in atherosclerotic lesions would be expected to reduce foam cell formation; however, the modified lipoproteins left in the lesion may themselves be toxic. Likewise, competition of sAPP for the uptake of the Aβ peptide by microglia could contribute to the increased concentration of extracellular Aβ and to the development of AD. Whether or not sAPP competes for the SR-A-mediated binding and internalization of ligands *in vivo* and the biological implications of this interaction remain to be determined.

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