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Serum Amyloid P Component Associates with High Density Lipoprotein as well as Very Low Density Lipoprotein but Not with Low Density Lipoprotein[1]

Xiang-An Li,*,†,[2] Chikao Yutani,† and Kentaro Shimokado†

**Department of Biochemistry, Taishan Medical College, Taian, Shandong 271000, P. R. China; and*
†National Cardiovascular Center, 5-7-1 Fujishirodai, Suita, Osaka 565-8565, Japan

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Serum amyloid P component (SAP) is a glycoprotein in human plasma. We previously showed that SAP is specifically localized in human atherosclerotic lesions, suggesting that SAP may play a role in atherogenesis. In this study, the interactions between human SAP and high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) were investigated by using a solid phase plate assay. Biotinylated SAP bound to immobilized HDL and VLDL in a calcium-dependent, saturable manner. The SAP–HDL and SAP–VLDL bindings reached saturation at 4 nM and 16 nM of SAP, respectively. The bindings were inhibited by native SAP in a dose-dependent manner. No binding between SAP and LDL was found in the presence of calcium or EDTA, which indicates the specificity of SAP–lipoproteins interactions. These results suggest that the function of SAP is related to its capability to interact with lipoproteins and this may have important implications in atherosclerosis and in amyloidosis.

Key Words: **amyloid P component; pentraxin; lipoprotein; binding; atherosclerosis; amyloidosis.**

Serum amyloid P component (SAP) is a main member of the pentraxin family of human plasma, which occurs as complexes of ten identical 25 kDa subunits non-covalently associated in two pentameric rings interacting face-to-face (1, 2). SAP is synthesized by the liver and circulates in human blood at 30-45 mg/L (2). It is found in all types of amyloid deposits (3), including plaque from Alzheimer's disease (4), in glomerular basement membrane and in elastic fibers in blood vessels (5, 6). SAP binds to a variety of ligands in a calcium-dependent manner, including fibronectin (7), C4b-binding protein (7, 8), glycosaminoglycans (9, 10), DNA and chromatin (11, 12), complement components (13-15), aggregated IgG (16), C-reactive protein (CRP) (17, 18), saccharides (19, 20), amyloid fibrils and Alzheimer's β-peptides (21, 22), collagen (23), and laminin (24). No deficiency of SAP has been reported. The association of SAP with many ligands and its stable evolutional conservation imply that it has important functions. However, its biological role is not clear.

We previously showed that SAP specifically localized in human atherosclerotic lesions, and that part of the SAP accumulated in the lesions via calcium-dependent binding to some ligands in the lesions (25, 26). The identification of the ligands may provide more insight into the mechanism of atherosclerosis. Our recent study showed that the amount of SAP in the human aorta positively correlated with the degree of atherosclerosis (Li, X. A. et al., unpublished data). In addition, our study showed that the amount of SAP in human plasma positively correlated with the level of very low density lipoprotein (VLDL), and negatively correlated with the level of high density lipoprotein (HDL) (Li, X. A. et al., unpublished data). Taken together, these findings suggest that SAP may play a role in the pathogenesis of atherosclerosis, probably related to the metabolism of lipoproteins. Prompted by these recent findings, we investigated the SAP-lipoprotein interactions by using a solid phase plate assay method. We found that SAP specifically bound to human HDL and VLDL, but did not bind to low density lipoprotein (LDL). Our present results provide more insight into the function of SAP.

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[2] To whom correspondence should be addressed at National Cardiovascular Center Research Institute, 5-7-1 Fujishirodai, Suita, Osaka 565-8565, Japan. Fax: 81-6-872-7485; E-mail: xiangali@res.ncvc.go.jp.

The abbreviations used are: SAP, serum amyloid P component; CRP, C-reactive protein; HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein; BSA, bovine serum albumin.

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MATERIALS AND METHODS

Purification of SAP and production of antiserum against SAP. SAP was purified from normal human serum by Sepharose 4B (Pharmacia) affinity adsorption followed by chromatography on DEAE-Sepharose (Pharmacia) as previously reported (2). The purified SAP showed a single band identical to standard SAP (Sigma) when analyzed by reduced sodium dodecyl sulfate-12% polyacrylamide gel electrophoresis. The concentration of SAP was estimated by A_{280} (1%)=18.2 (27). Rabbit anti-human SAP antiserum was raised by immunizing New Zealand white rabbit with purified SAP as previously described (2). The antiserum was monospecific against human serum when checked by crossed immunoelectrophoresis (10).

Biotinylation of SAP. 0.5 mg of purified SAP was conjugated to biotinamidocaproate-N-hydroxy-sulfosuccinimide ester to a ratio of 1:10 using an ImmunoProbe Biotinylation Kit (Sigma) following the instructions. The biotinylation proceeded on an ice bath for 2 hours, and biotin-SAP was purified from the mixture by FPLC on a Superose 6 HR 10/30 column (Pharmacia) which equilibrated with 50 mM Tris, 140 mM NaCl, pH 7.4 (TBS) containing 1 mM EDTA. The concentration of biotin-SAP was estimated by A_{280} (1%)=18.2.

Preparation of lipoproteins from human plasma. Human VLDL (density <1.006 g/ml), LDL (density 1.019–1.063 g/ml) and HDL (density 1.063-1.21 g/ml) were isolated from EDTA-containing plasma by sequential ultracentrifugation in solution of potassium bromide (28). The potassium bromide was removed by dialysis against TBS/1 mM EDTA. Each purified lipoprotein fraction gave a single peak in FPLC on a Superose 6 HR 10/30 column. In each lipoprotein fraction, no SAP was detected by Laurell rocket electrophoresis and by Western Blotting using the rabbit anti-human SAP antiserum (25). For binding assay in the presence of calcium, the lipoproteins were dialyzed against TBS/1 mM $CaCl_2$ buffer overnight before use. The total protein concentration of lipoprotein was measured by using a Micro BCA Protein Assay Kit with bovine serum albumin (BSA) as a standard (Pierce Chemicals).

Solid phase plate assay. The interactions between lipoproteins and their ligands were analyzed using a solid phase plate assay as described by Choi et al. (29). Briefly, microtiter plates (Sumitomo Bakelite) were coated with 100 μl of human HDL, LDL, VLDL or BSA (Sigma) (50 mg of protein/ml) diluted in 50 mM TBS/1 mM $CaCl_2$, pH 7.4, by incubating overnight at 4 °C. After 4×3 min washing with 0.3% BSA/TBS/1 mM $CaCl_2$, the plates were blocked with 3% BSA/TBS/1 mM $CaCl_2$ for 3 hours at room temperature and then incubated with increasing concentrations of biotin-SAP (0-32 nM) diluted in blocking buffer overnight at 4 °C. After being washed four times again with 0.3% BSA/TBS/1 mM $CaCl_2$, the plates were incubated with avidin-peroxidase (Sigma) diluted 40,000 times in 0.3% BSA/TBS/1 mM $CaCl_2$ for 30 min at room temperature. The plates were washed four times with TBS/1 mM $CaCl_2$ and detected with a TMBZ substrate kit (Sumitomo Bakelite). Absorbance at 450 mm was measured. Data are mean values of three experiments.

The protocol used to assess the SAP binding to lipoproteins in the presence of EDTA was identical to the one described above except that 1 mM $CaCl_2$ was substituted by 1 mM EDTA.

For competition studies, microtiter plates were coated with 100 μl of human HDL, VLDL or BSA (50 mg of protein/ml) diluted in 50 mM TBS/1 mM $CaCl_2$, pH 7.4, by incubating overnight at 4 °C. After 4×3 min washing with 0.3% BSA/TBS/1 mM $CaCl_2$, the plates were blocked with 3% BSA/TBS/1 mM $CaCl_2$ for 3 hours at room temperature and then incubated with 16 nM of biotin-SAP along with increasing concentrations of native SAP (0-256 nM) diluted in blocking buffer overnight at 4 °C. After being washed four times again with 0.3% BSA/TBS/1 mM $CaCl_2$, the plates were incubated with avidin-peroxidase diluted 40,000 times in 0.3% BSA/TBS/1 mM $CaCl_2$ for 30 min at room temperature. The plates were washed four times with TBS/1 mM $CaCl_2$ and detected with the TMBZ substrate kit. Absorbance at 450 mm was measured. The binding of biotin-SAP to BSA-coated wells was used as nonspecific binding and subtracted from the total binding. The wells containing only 16 nM of Biotin-SAP were used as controls. Data are mean values of three experiments.

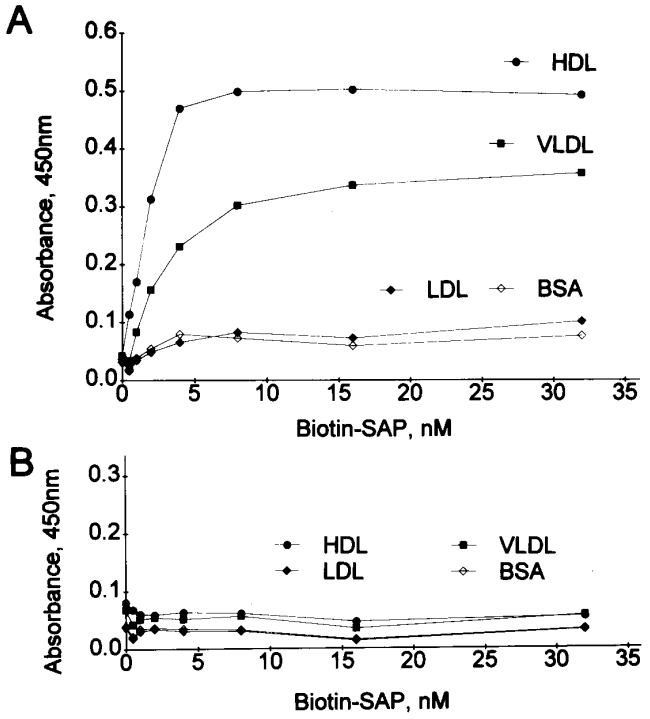

FIG. 1. Binding of SAP to lipoproteins. (A) Dose-dependent SAP binding to lipoproteins in the presence of calcium. Microtiter plates were coated with human HDL, LDL, VLDL or BSA (50 mg of protein/ml) diluted in 50 mM TBS/1 mM $CaCl_2$, pH 7.4, by incubating overnight at 4 °C. After 4×3 min washing with 0.3% BSA/TBS/1 mM $CaCl_2$, the plates were blocked with 3% BSA/TBS/1 mM $CaCl_2$ for 3 hours at room temperature and then incubated with increasing concentrations of biotin–SAP (0–32 nM) diluted in blocking buffer overnight at 4 °C. After being washed four times again with 0.3% BSA/TBS/1 mM $CaCl_2$, the plates were incubated with avidin–peroxidase diluted in 0.3% BSA/TBS/1 mM $CaCl_2$ for 30 min at room temperature. The plates were washed four times with TBS/1 mm $CaCl_2$ and detected with the TMBZ substrate kit. Absorbance at 450 mm was measured. Data are mean values of three experiments. (B) SAP binding to lipoproteins in the presence of EDTA. The protocol used in this experiment was identical to the one described above except that 1 mM $CaCl_2$ was substituted by 1 mM EDTA

RESULTS AND DISCUSSION

The SAP-lipoprotein interactions were investigated by using a solid phase plate assay. As shown in Fig. 1A, in the presence of a physiological concentration of calcium, biotin-SAP selectively bound to immobilized human HDL and VLDL in a dose-dependent manner. The bindings reached saturation at 4 nM of SAP for the SAP-HDL binding and 16 nM of SAP for the SAP-VLDL binding. Both the SAP-HDL and SAP-VLDL bindings

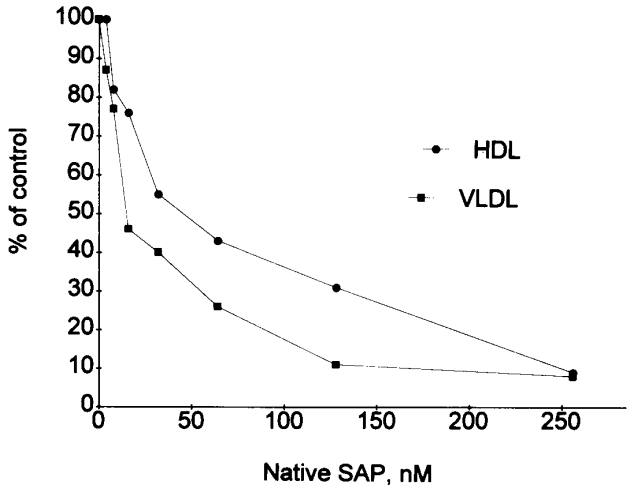

FIG. 2. Competitive inhibition of SAP binding to HDL and VLDL by native SAP. Microtiter plates were coated with human HDL, VLDL or BSA (50 mg of protein/ml) diluted in 50 mM TBS/1 mM $CaCl_2$, pH 7.4, by incubating overnight at 4 °C. After 4×3 min washing with 0.3% BSA/TBS/1 mM $CaCl_2$, the plates were blocked with 3% BSA/TBS/1 mM $CaCl_2$ for 3 hours at room temperature and then incubated with 16 nM of biotin–SAP along with increasing concentrations of native SAP diluted in blocking buffer overnight at 4 °C. After being washed four times again with 0.3% BSA/TBS/1 mM $CaCl_2$, the plates were incubated with avidin–peroxidase diluted in 0.3% BSA/TBS/1 mM $CaCl_2$ for 30 min at room temperature. The plates were washed four times with TBS/1 mm $CaCl_2$ and detected with the TMBZ substrate kit. Absorbance at 450 mm was measured. The binding of biotin–SAP to BSA-coated wells was used as nonspecific binding and subtracted from the total binding. The wells containing only 16 nM of biotin-SAP were used as control. Data are mean values of three experiments.

were of high affinity, and the binding reached half saturation at 1.5 nM of SAP for the SAP-HDL binding and 2 nM of SAP for the SAP-VLDL binding. These data indicate that SAP has higher affinity for HDL than for VLDL. The SAP-HDL and SAP-VLDL binding were calcium-dependent, and no binding was observed in the presence of EDTA (Fig. 1B). To assess the specificity of the binding, the binding of biotin-SAP to BSA-coated plates was assayed and used as an estimate of nonspecific binding. No binding between biotin-SAP and immobilized BSA was found (Fig. 1A). The specificity of the binding was further demonstrated by competitive inhibition with native SAP. As shown in Fig. 2, the biotin-SAP-HDL and biotin-SAP-VLDL bindings were competitively inhibited by native SAP in a dose-dependent manner, the SAP-HDL and SAP-VLDL bindings were blocked by about 90% by a 16 and 8-fold molar excess of unlabelled SAP, respectively. Interestingly, no SAP-LDL binding was observed in the presence of calcium or EDTA (Fig. 1A and B), indicating that the SAP-lipoprotein interactions require both of protein and lipids to participate, not merely a simple protein-protein interaction nor a simple protein-lipids interaction.

SAP attends to aggregate in the presence of calcium. The aggregation closely relates to the concentration of SAP (30). The concentration of SAP used in our experiment was much lower than that needed for the self aggregation of SAP, and therefore no aggregation could occur. The finding that no SAP bound to the BSA-coated plates (Fig. 1) also indicated that no aggregation took place.

SAP is one of the two pentraxins in human plasma. Another is CRP. The two pentraxins show great homologies in their amino acid sequence and molecular configurations (1). CRP has been shown to bind to lipoproteins (31-36). CRP interacts with LDL and abnormal VLDL, while SAP interacts with normal VLDL and HDL. Pentraxins and lipoproteins have been shown to present in human atherosclerotic lesions (25, 26, 37-39). Thus, it is likely that pentraxins interact with lipoproteins at the site of atherosclerotic lesions and interrupt the metabolism of lipoproteins. The present results give credence to our hypothesis that pentraxins contribute to the development of atherosclerosis.

SAP is present in all types of amyloid deposits (3), including Alzheimer's disease (4). Previous reports have suggested that SAP may play a role in amyloid formation: for example, SAP binds to amyloid fibrils and protects the amyloid fibrils from proteolytic degradation (21, 40), and SAP binds to Alzheimer's β-peptides and enhances/inhibits the b-peptide fibril formation (22, 41, 42). Lipoproteins are also present in amyloid deposits (43). Therefore, the elucidation of direct SAP-lipoprotein interactions in amyloid deposits may provide further insight into the role of SAP on amyloid formation.

In conclusion, the present results showed that human SAP bound to HDL and VLDL with high affinity, but did not bind to LDL. Our data suggest that the function of SAP is related to its capacity to interact with lipoproteins and the interactions may have important implications both for its normal physiology and for its pathophysiology in relation particularly to atherosclerosis and amyloidosis. The potential significance of the association between SAP and lipoproteins merits further evaluation.

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