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Partial amino acid sequence of apolipoprotein(a) shows that it is homologous to plasminogen

(atherosclerosis)

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ABSTRACT **Apolipoprotein(a) [apo(a)] is a glycoprotein with M_r ≈280,000 that is disulfide linked to apolipoprotein B in lipoprotein(a) particles. Elevated plasma levels of lipoprotein(a) are correlated with atherosclerosis. Partial amino acid sequence of apo(a) shows that it has striking homology to plasminogen. Plasminogen is a plasma serine protease zymogen that consists of five homologous and tandemly repeated domains called kringles and a trypsin-like protease domain. The amino-terminal sequence obtained for apo(a) is homologous to the beginning of kringle 4 but not the amino terminus of plasminogen. Apo(a) was subjected to limited proteolysis by trypsin or V8 protease, and fragments generated were isolated and sequenced. Sequences obtained from several of these fragments are highly (77–100%) homologous to plasminogen residues 391–421, which reside within kringle 4. Analysis of these internal apo(a) sequences revealed that apo(a) may contain at least two kringle 4-like domains. A sequence obtained from another tryptic fragment also shows homology to the end of kringle 4 and the beginning of kringle 5. Sequence data obtained from two tryptic fragments show homology with the protease domain of plasminogen. One of these sequences is homologous to the sequences surrounding the activation site of plasminogen. Plasminogen is activated by the cleavage of a specific arginine residue by urokinase and tissue plasminogen activator; however, the corresponding site in apo(a) is a serine that would not be cleaved by tissue plasminogen activator or urokinase. Using a plasmin-specific assay, no proteolytic activity could be demonstrated for lipoprotein(a) particles. These results suggest that apo(a) contains kringle-like domains and an inactive protease domain.**

For the past decade a significant correlation has been established between elevated lipoprotein(a) levels, coronary artery disease, and progression of atherosclerotic lesions (1–7). These studies led to the conclusion that the presence of lipoprotein(a) should be considered as a high-risk factor for coronary artery disease and atherosclerosis. Although the function of lipoprotein(a) is unknown, the composition of lipoprotein(a) has been elucidated. The protein component of lipoprotein(a) is comprised of apolipoprotein B-100 (apoB-100) and a second protein called apolipoprotein(a) [apo(a)] (8–10). These two proteins are covalently linked by disulfide bonds that can be readily reduced to yield, upon centrifugation, apo(a) and a lipoprotein particle containing apoB-100 that has properties similar to low density lipoprotein (LDL) (11–13). There are two apo(a) molecules and one apoB molecule per lipoprotein(a) particle (3). It is thought that there are several isoforms of apo(a) having molecular weights ranging from 280,000 to 500,000 (10–15). All of these isoforms appear to be immunologically related (13). To elucidate the function of apo(a), we have begun to sequence apo(a). We report here the results of the partial amino acid sequence of apo(a). This sequence shows striking homology to human plasminogen.

MATERIALS AND METHODS

Isolation of Lipoprotein(a) and Apo(a). One healthy female subject with elevated lipoprotein(a) levels served as donor for lipoprotein(a) isolation. Lipoprotein(a) was separated from plasma by a combination of density gradient and rate zonal ultracentrifugation (13), and apo(a) was isolated from lipoprotein(a) using methods described (13). In brief, solutions of lipoprotein(a) containing ≈2 mg of protein per ml in 0.15 M NaCl, 0.01% Na_2EDTA, and NaN_3 (pH 7.0) was made 0.01 M with respect to dithiothreitol. Reduction proceeded for 1 hr at room temperature in the dark before the reduced lipoprotein(a) was carboxymethylated with 0.03 M iodoacetic acid for ≈20 min at a constant pH of 8.0. The modified lipoprotein(a) was then dialyzed against 35% (wt/vol) NaBr, 0.01% Na_2EDTA (pH 7.0). Reduced and carboxymethylated apo(a) was isolated from the lipoprotein particle by rate zonal centrifugation in a Beckman 60Ti rotor at 59,000 rpm for 60–75 min at 20°C. After dialysis against 0.15 M NaCl/0.01% Na_2EDTA/NaN_3, pH 7.0, the apolipoprotein was passed over anti-apoB- and anti-apolipoprotein A-I-Sepharose columns as a precautionary step to remove any contaminating apolipoprotein A-I or apoB peptides. Anti-apoB and anti-apolipoprotein A-I antibodies were raised as described (13). The pure apo(a) was then dialyzed against 0.20 mM *N*-ethylmorpholine (pH 7.0), lyophilized, and stored at −70°C. The isolated apo(a) has a mobility faster than apoB-100 as determined by $NaDodSO_4$ gradient gel electrophoresis on 2.0–16% gels; the estimated molecular weight is 280,000 (10, 13).

Amino Acid Sequencing. Purified apo(a) (≈0.2 nmol, ≈50 μg) was subjected to amino-terminal amino acid sequencing using an Applied Biosystems (Foster City, CA) gas-phase sequencer (16). Limited proteolysis of apo(a) was carried out by incubating 500 μg of reduced and carboxymethylated apo(a) in 0.05 M Tris·HCl, pH 7.4/0.15 M NaCl with either trypsin at 5 μg or V8 protease at 5 μg for 15 min at 37°C. Fragments generated by proteolysis of apo(a) were isolated either by $NaDodSO_4$/PAGE followed by gel elution (17) or by reverse-phase HPLC. HPLC separation was performed on a Synchrome (Linden, IN) RP-4 column (15 cm × 4.6 mm). The column was developed with a gradient of propanol [1–50% (vol/vol) in 60 min] in 0.1% trifluoroacetic acid. Absorbance

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Abbreviations: apo(a), apolipoprotein(a); apoB-100, apolipoprotein B-100; LDL, low density lipoprotein; t-PA, tissue plasminogen activator.

was monitored at 210 and 280 nm. Two V8 peptides (fragments 1 and 2) and four tryptic fragments (tryptic peptides 1, 4–6) were isolated by gel elution, while tryptic fragments 2 and 3 were isolated by HPLC.

Affinity Chromatography. Lysine-Sepharose with a 6-aminohexanoic acid spacer arm was a gift from Robert Wohl of the University of Chicago. Before application, lipoprotein(a) or LDL were dialyzed against 0.1 M sodium phosphate, pH 7.4, containing 0.01% Na_2EDTA and 0.01% NaN_3. Samples were applied to a column (1.5 × 10 cm), equilibrated in the same buffer, at a flow rate of 10 ml/hr and successively washed with 0.1 M sodium phosphate, with 0.5 M sodium phosphate, and again with 0.1 M sodium phosphate. Bound material was eluted with 0.2 M ε-aminocaproic acid containing 0.1 M sodium phosphate (pH 7.4), 0.01% Na_2EDTA, and 0.01% NaN_3.

Immunoblotting. Gradient gel electrophoresis in the presence of $NaDodSO_4$ on 2.0–16% polyacrylamide gels (Pharmacia) was carried out according to methods described (10). For immunologial characterization, proteins were transferred to nitrocellulose using the Bio-Rad Trans-Blot cell as described (10). Transfer was carried out at 50 V, overnight with cooling. Antigens were identified with a double-antibody technique using anti-mouse or anti-rabbit IgG coupled to horseradish peroxidase (Miles) using 4-chloronaphthol as the chromogen. Antiserum to apo(a) was prepared according to described procedures (10). Antiserum to human plasminogen (Glu form) was a gift of Robert Wohl of the University of Chicago. The monoclonal antibody to kringle 4 of human plasminogen was a gift of F. J. Castellino at the University of Notre Dame; the plasminogen was a gift of Robert Byrne of the University of Chicago.

RESULTS

Partial Amino Acid Sequence of Apo(a). The amino-terminal amino acid sequence (15 residues) obtained for highly purified apo(a) is shown in Fig. 1. Computer-aided analysis of this sequence revealed that it has striking homology with the serine protease zymogen plasminogen. Plasminogen consists of five homologous and tandemly repeated regions called kringles followed by a trypsin-like domain at its carboxyl terminus. The kringle domains contain specific binding regions for fibrin, α_2-antiplasmin, and lysine (18–21). The plasma proteases prothrombin, tissue plasminogen activator (t-PA), urokinase, and factor XII also contain kringle-like domains that have homology with plasminogen kringles (22–25). Of the 15 amino-terminal residues of apo(a), 12 were identical matches with plasminogen residues 349–363. This region of plasminogen is not its amino terminus, but rather the beginning of kringle 4. It is unlikely that the sequence obtained for apo(a) is due to contamination since quantitative recovery from the sequencer was 70 and 50% on the first two cycles, respectively. Also, reduced apo(a) migrates as a single band with M_r ≈280,000 by $NaDodSO_4$/PAGE with purity of 95% and no apparent bands with the molecular weights of plasminogen (92,000) or plasmin (62,000 and 25,000), nor were bands apparent with the molecular weight of a plasminogen fragment consisting of kringles 4, 5, and the protease domain. Furthermore, apo(a) that had been eluted from a polyacrylamide gel gave the same amino-terminal sequence (data not shown).

To obtain further peptide sequence, apo(a) was subjected to limited proteolysis by trypsin or V8 protease. Fragments generated were isolated by either $NaDodSO_4$/PAGE gel elution (17) or HPLC. As shown in Fig. 1, six trypsin and two V8 protease fragments yielded sequences that have striking homology to plasminogen. The amino-terminal sequences of V8 fragment 1 and tryptic fragments 1–3 were overlapping sequences that corresponded to residues 391–425 of plasminogen kringle 4. The amino-terminal sequence of V8 fragment 1 and tryptic peptide 1 were identical to the corresponding plasminogen sequence, whereas the sequence obtained for tryptic peptides 2 and 3 were slightly different (Fig. 1). Of the 31 amino acids identified by tryptic peptides 2 and 3, 7 residues varied from the corresponding plasminogen kringle-4 sequence (77% homology); two of the seven differences were conservative substitutions. The sequence of tryptic peptides 2 and 3 has a lower degree of homology to kringles 1, 2, 3, and 5 of plasminogen (61%, 55%, 55%, and 64%, respectively). This result suggests that tryptic peptides 2 and

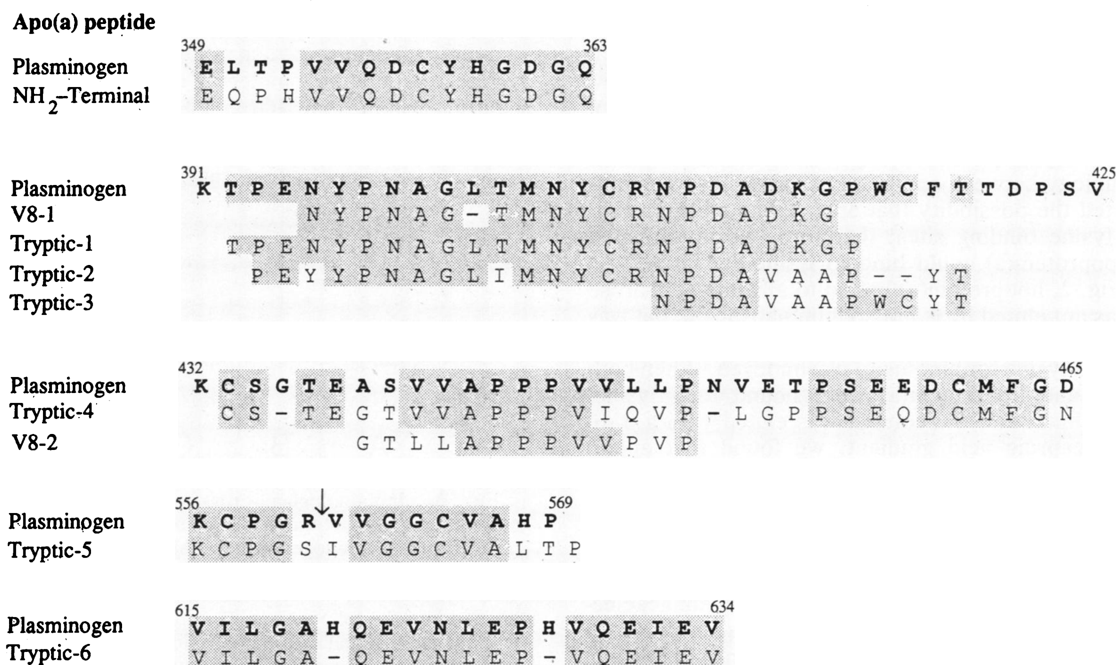

FIG. 1. Homology of partial apo(a) amino acid sequences with plasminogen. –, residue(s) not identified. Identical matches are shown in shaded areas. Plasminogen sequences were from ref. 18.

3 may represent sequence obtained from a duplicated kringle 4-like domain within apo(a).

Amino-terminal sequences of tryptic peptide 4 and V8 peptide 2 were homologous to plasminogen residues 432–465. This region of plasminogen represents the end of kringle 4 (residue 433), a linker region (residues 434–460), and the beginning of kringle 5 (residue 461). The amino-terminal sequence of tryptic fragment 4 showed 67% homology with residues 432–467 of plasminogen and 90% homology if conservative substitutions are taken into account. Significantly, the V8 peptide 2 sequence was slightly different than the analogous tryptic peptide 4 sequence, which may indicate another duplicated domain within apo(a). Tryptic fragments 1–4 and V8 fragments 1 and 2 also share some homology with the kringles of prothrombin, t-PA, urokinase, and factor XII. This homology, however, is considerably less than that observed for plasminogen kringles.

Apo(a) tryptic fragments 5 and 6 were found to have homology to the protease region of plasminogen. Tryptic peptide 6 sequence shares 100% homology with plasminogen residues 615–627, while the amino-terminal sequence of tryptic peptide 5 has 69% homology to plasminogen residues 556–569. It is noteworthy that arginine-560 is the site at which tissue plasminogen activator and urokinase cleave plasminogen to yield active plasmin. The analogous residue in apo(a) tryptic peptide 5 sequence is a serine. This suggests that apo(a) might contain a protease domain that cannot be activated. To test this hypothesis, we found that lipoprotein(a) (purified in the absence of diisopropyl fluorophosphate) did not bind [^{3}H]diisopropyl fluorophosphate, even after treatment with urokinase or t-PA. Nor did lipoprotein(a) exhibit any proteolytic activity in plasmin-specific assays after incubation with urokinase or t-PA (in the presence of fibrin) (D.L.E., data not shown). By NaDodSO$_4$/PAGE we also observed that urokinase and t-PA did not proteolyze apo(a) associated with lipoprotein(a) particles. Streptokinase, which forms a 1:1 molar complex with plasminogen and activates it via a nonproteolytic mechanism, also did not result in the generation of proteolytic activity when incubated with lipoprotein(a). These observations support the hypothesis that lipoprotein(a) has no intrinsic proteolytic activity. By mimicking plasminogen the apo(a) component of lipoprotein(a) could act as an inhibitor of t-PA. However, we found this not to be the case. Lipoprotein(a) did not inhibit either the activation of plasminogen or the cleavage of the peptide substrate S-2288 (Helena Laboratories, Beaumont, TX) by t-PA.

Binding of Lipoprotein(a) to Lysine-Sepharose. The observed homology between apo(a) and kringle 4 of plasminogen suggested the possibility that apo(a), like plasminogen, possesses lysine binding sites; therefore, we investigated whether lipoprotein(a) could bind to lysine-Sepharose. As shown in Fig. 2, lipoprotein(a) bound to a lysine-Sepharose column, was not eluted by 0.5 M sodium phosphate, but was eluted by 0.2 M ε-aminocaproic acid. ε-Aminocaproic acid competes with lysine for binding to plasminogen. When both plasminogen and lipoprotein(a) were bound to a lysine-Sepharose column and the column was eluted with a 0–10 mM ε-aminocaproic acid gradient, we found that apo(a) eluted earlier ($\approx$1.8 mM) than plasminogen ($\approx$3.8 and 5.3 mM). LDL did not bind to lysine-Sepharose.

Immunological Cross-Reactivity of Lipoprotein(a) and Plasminogen. To test the possibility that apo(a) and plasminogen shared common antigenic determinants, lipoprotein(a) and plasminogen were subjected to NaDodSO$_4$ gradient gel electrophoresis followed by immunoblotting. As seen in Fig. 3, lipoprotein(a) reacted with an anti-plasminogen antibody and conversely plasminogen reacted with an anti-apo(a) antibody indicating that apo(a) and plasminogen share common epitopes. In addition, a monoclonal antibody (10-F-1) known to

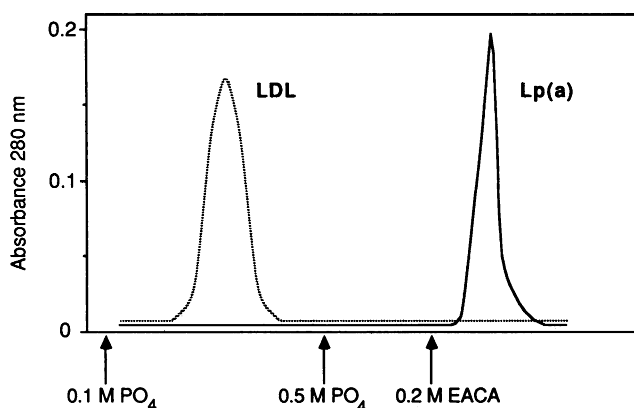

FIG. 2. Lysine-Sepharose chromatography of purified lipoprotein(a) and LDL. Lipoprotein(a) [Lp(a)] and LDL (2 mg of protein) in 0.1 M sodium phosphate (pH 7.4) were applied to a lysine-Sepharose column (1.5 × 10 cm) equilibrated in 0.1 M sodium phosphate (pH 7.4). The column was subsequently washed with 0.5 M sodium phosphate (pH 7.4), and lipoprotein(a) was eluted with 0.2 M ε-aminocaproic acid (EACA).

recognize an epitope in the kringle-4 region of human plasminogen reacted with lipoprotein(a) (Fig. 3*A*). Neither factor VIII or t-PA antibodies detected apo(a).

DISCUSSION

Apo(a), a glycoprotein with a molecular weight ranging from 280,000 to 500,000, is disulfide linked to apoliprotein B of an LDL-like particle to yield the so-called lipoprotein(a) particle (10-15). Partial amino acid sequencing of apo(a) revealed significant homology to plasminogen, a serine protease zymogen of M_r 92,000. Plasminogen consists of five homologous and tandemly repeated domains called kringles and a trypsin-like protease domain (18). The kringle domains have specific binding regions for fibrin, α_2-antiplasmin, lysine, and ω-aminocarboxylic acids (19–21). Plasminogen kringles 1–4 bind lysine, with kringles 1 and 4 exhibiting the highest binding affinity for lysine (19). Urokinase and t-PA activate plasminogen by cleaving a single peptide bond yielding active two-chain plasmin, which subsequently cleaves itself at lysine-77 generating a molecule of M_r 87,000 (26, 27). Significantly, of more than 300 residues identified for apo(a), no unique sequences were revealed. However, the apo(a) iso-

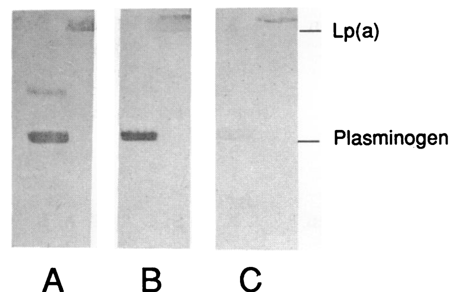

FIG. 3. Immunological cross-reactivity of lipoprotein(a) and plasminogen as determined by immunoblotting. Lipoprotein(a) [Lp(a)] and plasminogen were subjected to electrophoresis on a 2–16% polyacrylamide/NaDodSO$_4$ gradient gel and transferred electrophoretically to nitrocellulose. The replicate nitrocellulose was divided into the following three sections: section A was developed with the monoclonal antibody to kringle 4 of plasminogen, section B with anti-plasminogen, and section C with anti-apo(a). Plasminogen concentrations in sections A, B, and C were 16, 2, and 16 μg, respectively, and lipoprotein(a) concentrations in sections A, B, and C were 10, 20, and 2 μg, respectively.

form sequenced here was the smallest (M_r, 280,000) form reported to date (10, 11, 13). The higher molecular weight isoforms of apo(a) may yield unique sequences.

The results shown here suggest that apo(a) contains regions that share homology with kringle 4, and perhaps 5, as well as the protease domain of plasminogen. Analysis of the apo(a) sequences also indicates that apo(a) may contain at least a repeated kringle 4-like domain and possibly a kringle 4-kringle 5 domain repeat. Since kringle 4 of plasminogen binds lysine, it was not surprising to observe that lipoprotein(a) bound to lysine-Sepharose. The complete amino acid sequence of apoB has been determined and shown to contain lysine- and arginine-rich regions (22). Thus, apo(a) may interact with these regions facilitating its covalent linking with apoB. Plasminogen kringle 4 has also been implicated in the binding of plasminogen to fibrin (20). The binding of lipoprotein(a) to fibrin has not yet been determined. It is of interest to note that, while isolating plasminogen, Gilmore and Moroz (28) identified a very low density lipoprotein-like particle that bound lysine-Sepharose. Similar to lipoprotein(a), this particle had pre-β electrophoretic mobility, contained apoB, and eluted from lysine-Sepharose at a lower ε-aminocaproic acid concentration than plasminogen (1–3, 10).

The data presented here also suggest that if apo(a) contains a complete protease domain, it may be either inactive or not activatable by streptokinase, urokinase, or t-PA. Plasminogen is activated by cleavage by urokinase or t-PA at arginine-560. The sequence around this site is Lys-Cys-Pro-Gly-Arg-Val-Val-Gly-Gly. The analogous apo(a) sequence is Lys-Cys-Pro-Gly-Ser-Ile-Val-Gly-Gly. Thus serine and not arginine is present at the proposed activation site of the protease domain of apo(a). That the proposed apo(a) protease may be inactive is also supported by the observations that apo(a) does not bind diisopropyl fluorophosphate, is not cleaved by either urokinase or t-PA, nor is it active in plasmin-specific assays. However, based on the size of apo(a) and the proposal that the kringle 4-like domain may be repeated, it is possible that the protease domain may also be duplicated with at least one being active. Furthermore, the assays performed here may not be sensitive to the true proteolytic activity of apo(a). However, the production of a serine protease-like molecule with an altered activation site having no proteolytic activity is not unprecedented. The α and γ subunits of 7S nerve growth factor have homology with the serine protease kallikrein (29). However, the γ subunit is active while the α subunit is not. Sequence analysis shows that the γ subunit contains arginine at its activation site whereas the α subunit has a glutamine at this position.

The homology of apo(a) and plasminogen is underscored by immunological cross-reactivity. By immunoblot analysis apo(a) was detected by both a plasminogen polyclonal antibody and a plasminogen kringle 4 monoclonal antibody, and apo(a) polyclonal antibodies detected plasminogen. This immunological cross-reactivity should be taken into account when determining plasma lipoprotein(a) levels from data based on antibody binding alone. In particular, estimates of low levels of lipoprotein(a) in so-called lipoprotein(a)-negative subjects could be confounded by this cross-reactivity unless size, density, or other features of the protein are determined, which would eliminate false positives.

The correlation of high lipoprotein(a) levels with coronary artery disease and atherosclerosis has tempted some to consider lipoprotein(a) as an atherogenic agent. However, the mechanism(s) by which lipoprotein(a) exerts this proposed effect are unknown. It has been proposed that the lysine- and arginine-rich regions within the apoB molecule may, in part, mediate the binding of apoB to the LDL receptor (30, 31). The binding of lipoprotein(a) to lysine-Sepharose leads to the speculation that apo(a), by interacting with the basic amino acid-rich regions of apoB, may prevent the binding of lipoprotein(a) to the LDL receptor. Thus, apo(a) could exert its atherogenic effect by directing lipoprotein(a) to cellular receptors other than the LDL receptor, such as the macrophage acetyl-LDL receptor. Maartmann-Moe and Berg (32) suggest that human fibroblasts bind and internalize lipoprotein(a) particles by mechanism(s) other than the LDL receptor. In addition, Armstrong et al. (12) have found that the binding of lipoprotein(a) particles to the LDL receptor is significantly increased when apo(a) is removed by reduction. These studies, however, are in contrast to Albers and coworkers (34) who suggest that the LDL receptor does bind lipoprotein(a) particles. Plasminogen has been shown to specifically bind human endothelial cells. ε-Aminocaproic acid competes with this binding, suggesting that the kringle domains of plasminogen mediate its binding to these cells (33). This result demonstrates the possible presence of cell surface receptors that recognize protein kringle domains. Since kringle 4 of plasminogen has been implicated in fibrin binding, apo(a) may also bind fibrin. Apo(a) could, therefore, direct lipoprotein(a) particles to sites of fibrin deposition (vascular injury) and thus provide a concentrated substrate for the acetyl-LDL receptor of macrophages.

Apart from the limited binding studies on lipoprotein(a), the normal physiological function of apo(a) also remains unknown. Even the existence or level of lipoprotein(a) in lipoprotein(a)-negative subjects remains uncertain. Lipoprotein(a)-positive subjects might over express a normal gene product, or apo(a) might result from a mutant plasminogen-like gene product that is susceptible to cross-linking to apoB. It is hoped that the data presented here, and the subsequent cloning of the apo(a) gene, will help to elucidate the function of lipoprotein(a) in normal lipid metabolism and in the development of atherosclerosis.

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1. Dahlen, G., Ericson, G., Furberg, C., Lundkuist, L. & Svärdsudd, K. (1972) *Acta Med. Scand.* Suppl. 531, 1–29.
2. Berg, K., Pahlin, G. & Frick, M. H. (1974) *Clin. Genet.* **6**, 230–235.
3. Dahlen, G., Berg, K., Gillnas, T. & Ericson, C. (1975) *Clin. Genet.* **7**, 334–341.
4. Albers, J. J., Adolphson, J. L. & Hazzard, W. R. (1977) *J. Lipid Res.* **18**, 331–338.
5. Dahlen, G. H., Attar, M., Guyton, J. R., Kautz, J. A. & Gotto, A. M. (1983) *Arteriosclerosis (Dallas)* **3**, 478 (abstr.).
6. Koltringer, P. & Jurgens, G. (1985) *Atherosclerosis* **58**, 187–198.
7. Dahlen, G. H., Guyton, J. R., Attar, M., Farmer, J. A., Kautz, J. A. & Gotto, A. M. (1986) *Circulation* **74**, 758–765.
8. Gaubatz, J. W., Heideman, C., Gotto, A. M., Jr., Marrisett, J. D. & Dahlen, G. H. (1983) *J. Biol. Chem.* **258**, 4582–4589.
9. Utermann, G. & Weber, W. (1983) *FEBS Lett.* **154**, 357–361.
10. Fless, G. M., Rolih, C. A. & Scanu, A. M. (1984) *J. Biol. Chem.* **259**, 11470–11478.
11. Fless, G. M., ZumMallen, M. & Scanu, A. M. (1985) *J. Lipid Res.* **26**, 1224–1229.
12. Armstrong, V. W., Walli, A. K. & Seidel, D. (1985) *J. Lipid Res.* **26**, 1314–1323.
13. Fless, G. M., ZumMallen, M. & Scanu, A. M. (1986) *J. Biol. Chem.* **261**, 8712–8718.

14. Seman, L. J. & Brekenridge, W. C. (1986) *Biochem. Cell Biol.* **64**, 999–1009.
15. Gaubatz, J., Chari, M. V., Nava, M. L., Guyton, J. R. & Morrisett, J. D. (1987) *J. Lipid Res.* **28**, 69–79.
16. Hewick, R. M., Hunkapiller, M. W., Hood, L. E. & Preyer, W. J. (1982) *J. Biol. Chem.* **256**, 7990–7997.
17. Hunkapiller, M. W., Lujan, E., Ostrander, F. & Hood, L. E. (1983) *Methods Enzymol.* **91**, 227–247.
18. Castellino, F. J. (1981) *Chem. Rev.* **81**, 431–446.
19. Lerch, P. G. & Rickli, E. E. (1980) *Biochim. Biophys. Acta* **625**, 374–378.
20. Collen, D. (1980) *Thromb. Haemostasis* **43**, 77–89.
21. Wiman, B. & Collen, D. (1979) *Biochim. Biophys. Acta* **579**, 142–146.
22. Magnusson, S., Peterson, T. E., Sottrup-Jensen, L. & Claeys, H. (1975) in *Proteases and Biological Control*, eds. Reich, E. & Rifkin, D. B. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 123–149.
23. Pennica, D., Holmes, W. E., Kohr, W. J., Harkins, R. N., Vehar, G. A., Ward, C. A., Bennett, W. F., Yelverton, E., Seeburg, P. H., Heyneker, H. L., Goeddel, D. V. & Collen, P. (1983) *Nature (London)* **301**, 214–221.
24. Gunzler, W. A., Steffens, G. J., Otting, F., Kim, S.-M., Frankus, E. & Flohe, L. (1982) *Hoppe-Seyler's Z. Physiol. Chem.* **363**, 1155–1165.
25. Cool, D. E., Edgell, C.-J. S., Louie, G. V., Zoller, M. J., Brayer, G. D. & MacGillivray, R. T. A. (1985) *J. Biol. Chem.* **260**, 13666–13676.
26. Sodetz, J. M., Brockway, W. J. & Castellino, F. J. (1972) *Biochemistry* **11**, 4451–4457.
27. Robbins, K. C., Summaria, L., Hsieh, B. & Shah, R. J. (1967) *J. Biol. Chem.* **242**, 2333–2342.
28. Gilmore, M. J. & Moroz, L. A. (1983) *Thromb. Res.* **31**, 863–874.
29. Bradshaw, R. A., Dunbar, J. C. & Isackson, P. J. (1986) *J. Cell. Biochem.* Suppl. 10a, 233 (abstr.).
30. Knott, T. J., Rall, S. C., Innerarity, T. L., Jacobson, S. F., Urdea, M. S., Levy-Wilson, B., Powell, L. M., Pease, R. J., Eddy, R., Nakai, H., Priestley, L. M., Robertson, E., Rall, L. B., Betsholtz, C., Shows, T. B., Mahley, R. W. & Scott, J. (1985) *Science* **230**, 37–43.
31. Knott, T. J., Pease, R. J., Powell, L. M., Wallis, S. C., Rall, S. C., Innerarity, T. L., Blackhart, B., Taylor, W. H., Marcel, Y., Milne, R., Johnson, D., Fuller, M., Lusis, A. J., McCarthy, B. J., Mahley, R. W., Levy-Wilson, B. & Scott, J. (1986) *Nature (London)* **323**, 734–737.
32. Maartmann-Moe, K. & Berg, K. (1981) *Clin. Genet.* **20**, 352–362.
33. Hajjar, K. A., Harpel, P. P., Jaffe, E. A. & Nachman, R. L. (1986) *J. Biol. Chem.* **261**, 11650–11656.
34. Floren, C.-H., Albers, J. J. & Bierman, E. L. (1981) *Biochem. Biophys. Res. Commun.* **102**, 636–639.