Ligand-Induced Conformational Change of Lipoprotein(a)†

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ABSTRACT: Lipoprotein(a) undergoes a dramatic, reversible conformational change on binding 6-aminohexanoic acid (6-AHA), as measured by a decrease in the sedimentation rate, the magnitude of which is directly proportional to apo(a) mass. A similar reversible transition from a compact to an extended form has been shown to occur in plasminogen on occupation of a weak lysine binding site. The magnitude of the change in Lp(a) with large apo(a) is about 2.5 times that seen for plasminogen, however. Regardless of apo(a) size, binding analysis indicated that 1.4−4 molecules of 6-AHA bound per Lp(a) particle; the midpoint of the conformational change occurs at 6-AHA concentrations of 100−200 mM. Since rhesus Lp(a), which lacks both kringle V and the strong lysine binding site on kringle IV 10, also undergoes a similar conformational change, the phenomenon may be attributable to weak sites, possibly located in K-IV 5−8. Compact Lp(a), i.e., native Lp(a), had a frictional ratio (f/f_o) of 1.2 that was independent of apo(a) mass, implying constant shape and hydration. For Lp(a) in saturating 6-AHA, f/f_o ranged from 1.5 to over 2.1 for the largest apo(a) with 32 K-IV, indicating a linear relationship between hydrodynamic volume and number of kringles, as expected for an extended conformation. However, only the variable portion of apo(a) represented by the K-IV 2 domains, participates in the conformational change; the invariant K-IV 3−9 domains remain close to the surface. These results suggest that apo(a) is maintained in a compact state through interactions between weak lysine binding sites and multiple lysines on apoB and/or apo(a), and that these interactions can be disrupted by 6-AHA, a lysine analog.

Lipoprotein(a) [Lp(a)][1] is a cholesteryl ester rich lipoprotein particle present in human plasma that has recently attracted considerable attention because of its possible role in the generation of atherosclerosis and thrombosis (Scanu & Fless, 1990; Utermann, 1989). Structurally, Lp(a) is similar to LDL, but has a slightly larger lipid complement and has a high molecular weight glycoprotein, called apo(a), disulfide-linked to apoB-100 (Fless et al., 1984, 1985, 1986; Gaubatz et al., 1983; Utermann & Weber, 1983). Apo(a) is polymorphic in molecular weight, containing from 12 to 51 kringle IV domains as well as a kringle V and a protease domain all homologous to the respective domains of plasminogen (Lackner et al., 1993; McLean et al., 1987). The kringle IV domains of apo(a) are not identical (McLean et al., 1987), and have been classified into 10 types (Morrisett et al., 1990). Heterogeneity of apo(a) is caused by variation in the number of K-IV 2 domains, whereas the other K-IV types are invariable (Koschinsky et al., 1990; Vanderhoek et al., 1993). Like plasminogen, Lp(a) has a strong lysine binding site (LBS), that enables it to bind to lysine−Sepharose (Armstrong et al., 1990; Eaton, 1987; Fless & Snyder, 1994; Hoover-Plow et al., 1993). In apo(a), this LBS is located on K-IV 10 (Lograsso et al., 1994; Snyder & Fless, 1994), which is the only K-IV domain of apo(a) having both Asp^{55} and Asp^{57} of the anionic center intact. Weaker LBS may exist on K-IV 5−8 where Asp^{57} is replaced by Glu^{57} (Ernst et al., 1995; Frank et al., 1994a,b; Guevara et al., 1992).

Through LBS located on three of its five kringle domains, plasminogen is thought to interact with fibrin (Lucas et al., 1983; Sottrup-Jensen et al., 1978; Thorsen, 1975; Wu et al., 1990). The act of binding elicits a conformational change in Glu-plasminogen that facilitates its activation to plasmin (Peltz et al., 1982; Walther et al., 1975). A similar change can be detected in solution, where saturating amounts of lysine analogs such as 6-AHA or t-AMCHA transform the "native" closed form of Glu-plasminogen into an open or extended structure (Abiko et al., 1969; Alkjaersig, 1964; Christensen & Molgaard, 1992; Mangel et al., 1990; Markus et al., 1978; Marshall et al., 1994; Ponting et al., 1992; Violand et al., 1978). The conformational change alters some of the physical properties of Glu-plasminogen, including its sedimentation rate which decreases reversibly from 5.75 to 4.85 S (Violand et al., 1978). Recent studies have indicated that the LBS of apo(a) are functionally similar to those of plasminogen as is evidenced by the ability of Lp(a) to compete with plasminogen for lysine−Sepharose (Armstrong et al., 1990; Eaton, 1987; Fless & Snyder, 1994; Hoover-Plow et al., 1993), fibrin (Fless & Snyder, 1994; Fleury & Angles-Cano, 1991; Harpel et al., 1989; Leerink et al., 1994;

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[1] Abbreviations: Lp(a), lipoprotein(a); apo(a), apolipoprotein(a); apoB, apolipoprotein B; LDL, low-density lipoprotein; Lp(a-), remnant lipoprotein particle left after reduction and carboxymethylation of Lp(a) and removal of apo(a); t-PA, tissue plasminogen activator; PAI-1, plasminogen activator inhibitor; K, kringle; K-IV 1−10, apo(a) kringle domain of subtypes 1−10, all with homology to kringle 4 of plasminogen; Asp^{55}, numbering of amino acids of K-IV follows the convention of plasminogen K-V; LBS, lysine binding site; 6-AHA, 6-aminohexanoic acid; t-AMCHA, *trans*-4-(aminomethyl)cyclohexanecarboxylic acid; Na_2EDTA, sodium salt of ethylenediaminetetraacetic acid; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; HEPES, sodium salt of *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; HBS, HEPES-buffered saline (10 mM HEPES, 150 mM NaCl, 0.01% Na_2EDTA, 0.01% NaN_3, pH 7.4); M_r, relative molecular mass.

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Loscalzo et al., 1990; Rouy et al., 1992), and cellular receptors (Gonzales-Gronow et al., 1989; Hajjar et al., 1989; Miles et al., 1989, 1995). In light of the similarities between the two molecules, it was therefore of interest to investigate whether Lp(a) undergoes a conformational change in response to the saturation of apo(a)−LBS by the lysine analog 6-AHA.

EXPERIMENTAL PROCEDURES

Preparation of Lipoproteins. Autologous Lp(a) and LDL were purified from the plasma of six human subjects, all of whom gave informed consent prior to blood donation or plasmapheresis. Lipoproteins were isolated from plasma by a combination of lysine−Sepharose chromatography and density gradient centrifugation using previously described methods (Fless & Snyder, 1994; Fless et al., 1986; Snyder et al., 1992). Lp(a-) was also prepared from Lp(a) by procedures described previously (Fless et al., 1985, 1986). Lipoprotein purity was established by SDS−PAGE using 3.5% T, 2.66% C, 1.5 mm polyacrylamide gels in a Novex X Cell II gel electrophoresis apparatus (Novex, San Diego, CA). Lp(a) particles with apo(a) molecular weight less than 500 000 were phenotyped by electrophoresis using the Phast system (Pharmacia) and the conditions described by Molinari et al. (1990). Those with larger molecular weight apo(a)s were phenotyped using the 1.5% agarose submarine gel system described by Kamboh et al. (1991). Both gel systems were calibrated using four different homogeneous Lp(a) particles of which the apo(a) molecular weight, ranging from 2.89×10^5 to 4.88×10^5, had been determined previously by sedimentation equilibrium (Fless et al., 1994). The number of kringle IV domains in each apo(a) polymorph was calculated from the protein molecular weight obtained by subtracting the portion (23%) due to carbohydrate (Fless et al., 1994). Nondenaturing gel electrophoresis of LDL and Lp(a) was carried out using previously described methods (Fless et al., 1984).

Analytical Centrifugation. Sedimentation and flotation equilibrium experiments were performed using a Beckman Optima XLA ultracentrifuge interfaced to an IBM PS/2 Model 55SX personal computer, an An-60 Ti four-place rotor, and analytical cells equipped with six-channel charcoal-filled centerpieces. Equilibrium ultracentrifugation of Lp(a), Lp(a-), and LDL was conducted using three different solutions of NaBr that varied between 1.5 and 12 wt % in concentration as previously described (Fless et al., 1994, 1976, 1986). By conducting equilibrium experiments at several densities, it was possible to evaluate simultaneously lipoprotein molecular weight and partial specific volume.

Sedimentation velocities for each lipoprotein of interest were measured in the Beckman Optima XLA analytical centrifuge using the An-60 Ti rotor and double-sector cells with aluminum-filled epon centerpieces and quartz windows. Rotor speed was 40 000 rpm, and the temperature was controlled at 20 °C. Diffusion experiments were carried out at 3500 rpm and 20 °C using a synthetic boundary centerpiece to create a sharp boundary by layering solvent over lipoprotein solution. Both rotor and cells were preequilibrated to 20 °C, and neither experiment was initiated before that temperature was attained.

The lipoproteins were dialyzed extensively against 10 mM HEPES, 0.01% Na_2EDTA, and 0.01% NaN_3, pH 7.4, and were then adjusted by dilution to a protein concentration of approximately 0.2−0.3 mg/mL and the desired concentration of 6-AHA. All solutions of Lp(a) containing 6-AHA were prepared in the presence of 10 mM HEPES, 100 mM NaCl, 0.01% Na_2EDTA, and 0.01% NaN_3, pH 7.4. Lp(a) without 6-AHA was adjusted to 0.15 M NaCl, 10 mM HEPES, 0.01% Na_2EDTA, and 0.01% NaN_3, pH 7.4. The cells were scanned at 8 min intervals at wavelengths giving the best signal to noise ratios. Solutions with low concentrations of 6-AHA were scanned at wavelengths closer to 220 nm, whereas those with high concentrations were scanned at 280 nm at which 6-AHA does not absorb. Usually 16 data sets were collected after a 16 or 24 min delay.

Data from sedimentation equilibrium and sedimentation velocity experiments were respectively analyzed by the XLAEQ and XLAVEL software programs (Beckman, Palo Alto, CA). Diffusion coefficients were analyzed from data originating either from low-speed diffusion or sedimentation velocity experiments using the second moment boundary spreading method of the Optima XL-A Data Analysis software (Beckman).

Solvent density was measured with a Precision Density Meter DMA-02-C (Mettler/Paar). The instrument was calibrated with distilled water and dry air at known barometric pressure. The temperature of the vibration density meter cell was controlled to 20 ± 0.01 °C with an external water bath. Solvent viscosity was measured with Cannon−Manning semimicro calibrated viscometers (Cannon Instrument Co., State College, PA) using a Cannon Instrument Co. constant-temperature water bath controlled to 20 ± 0.01 °C. The sedimentation and diffusion coefficients were both corrected to standard conditions of water at 20 °C:

$$s_{20,w} = s_{obs}\frac{\eta_s(1 - \bar{\nu}\rho_w)}{\eta_w(1 - \bar{\nu}\rho_s)}$$

where η_s and η_w are, respectively, the viscosities of the solvent and water at 20 °C; ρ_w and ρ_s are, respectively, the densities of pure water and solution at 20 °C; and $\bar{\nu}$ is the lipoprotein partial specific volume. There was no need to correct the sedimentation coefficient of Lp(a) in HBS for concentration dependence (see Figure 1), although this was necessary for Lp(a) dissolved in 6-AHA. We corrected it by assuming that the difference in the magnitude between the observed sedimentation velocity and its value at infinite dilution [e.g., −3.1% at saturating levels of 6-AHA and a Lp(a) concentration of 0.3 mg/mL] was directly proportional to the percentage change in conformation or $s_{20,w}$ of Lp(a) induced by the addition of 6-AHA.

Determination of the Frictional Ratio. The translational friction coefficient, *f*, was calculated using the molecular weights and partial specific volumes obtained by sedimentation equilibrium, and the Svedberg equation:

$$f = \frac{M(1 - \bar{\nu}\rho)}{Ns}$$

where M is the molecular weight, $\bar{\nu}$ the partial specific volume, and s the sedimentation coefficient of the lipoprotein; ρ is the solvent density and N is Avogadro's number. The value of f is compared with f_o, the minimum possible friction coefficient of a spherical anhydrous protein molecule. The volume V_p of such a particle could be calculated from

M and $\bar{\nu}$:

$$V_p = \frac{4\pi}{3} R_p^{\,3} = \frac{M\bar{\nu}}{N}$$

where R_p is the radius of the anhydrous sphere. It has been observed, however, that the following empirical relationship is more accurate:

$$R_p = (6.723 \times 10^{-9}) M^{1/3}$$

(Teller et al., 1979; Waxman et al., 1993). Taking hydration into account, the volume of a spherical particle of molecular weight M and partial specific volume $\bar{\nu}$ is

$$V = V_p \frac{\bar{\nu} + \delta}{\bar{\nu}}$$

where δ is the hydration of the protein in grams of H_2O per gram of protein. For this spherical molecule, the hypothetical frictional coefficient is called f_o, and is given by Stoke's law:

$$f_o = 6\pi\eta R_{sphere} = \left\{ \frac{3}{4\pi} \left[V_p \frac{\bar{\nu} + \delta}{\bar{\nu}} \right] \right\}^{1/3}$$

where η is the solvent viscosity and R_{sphere} is the radius of the hydrated spherical molecule. As a first approximation, the hydration of Lp(a) was estimated from the sum of the hydrations of Lp(a-) and apo(a). We further assumed a value for the hydration of Lp(a-) as that of LDL, which Fisher et al. determined to be 0.34 g of H_2O/g of lipoprotein (Fisher et al., 1971). The hydration of apo(a) was calculated from its amino acid and carbohydrate composition as suggested by Kuntz and Kauzmann (1974). We obtained a value of 0.45 g of H_2O/g of apo(a) for all human apo(a) polymorphs examined in this study. Using molecular weights of four pairs of Lp(a) and Lp(a-) that were determined previously (Fless et al., 1994), we determined the percentage of apo(a) in Lp(a) from the difference in mass between Lp(a) and Lp(a-) (Fless et al., 1994). This gave values of hydration that varied from 0.36 to 0.37 g of H_2O/g of Lp(a) for Lp(a) particles with apo(a) polymorphs having 15−27 K-IV domains. For the calculation of f/f_o, δ was estimated at 0.37 g of H_2O/g of Lp(a) for all Lp(a) particles.

As an estimate of the effective hydrodynamic radius of the Lp(a) particles in various solvents, the frictional coefficient obtained from sedimentation velocity was used in the Stoke's equation:

$$R_h = \frac{f}{6\pi\eta}$$

where R_h is the effective hydrodynamic radius.

Chemical Analysis. Protein content was determined by the method of Lowry et al. (1951) as modified by Markwell et al. (1978) using bovine serum albumin as standard, as previously described (Fless et al., 1986).

RESULTS

Concentration and Ionic Strength Dependence of the Sedimentation Coefficient. Since the sedimentation coefficient is usually concentration dependent, we measured the magnitude of this effect with Lp(a) in order to determine if

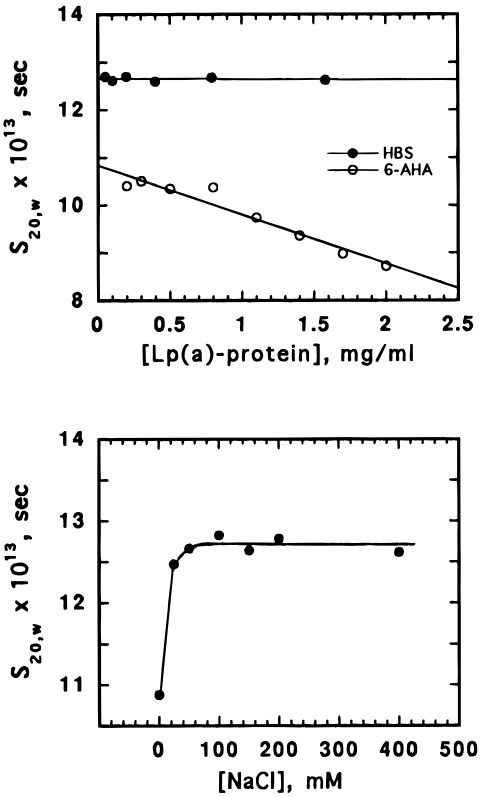

FIGURE 1: Upper panel: Concentration dependence of the sedimentation coefficient of Lp(a) (15 K-IV) in HBS and 500 mM 6-AHA. The sedimentation coefficient was corrected for the viscosity and density of the solvent. The equation for the least-squares line obtained with Lp(a) in HBS is $y = 12.7 - 0.0191x$, and for Lp(a) in 6-AHA, $y = 10.8 - 1.040x$. Lower panel: Effect of ionic strength on the sedimentation coefficient of Lp(a) (15 K-IV). The ionic strength was changed by increasing the concentration of NaCl from 0 to 0.4 M while maintaining a constant concentration of 10 mM HEPES, 0.01% Na_2EDTA, 0.01% NaN_3, pH 7.4.

sedimentation rates obtained at protein concentrations ranging between 0.2 and 0.5 mg/mL differed significantly from rates at infinite dilution. As shown in Figure 1a (top panel), the sedimentation coefficient of Lp(a) in HBS exhibited minimal concentration dependence, with the coefficients determined at 0.2 and 0.5 mg/mL, respectively, differing from the value at zero concentration by only −0.03 and −0.08%. Therefore, values of $s_{20,w}$ determined at these concentrations are essentially equivalent to $s^0_{20,w}$ and were not corrected. In contrast, the sedimentation coefficient of Lp(a) in the presence of 500 mM 6-AHA, 100 mM NaCl showed a pronounced concentration dependence (Figure 1a). The magnitude of $s_{20,w}$ determined at a concentration of 0.3 mg/mL differed appreciably (−3.1%) from the one extrapolated to infinite dilution. Sedimentation coefficients of Lp(a) in 6-AHA were therefore corrected for concentration dependence. A pronounced effect is usually observed with highly extended macromolecules, whereas the concentration dependence of globular proteins is much less (Van Holde, 1971). We also investigated the effect of ionic strength on the sedimentation rate of Lp(a) and found that above an ionic strength of 0.1, or 0.1 M NaCl, Lp(a) sedimentation rates were essentially constant. At zero concentration of NaCl with Lp(a) dissolved in 10 mM HEPES, the sedimentation rate decreased by 14% and is probably due to unfolding caused by increased charge repulsion.

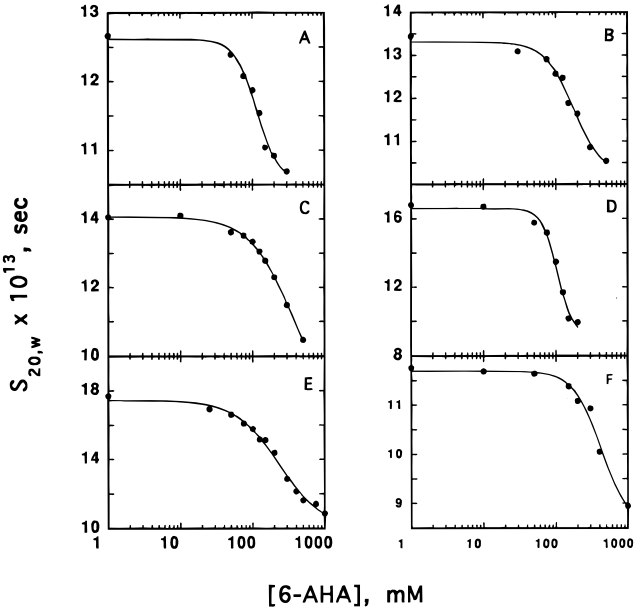

FIGURE 2: Effect of 6-AHA on the $s_{20,w}$ of human and rhesus Lp(a). (A) 15 K-IV; (B) 18 K-IV; (C) 18 K-IV; (D) 27 K-IV; (E) 32 K-IV; and (F) 14 K-IV rhesus Lp(a). The solid line represents the best fit theoretical curve calculated as described under Experimental Procedures.

Effect of 6-AHA on the Sedimentation Coefficient of Lp(a). The effect on Lp(a) conformation by the lysine analog, 6-AHA, was examined in seven different Lp(a) particles having essentially six different apo(a) polymorphs that ranged in mass from 289 000 to 558 000 daltons, or 15−32 kringle IV domains. Two subjects having respectively a 20 and a 22 kringle IV domain apo(a) had such low Lp(a) concentrations that even after plasmapheresis it was not possible to prepare enough Lp(a) to allow generation of a complete titration curve. For these two samples, we determined only the end point sedimentation rate in 0.15 M NaCl and 500 mM 6-AHA/100 mM NaCl.

As shown in Figure 2, each human Lp(a) sample exhibited a profound reduction in sedimentation rate on exposure to 6-AHA; however, the concentration of 6-AHA needed to effect a decrease was large, since 100−200 mM 6-AHA was required to produce half of the sedimentation velocity change. This suggested the involvement of weak LBS since the affinity of the strong LBS, which is presumably located on K-IV 10, is much stronger for 6-AHA. For example, the dissociation constant for the binding of 6-AHA to recombinant apo(a) K-IV is 10−20 μM (Lograsso et al., 1994), and IC_{50} values for the inhibition of the binding of Lp(a) to lysine−Sepharose by 6-AHA are in the range of 0.7−2.1 mM (Fless & Snyder, 1994; Hoover-Plow et al., 1993). This hypothesis was tested by measuring the effect of 6-AHA on the sedimentation rate of rhesus Lp(a). This lipoprotein does not bind to lysine−Sepharose, because its K-IV 10 has Trp^{72} substituted by Arg, thus rendering its strong lysine binding site nonfunctional (Scanu et al., 1993). Despite this deficiency, rhesus Lp(a) exhibited the same decrease in sedimentation velocity when treated with 6-AHA (Figure 2F).

Analysis of Sedimentation in Terms of 6-AHA Binding. The binding of saturating 6-AHA results in a dramatic conformational change from a compact form, called $Lp(a)_c$, to a more extended form, called $Lp(a)_e$. The sigmoidal shape of the curves suggested that, as a first approximation, the data could be analyzed according to a two-state model in which saturating 6-AHA induces a conformational change from $Lp(a)_c$ to $Lp(a)_e$. Thus, binding of 6-AHA was analyzed as a reversible equilibrium in which 6-AHA binds to n sites. The binding to each site was not measured directly, and might be independent or cooperative. However, we will show below that the conformational change which results from the binding of n molecules of ligand is cooperative as it obeys the Hill equation (Cantor & Schimmel, 1980). That is, it requires n molecules of bound ligand per Lp(a) particle. Accordingly, for the reaction

$$[\mathrm{Lp(a)_c}] + n[\mathrm{6AHA}] \rightleftarrows [\mathrm{Lp(a)_e{\cdot}6AHA}_n]$$

an equilibrium constant, $K_{1/2}$, can be defined as the ligand concentration at which the value of the sedimentation constant is half-way between that of the compact and extended forms of Lp(a). $K_{1/2}$ would thus be given by the expression:

$$K_{\mathrm{d,app}} = (K_{1/2})^n = \frac{[\mathrm{Lp(a)_c}][\mathrm{6AHA}]^n}{[\mathrm{Lp(a)_e{\cdot}6AHA}_n]}$$

where $[\mathrm{Lp(a)_e{\cdot}6AHA}_n]$ represents a complex between the extended form of Lp(a) and n molecules of 6-AHA. The term $(K_{1/2})^n$ for this cooperative conformational change is an apparent K_d ($K_{d,app}$), equivalent to the term K^n from the Hill equation. If x = the fraction of Lp(a) in the extended form, then the above equation becomes

$$K_{\mathrm{d,app}} = \frac{(1-x)[\mathrm{6AHA}]^n}{x}$$

The sedimentation data can be used to determine the fractions of Lp(a) in the compact and extended forms by letting S_c equal the sedimentation rate of Lp(a) in the compact or native state and S_e equal the sedimentation velocity of extended Lp(a) in saturating amounts of 6-AHA. If S is the experimental sedimentation coefficient, then $(S - S_e)/(S_c - S_e) = x$ or the fraction of extended Lp(a) and $1 - [(S - S_e)/(S_c - S_e)] = 1 - x$ or the fraction of compact Lp(a). On substitution

$$K_{\mathrm{d,app}} = \frac{[1 - [(S-S_e)/(S_c - S_e)][\mathrm{6AHA}]^n}{(S-S_e)/(S_c-S_e)}$$

which can be simplified as

$$S = \frac{(S_c - S_e)[\mathrm{6AHA}]^n}{K_{\mathrm{d,app}} + [\mathrm{6AHA}]^n} + S_e$$

Analysis of the titration curves indicated that binding of 6-AHA by Lp(a) involved multiple LBS, in that the number of 6-AHA molecules bound by a particular Lp(a) particle at saturation ranged from 1.4 to 4 (see Table 1). The magnitude of the phenomenological dissociation constants varied greatly (from 0.3 M to 1×10^{-5} M) and was a reflection of the fact that the $K_{d,app}$ is proportional to the 6-AHA concentration raised to the nth power, where n is equal to the number of bound 6-AHA molecules per Lp(a) particle. However, there was no correspondence between the number of K-IV domains and strength of binding.

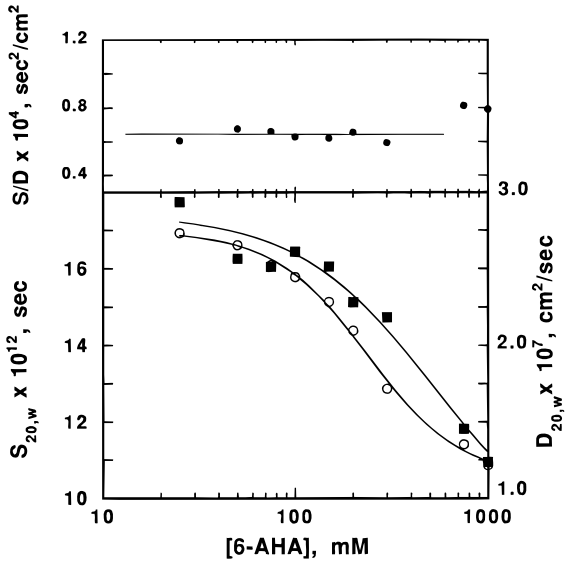

FIGURE 3: Effect of 6-AHA on the ratio of the sedimentation to the diffusion coefficient of 32 K-IV Lp(a) (upper panel). The lower panel shows the sedimentation (○) and diffusion coefficients (■) of Lp(a) (32 K-IV) as a function of 6-AHA concentration.

Lack of Effect of 6-AHA on the Molecular Weight of Lp-(a). The results shown in Figure 2 suggested that 6-AHA induces a conformational change of Lp(a) to a more extended form. An alternative explanation for the results shown in Figure 2 is that 6-AHA causes dissociation of components of Lp(a), i.e., a decrease in molecular weight. Accordingly, the ratio of $s_{20,w}/D_{20,w}$ was measured for a 32 K-IV Lp(a), since this value is proportionate to the molecular weight. As shown in Figure 3, it is clear that besides the decrease in sedimentation coefficient, there is a parallel decrease in the diffusion coefficient of Lp(a) with increasing concentrations of 6-AHA, while the ratio s/D remains constant. This implies that the observed decrease in the values of both $s_{20,w}$ and $D_{20,w}$ involves an increase in the frictional coefficient of Lp-(a) rather than a change in the molecular weight. Furthermore, the magnitude of change in the sedimentation coefficient is so great that to account for it by a reduction in molecular weight would require the dissociation of a substantial portion of Lp(a), most likely the apo(a) moiety. Such a process would introduce heterogeneity which would probably be detected by the skewing of the sedimenting boundary or even by the presence of multiple boundaries. However, in none of the sedimentation velocity experiments was such a phenomenon detected.

We also used nondenaturing polyacrylamide gel electrophoresis to determine whether 6-AHA dissociated part of the protein moiety of Lp(a). Examination of Figure 4 reveals that the same characteristic difference in mobility observed between LDL and L(a) was also seen in the presence of 6-AHA. The lower electrophoretic mobility of Lp(a) is caused by the presence of apo(a) on the Lp(a) particle, and there is no evidence that apo(a) dissociated from the lipoprotein particle since only one band was visualized. Dissociation would imply the existence of two bands: one due to Lp(a-) with a mobility equal to that of LDL and a faster moving apo(a) band located about one-third down the length of the gel (Fless et al., 1985). We conclude, therefore, that 6-AHA causes a conformational change in Lp(a), and not disaggregation of a portion of the particle.

FIGURE 4: Nondenaturing gel electrophoresis of 15 K-IV Lp(a) and autologous LDL dissolved in HBS (left gel) and 500 mM 6-AHA (right gel). A 2.5−16% gradient polyacrylamide gel (Isolab) was cut in half; the left half was dialyzed against 90 mM TRIS, 90 mM boric acid, 2 mM Na_2EDTA, pH 8.3, and the right half was dialyzed against the same buffer containing 500 mM 6-AHA. Samples (20 μg of protein) were subjected to 150 V for 16 h at 4 °C and stained with Coomassie blue R250.

Reversibility of the Conformational Change. To test the reversibility of the conformational change, we measured the sedimentation coefficient of native 15 K-IV Lp(a) (12.66 S), and compared it to the values obtained with Lp(a) in 500 mM 6-AHA (10.69 S) and after dialysis back into HBS (12.58 S). This result clearly indicates that the process is reversible.

We also assessed whether the change in the conformation of Lp(a) elicited by the binding of 6-AHA was related to the presence of apo(a) by determining the sedimentation coefficient of LDL and Lp(a-) in the presence and absence of 200 mM 6-AHA. This concentration of 6-AHA caused a reduction in sedimentation velocity of the Lp(a) species characterized in Figure 2A,B,D that ranged from 13.5 to 40%. In contrast, the corresponding effect of 6-AHA on autologous LDL and Lp(a-) was minimal in that the presence of the ligand changed their mean sedimentation coefficients by only 1.4 and 1.1%, respectively.

Conformation of Apo(a), and the Relationship of $s_{20,w}$ and f/f_o to Apo(a) Mass. To get a better understanding of the conformational change initiated by the binding of 6-AHA by Lp(a), we determined the effect of apo(a) mass on the sedimentation coefficient of Lp(a); these data are presented in Table 2. Mass was expressed in terms of the number of apo(a) K-IV domains, and this variable was related to the sedimentation coefficient of Lp(a) either in HBS or in saturating concentrations of 6-AHA. Depending on the particular Lp(a) species, the latter ranged in concentration from 0.2 to 1 M (see Figure 2). The results obtained with seven human Lp(a) species are shown in Figure 5 along with data derived from rhesus Lp(a) and human LDL and Lp(a-). There was a strong correlation between the sedimentation coefficient of human Lp(a) in HBS and the number of kringle IV domains whereas the sedimentation coefficient of Lp(a) in saturating 6-AHA was practically constant and not affected

Table 1: Binding Parameters of Lp(a)

Lp(a)	no. of K-IVs	n^a	$K_{d,app}{}^a$ (M)	R
human	15	2.9 ± 0.7	1.28 ± 3.91 E-3	0.993
human	20	2.2 ± 0.6	1.32 ± 2.95 E-3	0.993
human	20	1.4 ± 0.2	2.09 ± 1.26 E-1	0.999
human	27	4.0 ± 1.0	0.92 ± 3.99 E-5	0.994
human	32	1.5 ± 0.2	3.28 ± 3.02 E-1	0.996
rhesus	14	2.3 ± 0.7	1.08 ± 4.02 E-3	0.992

[a] The parameters n and $K_{d,app}$ are defined by the equation: $K_{d,app} = \{[1 - [(S - S_e)/(S_c - S_e)]][6AHA]^n\}/[(S - S_e)/(S_c - S_e)]$ where n is the number of bound 6-AHA molecules per Lp(a) particle and $K_{d,app}$ is the phenomenological dissociation constant; S is the observed sedimentation coefficient; S_e is the sedimentation coefficient of the extended form of Lp(a) in saturating amounts of 6-AHA, and S_c is the sedimentation coefficient of the compact form of Lp(a) in HBS. R is the regression coefficient. For additional detail, see Experimental Procedures.

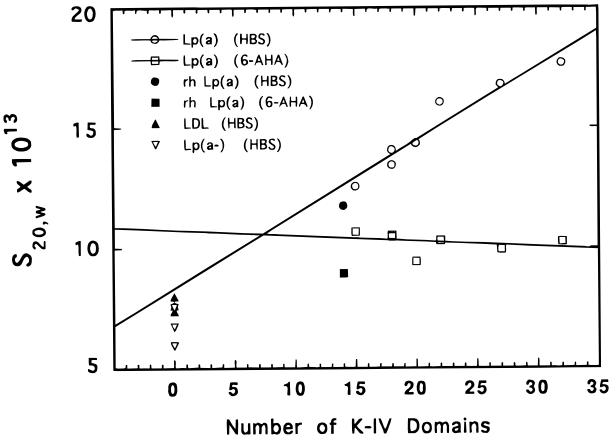

FIGURE 5: Dependence of the sedimentation coefficient of Lp(a) on the number of K-IV domains or mass of apo(a). The solid lines represent the best-fit least-squares lines obtained with human Lp(a) in either HBS ($y = 10.8 - 0.0238x$) or saturating amounts of 6-AHA ($y = 8.32 + 0.307x$).

by apo(a) mass. The least-squares line fitted through the experimental data points obtained with Lp(a) in HBS came very close to values of $s_{20,w}$ corresponding to human LDL while those of Lp(a-) deviated more. Rhesus Lp(a) had a smaller sedimentation coefficient in both solvents that may have been caused by slight structural differences from its human counterpart.

A striking aspect of these data is that the least-squares lines in Figure 5 intersect at a point corresponding to a

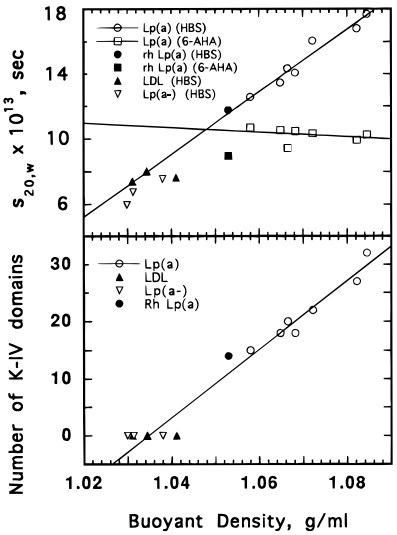

FIGURE 6: Dependence of the sedimentation coefficient of Lp(a) (upper panel) and apo(a) mass or number of K-IVs (lower panel) on the buoyant density of Lp(a). The solid lines represent the best-fit least-squares line obtained with human Lp(a). The equation describing the variation of $s_{20,w}$ with density for Lp(a) in HBS is $y = -201 + 202x$ and in 6-AHA $y = 25.5 - 14.2x$. The equation describing the variation of apo(a) kringle number with Lp(a) density is $y = -621 + 600x$.

hypothetical Lp(a) particle containing an apo(a) polymorph with 7.4 ± 1.6 K-IV domains consisting of the invariable K-IV 3−10 region of apo(a) inclusive of the four weak LBS located on K-IV 5−8. That is, such an Lp(a) particle would not experience a change in conformation, observable by this method, on binding 6-AHA. Thus, it appears that the variable K-IV 2 domains of apo(a) are responsible for the reduced sedimentation rate of Lp(a) in 6-AHA.

The Svedberg equation states that the sedimentation coefficient depends on three parameters: molecular weight, a buoyancy factor ($1 - \bar{\nu}\rho$), and the frictional coefficient. Of these, the molecular weight of Lp(a), which was relatively constant [(3.55 ± 0.4) × 10^6], did not correlate with the sedimentation coefficient or the number of K-IV domains. A possible reason for the lack of correlation is the variability in the size of the lipid moiety. However as shown in Figure 6 there is a clear relationship between the buoyant density of Lp(a), and hence its reciprocal, the partial specific volume, and the sedimentation coefficient in HBS. In addition, the buoyant density of Lp(a) correlated well with the number

Table 2: Hydrodynamic Properties of Lp(a), Lp(a-), and LDL in HBS and 6-AHA[a]

particle	no. of K-IVs	$M_r \times 10^{-6}$	$\bar{\nu}$ (cm³/g)	$s_{20,w}$ (in HBS) (S)	$s_{20,w}$ (in 6-AHA) (S)	$f_o \times 10^7$ (g/s)	f/f_o (in HBS)	f/f_o (in 6-AHA)	R_{sphere} (Å)	R_h (in HBS) (Å)	R_h (in 6-AHA) (Å)
h-Lp(a)	15	3.62	0.945	12.55	10.69	2.18	1.24	1.46	115	143	168
h-Lp(a)	18	3.72	0.939	13.44	10.54	2.20	1.31	1.67	116	152	194
h-Lp(a)	18	3.39	0.936	14.06	10.47	2.13	1.23	1.65	113	139	187
h-Lp(a)	20	3.33	0.938	14.33	9.44	2.12	1.17	1.77	112	131	199
h-Lp(a)	22	3.61	0.933	16.04	10.32	2.18	1.18	1.84	115	137	212
h-Lp(a)	27	3.60	0.924	16.80	9.94	2.18	1.27	2.14	115	146	247
h-Lp(a)	32	3.60	0.922	17.68	10.26	2.18	1.24	2.13	115	142	245
rh-Lp(a)	14	3.40	0.950	11.76	8.95	2.13	1.17	1.54	113	132	174
h-Lp(a-)		2.88	0.968	6.75	6.67	2.00	1.20	1.21	106	127	128
RCM-LDL		2.84	0.970	7.05		1.99	1.07		105	113	
h-LDL		2.52	0.966	7.68	7.79	1.93	1.02	1.01	102	104	100

[a] Hydrodynamic parameters are defined in the text. Values for LDL and Lp(a-) represents the means of three different samples. RCM-LDL represents a single sample. For clarity of presentation, the errors in the measurements have been omitted. The estimated errors in M_r, $\bar{\nu}$, and $s_{20,w}$ are ±3%, 0.3%, and 1%, respectively. Abbreviations: h, human; rh, rhesus; RCM, reduced and carboxymethylated.

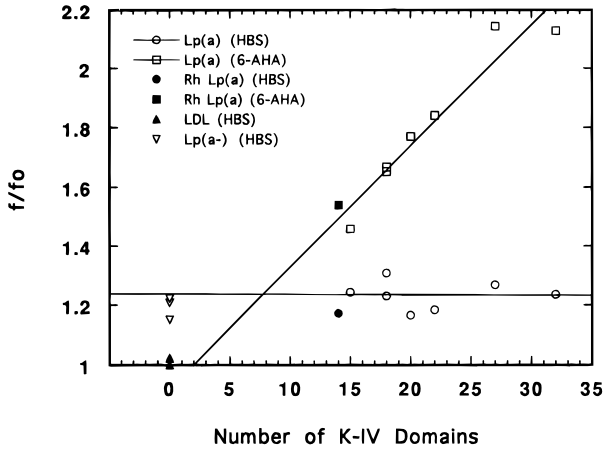

FIGURE 7: Effect of apo(a) mass or number of K-IVs on the frictional ratio of Lp(a) in HBS or saturating concentrations of 6-AHA. The frictional ratio of Lp(a) was calculated assuming a hydration of 0.37 g of H_2O/g of Lp(a) and 0.34 g of H_2O/g of Lp(a-) or LDL. The solid lines represent the best-fit least-squares line obtained with human Lp(a). The equation describing the line obtained with Lp(a) in HBS is $y = 1.24 - 0.000139x$, and the corresponding equation for Lp(a) in 6-AHA is $y = 0.993 + 0.0410x$.

of K-IV domains (Figure 6, bottom). Values obtained with LDL and Lp(a-) deviated only slightly from the least-squares lines fitted to the Lp(a) data points in Figure 6, thus validating the Lp(a) results. Therefore, as expected, the sedimentation coefficient also correlates with the number of K-IV domains. A somewhat surprising result, however, is obtained for different Lp(a)s present in saturating amounts of 6-AHA: the $s_{20,w}$ is essentially independent of the number of K-IV domains. Assuming that there is no change in the partial specific volume of Lp(a) on binding 6-AHA, and knowing that the molecular weight of Lp(a) is not affected by 6-AHA, it must be that with increasing number of K-IV domains there is a corresponding increase in the frictional coefficient which tends to lower $s_{20,w}$, and counterbalance any increase in the sedimentation coefficient of Lp(a) caused by a greater buoyant density.

To test this hypothesis, the frictional ratio of Lp(a) present in either HBS or saturating concentrations of 6-AHA was calculated along with those of rhesus Lp(a) and human LDL and Lp(a-) and plotted against number of K-IV domains in Figure 7. A striking aspect of this graph is the finding that the frictional ratio of Lp(a) in HBS is independent of apo(a) mass or number of K-IVs. Assuming a hydration of 0.37 g of H_2O/g of Lp(a), the mean value obtained for the seven Lp(a)s is 1.23 ± 0.05. A constant frictional ratio suggests that apo(a) of the compact form of Lp(a) must be confined to the lipoprotein surface instead of projecting into the aqueous medium, thereby preventing larger apo(a)s from increasing the frictional resistance to sedimentation. In contrast, saturating the LBS of Lp(a) with 6-AHA causes a huge increase in the frictional ratio of Lp(a) which depends strongly on the number of K-IV domains.

These results suggest that the binding of 6-AHA by Lp(a) releases the apo(a) molecule from the lipoprotein surface into the solvent, where it provides increased frictional resistance; the larger the apo(a), the greater the frictional ratio. The two least-squares lines fitted through the Lp(a) data intersect at a point corresponding to an apo(a) containing 7.7 ± 3 K-IV domains. This finding suggests, as did the data derived from the dependence of the sedimentation coefficient with K-IV number, that approximately eight K-IV domains are close to the lipoprotein surface and thus do not contribute to the frictional resistance. Unlike the three LDL which had a mean frictional ratio of 1.02 ± 0.02 at a hydration of 0.34 g of H_2O/g of LDL, the mean frictional ratio of three Lp(a-) was substantially higher, being 1.20 ± 0.04 on the assumption that they were hydrated to the same extent as LDL. Part, but not all, of the increase in the frictional ratio of Lp(a-) can be assigned to the process of reduction and carboxymethylation since the frictional ratio of LDL, which had been reduced and alkylated with iodoacetic acid, increased to a value of 1.07.

DISCUSSION

Lipoprotein(a) undergoes a global conformational change when exposed to the lysine analog 6-AHA—the largest of any ligand-induced conformational change known to date. The transition from a compact to an extended conformation is characterized by nearly a 50% decrease in the sedimentation coefficient of Lp(a) particles having relatively large apo(a) polymorphs. In comparison, a similar transition induced in Glu-plasminogen by 6-AHA, which was thought to be the largest conformational change detected in a protein (Mangel et al., 1990), is accompanied by only a 16% decrease in sedimentation velocity (Violand et al., 1978). The LBS of plasminogen responsible for the conformational change effected by 6-AHA are not the strong LBS located on K-I, but weaker ones situated on K-IV and K-V. The latter are thought to contribute to the compact configuration of Glu-plasminogen by mediating the interaction between the N-terminal peptide and K-V and the interkringle interaction involving K-III and K-IV (Marshall et al., 1994). Their disruption by 6-AHA induces the extended conformation of Glu-plasminogen. As observed with plasminogen, the conformational change in Lp(a) is brought about by the saturation of a small number of weak LBS with 6-AHA. These are probably located in K-IV 5−8 of apolipoprotein(a), and their low affinity for 6-AHA is exemplified by the concentration of ligand (100−200 mM) needed to effect a 50% change in the conformation of Lp(a). However, the interaction between apo(a) and apoB in Lp(a), or other lysine-containing proteins, is enhanced by the involvement of multiple LBS located on the concatenated K-IVs of apo(a). The different Lp(a) species studied showed considerable variation in the number of bound 6-AHA molecules (1.4 to 4) and magnitude of $K_{d,app}$ (0.3 M to 1×10^{-5} M) which was not related to apo(a) mass or number of K-IV domains. The variability in values of n and $K_{d,app}$ for different Lp(a) species may reflect heterogeneity in the conformation of apoB with different states of lipidation, or heterogeneity in primary structures of apo(a). There is also the possibility that differences in the state and level of glycosylation of apo(a) could account for differences in the number and affinity of 6-AHA molecules bound to Lp(a). All, however, including rhesus Lp(a), exhibited a characteristic reduction in sedimentation coefficient on exposure to 6-AHA. Unlike human Lp(a), rhesus Lp(a) does not bind to lysine−Sepharose, because Trp^{72} in the strong LBS of human K-IV 10 is replaced by Arg^{72} in rhesus Lp(a), rendering the strong LBS inactive (Scanu et al., 1993). Since human and rhesus Lp(a) show the same conformational change in saturating 6-AHA, this strong LBS is apparently not involved. The observation that concentrations greater than 30−50 mM

6-AHA were needed to effect a minimal change in sedimentation coefficient—much greater than concentrations needed to saturate the strong LBS—supports this conclusion. The magnitude of these threshold concentrations was much greater than the dissociation constant for the binding of 6-AHA to recombinant apo(a) K-IV 10, which was found to be 20 μM (Lograsso et al., 1994), or IC_{50} values of 0.7−2.1 mM obtained for the inhibition of the binding of Lp(a) to lysine−Sepharose by 6-AHA (Fless & Snyder, 1994; Hoover-Plow et al., 1993).

Since the sedimentation coefficient of Lp(a) depends on its mass, buoyancy, and frictional properties, it was necessary to show that the reduced sedimentation rate in 6-AHA was not caused by a decrease in the effective molecular weight of Lp(a), i.e., $M(1 - \bar{v}\rho)$. Measurement of both the sedimentation and diffusion coefficients of Lp(a) as a function of 6-AHA clearly indicated that the process occurring is one involving an increase in frictional coefficient and not a change in effective molecular weight. Both the sedimentation and diffusion coefficients decreased in parallel, and as a result the ratio of the sedimentation and diffusion coefficients, which is a measure of molecular weight, remained constant. Nondenaturing gel electrophoresis of Lp(a) in gels containing 500 mM 6-AHA also indicated no loss of protein from the lipoprotein particle. The conformational change was not caused by the increase in ionic strength resulting from the high 6-AHA concentrations, because $s_{20,w}$ values were unchanged between 0.1 and 0.4 M NaCl. Finally, the conformational change was shown to be reversible in that the sedimentation coefficient of native Lp(a) could be regained on the removal of 6-AHA by exhaustive dialysis. The reversibility of this process is also implied by the fact that Lp(a) was isolated by affinity chromatography on lysine−Sepharose with subsequent elution by 200 mM 6-AHA and dialysis to remove the ligand.

The data reported above have important implications for the structure and function of Lp(a). In agreement with the finding of Rainwater et al. (1995), we found a tight correlation between apo(a) mass and Lp(a) density ($r = 0.9677$). This dependence was mainly responsible for the excellent correlation of the sedimentation coefficient of Lp(a) with apo(a) mass ($r = 0.9628$), since there was no correlation of Lp(a) molecular weight ($r = 0.1633$) or frictional coefficient ($r = 0.0171$) with apo(a) mass or kringle IV number. In contrast, the sedimentation coefficient of Lp(a) in saturating 6-AHA was independent of kringle number, or apo(a) mass, since the effect of increasing apo(a) mass was offset by a corresponding increase in the frictional coefficient.

In HBS, the frictional coefficient of native Lp(a) is independent of apo(a) mass, and had a mean value of 1.23 at a calculated hydration of 0.37 g of H_2O/g of Lp(a). The value of f did not increase with additional K-IV domains, suggesting a compact structure in which apo(a) is closely apposed to the lipoprotein surface. Such an organization would be compatible with results from small-angle X-ray scattering suggesting that apo(a) is wrapped around the Lp(a) surface without major globular domains extending into the solvent (Prassl et al., 1995). The frictional coefficient of 1.23 observed for Lp(a) in HBS could indicate an asymmetric structure approximated either by an oblate or by a prolate ellipsoid of revolution having an axial ratio of 5. Such a model, however, is clearly not in agreement with results derived from electron microscopy (Sines et al., 1994) or small-angle X-ray scattering that suggest that Lp(a) is spherical (Prassl et al., 1995). On the other hand, if the high frictional coefficient of Lp(a) were attributable solely to hydration, the calculated water content of apo(a) would be 4.8 g/g of apo(a), an unacceptably high value. The value of f may have resulted in part from an underestimation of the hydration of Lp(a), and from the rugose character of the lipoprotein surface, because derivation of the frictional coefficient assumes that protein molecules are smooth (Teller et al., 1979). An additional surprising result was that the frictional ratio of Lp(a-) was not identical to that of reduced and carboxymethylated LDL (1.20 and 1.07, respectively). While to a first approximation Lp(a-) and LDL are similar, several differences have been noted. Lp(a-) has a larger and slightly different lipid core than autologous LDL (Fless et al., 1986) and is not immunochemically identical to LDL (Zawadzki et al., 1988), and the core lipids interact differently with the surface phospholipids (Prassl et al., 1995; Sommer et al., 1992). Thus, the conformations of apoB in LDL and Lp(a-) may not be identical: in the latter particle, the protein may form a more rugose surface, resulting in a greater frictional coefficient.

In contrast to compact Lp(a) in HBS, the frictional coefficient of Lp(a) in saturating 6-AHA was directly proportional to the number of K-IV domains, indicative of protrusion of these domains into the aqueous medium. This would require some portion of the apo(a) molecule to assume a more extended conformation. In this extended state, however, apo(a) is not completely unfolded, as indicated by the intersection of the two lines in Figure 7, which shows that for a hypothetical apo(a) containing 7.7 K-IV domains, there would be no difference in the frictional resistance of a Lp(a) particle in HBS or 6-AHA. Thus, a portion of apo(a), probably the invariable K-IV 3−10 domain, is held to the lipoprotein surface by forces that cannot be disrupted by 6-AHA. This ligand probably competes with lysine residues in either apoB or the K-V or protease domain of apo(a). Although these weak interactions might be either between apo(a) and apoB, or between portions of apo(a) itself, the former are more likely, *a priori*, in view of the multiple clusters of basic residues occurring on apoB and the paucity of lysine in apo(a) itself. The increased frictional resistance caused by unfolding is probably provided by the variable K-IV 2 domains and the penultimate K-IV 1.

Table 2 gives the values of the hydrodynamic parameters for human Lp(a), Lp(a-), and LDL, and rhesus Lp(a). From the values for M, $\bar{v}$, and s, the frictional coefficients, f, were calculated; and from f, values were obtained for the effective hydrodynamic radius, R_h. As shown in the Table, 6-AHA greatly increases the magnitude of R_h for human and rhesus Lp(a), but not for Lp(a-) or LDL. For these seven different human Lp(a) particles in 6-AHA, R_h is approximately 1.2−1.7-fold greater than the corresponding mean value for R_h of 141 Å, that was obtained in HBS. Since both sets of radii were calculated using the same values for the hydration [0.37 g of H_2O/g of Lp(a)], these data support the hypothesis that in saturating 6-AHA, Lp(a) adopts a greatly extended conformation, in which the variable portion of apo(a) protrudes into the solvent.

Our findings have relevance to the problem of Lp(a) assembly and the interaction of Lp(a) with apoB-containing lipoproteins. Recent studies on recombinant apo(a) peptides

of various length and kringle type, and with mutated LBS, have established that K-IV 5−8 (Ernst et al., 1995; Frank et al., 1994a,b), particularly K-IV 6 (Frank et al., 1994), are necessary for the binding of apo(a) to LDL, and that they contribute to the interaction of Lp(a) with lysine−Sepharose (Ernst et al., 1995). In contrast, K-IV 10, containing a strong LBS, was not necessary for Lp(a) formation, and K-IV 1−4 did not contribute to the assembly process. These results underscore our findings that K-IV 5−8 play a critical role in Lp(a) structure. In contrast, the repetitive K-IV 2 domains do not appear necessary for Lp(a) formation. These domains protrude into the solvent when K-IV 5−8 bind 6-AHA, and give rise to the dramatic frictional coefficient of the extended Lp(a).

Apo(a) is linked to Lp(a) by a disulfide bond between apoB and K-IV 9 of apo(a) (Brunner et al., 1993; Fless et al., 1984; Gaubatz et al., 1983; Koschinsky et al., 1993; McLean et al., 1987; Utermann & Weber, 1983). The present study demonstrates that additional interactions between apo(a) and apoB may contribute to the tight packing of the compact conformation of Lp(a). Thus, these studies are in agreement with the concept that noncovalent bonds are important in the interaction of apo(a) and apoB in Lp(a) (Ernst et al., 1995; Frank et al., 1994a,b; Phillips et al., 1993; Trieu et al., 1991). Despite the apparent closeness of apo(a) to the surface of the Lp(a) particle, however, Zawadski et al. observed that 27 different monoclonal antibodies against apoB reacted with Lp(a) without observable steric hindrance from apo(a) (Zawadzki et al., 1988). Similarly, the apoB in LDL and Lp(a) is equally accessible to proteolytic attack by thermolysin and cathepsin D (Huby et al., 1994). In addition, LDL and Lp(a) have high affinities for the LDL receptor on fibroblasts (Snyder et al., 1994), and have an accessible heparin binding domain on apoB (Dahlen et al., 1978; Edelberg & Pizzo, 1990; Edelberg et al., 1991; Makino et al., 1995; Williams et al., 1992).

The apparent inability of apo(a) to hinder access to apoB can be reconciled with the close apposition of apo(a) to apoB at the surface of the Lp(a) particle in terms of the observations presented in this paper. Because the interactions between the lysine binding sites and the lysines on apoB or apo(a) are weak, they can be disrupted readily by antibodies, proteases, or receptors, all of which involve higher affinity interactions than occur between weak LBS and lysines. In the absence of antibodies, proteases, or receptors, these multiple weak interactions allow for tight packing of apo(a) to the surface of the Lp(a) particle. Thus, in the compact state, the Lp(a) particle has a relatively symmetrical, globular form. The binding of 6-AHA disrupts these multiple weak interactions, thereby generating a more extended form of Lp(a) in which the apo(a) protrudes further into the aqueous medium. A compact structure would be transported efficiently through aqueous media because of a lower frictional coefficient. When binding a target surface such as presented by fibrin or endothelium through its weak LBS, Lp(a) would change conformation and thus greatly increase in size. There Lp(a) is thought to regulate fibrinolysis by inhibiting the activation of plasminogen by t-PA, and by suppressing PAI-1 inhibition of t-PA, mainly by sterically blocking the access of both plasminogen and PAI-1 to either surface (Edelberg & Pizzo, 1991). As a result, Lp(a) would be a more effective regulator of fibrinolysis in the extended state, e.g., when surface-bound, than when present in solution.

It is tempting to speculate as to the role such a conformational change might have in the biological functions of Lp(a). The conformational change induced by 6-AHA might mimic the changes occurring when Lp(a) binds to the high-affinity ligands for either of its proteins, for example, the LDL receptor for apoB, or fibrin for apo(a). Thus, the binding of apo(a) to fibrin might release the apoB for interactions with the LDL receptor. Similarly, the binding of Lp(a) to an LDL receptor might release apo(a) for interactions with other proteins, such as fibrin or other lysine-containing ligands. Such a scheme would allow for the simultaneous binding of multiple ligands. The two protein moieties of the Lp(a) particle—apo(a) and apoB—would then act as a cross-linking bridge between the ligands. At present, no biological function of Lp(a) is known, but a cogent proposal remains the original one of Brown and Goldstein that this particle may serve to target cholesterol to sites of wound healing, where fibrin deposition, cell proliferation, and hence membrane synthesis are profuse (Brown & Goldstein, 1987). The concentrations of ligand needed to induce the conformational changes observed in this study would never be obtained in the bulk solution phase of plasma. On the other hand, high local concentrations of lysines could be found on selected interfaces—and possibly with correct orientation—for simultaneous interactions with multiple weak LBS of apo(a), as in fibrin thrombi. The interaction of lysines at a surface with Lp(a) would be facilitated by the concatenation of K-IV domains in apo(a). The data presented in this paper—showing multiple weak interactions, potentially disruptible by multiple or higher affinity ones—are in accord with this proposed biological function.

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