Specificity of Ligand-Induced Conformational Change of Lipoprotein(a)†

Gunther M. Fless,*,‡ José Y. Santiago,‡ James Furbee, Jr.,‡ and Stephen C. Meredith§

Departments of Medicine and Pathology, University of Chicago, 5841 South Maryland Avenue, Chicago, Illinois 60637

Received March 25, 1997; Revised Manuscript Received July 15, 1997⊗

ABSTRACT: The conformation of Lp(a) was probed with a set of ω-aminocarboxylic acids and other analogs of 6-aminohexanoic acid (6-AHA). Using the viscosity-corrected sedimentation coefficient, six additional ligands were shown to induce a major conformational change in Lp(a), from a compact form to an extended form. These were *trans*-4-(aminomethyl)cyclohexanecarboxylic acid (t-AMCHA), proline, 4-aminobutyric acid, 8-aminooctanoic acid, N^{α}-acetyllysine, and glycine. Lysine, N^{ϵ}-acetyllysine, glutamic acid, and adipic acid were determined not to cause a conformational change. Urea and guanidine hydrochloride were ineffective at inducing this conformational change at concentrations at which the above ligands did unfold Lp(a). The conformational change was inhibited by 100 mM NaCl and to a lesser extent by 20 mM sodium glutamate. Despite the fact that these two salts have nearly the same ionic strengths, the greater inhibition of the unfolding by NaCl is consistent with a proposed stabilization of interkringle interactions by chloride ions. In 100 mM NaCl, which most closely resembles physiological conditions, only proline, 4-aminobutyric acid, 6-AHA, and t-AMCHA were effective ligands. By analyzing the dimensions of the conformation altering ligands, we propose that a critical variable in determining the effectiveness of a ligand in disrupting Lp(a) is the distance between the carboxyl and amine functions of the ligand. The optimal distance is approximately 6 Å, which agrees with the observed 6.6−6.8 Å separation of the cationic and anionic centers of known plasminogen and apo(a) lysine binding sites. These studies have implications for the mechanism of Lp(a) particle assembly.

Lipoprotein(a) [Lp(a)][1] is a large cholesteryl ester-rich, LDL-like lipoprotein particle of which the protein moiety consists of two large glycoprotein molecules, apoB and apo(a), linked by a disulfide bond (Fless et al., 1984, 1985, 1986). Apo(a) is a multidomain protein which is composed of a protease domain and a variable number of structural units, called kringles (K), that have extensive homology to similar domains occurring in plasminogen (McLean et al., 1987). It is polymorphic in nature and may contain from 12 to 51 K-IV domains in addition to a single K-V and a protease domain (McLean et al., 1987; Lackner et al., 1993). There are 10 types of K-IV (Morrisett et al., 1990), of which K-IV_{10} contains a strong lysine binding site (LBS) (Scanu et al., 1993, 1994; Lograsso et al., 1994; 1994; Hoover-Plow et al., 1996), and K-IV_{5-8} contain weaker LBS (Edelstein et al., 1995; Ernst et al., 1995; Frank et al., 1995; Gabel et al., 1996; Klezovitch et al., 1996). Polymorphism is due to variation in K-IV_2 domains whereas the other kringle domains are constant (Koschinsky et al., 1990; Vanderhoek et al., 1993).

Relatively little is known regarding the disposition of apo(a) on the lipoprotein surface. Results of a compositional analysis indicated that in comparison to other lipoproteins, the amount of protein needed to stabilize the lipoprotein surface was exceeded by Lp(a). This implied that apoB was imbedded in the surface lipid monolayer, and that apo(a) either made contact with apoB or extended into the medium (Fless et al., 1984). Small-angle X-ray scattering studies of Lp(a) supported a similar conclusion by placing the location of apo(a) above the surface, wrapped around the particle without major globular domains projecting into the aqueous medium (Prassl et al., 1995). This strongly suggested that besides the disulfide linkage, other noncovalent interactions between apo(a) and apoB were required to maintain apo(a) close to apoB or the lipoprotein surface. Such a structure was found to be consistent with the relatively low frictional coefficient (1.24) and radius (141 Å) obtained by a hydrodynamic analysis of Lp(a) (Fless et al., 1996). Since both parameters did not increase in magnitude with either apo(a) mass or kringle number, this again suggested a compact particle in which apo(a) is closely apposed to the lipoprotein surface.

Because apo(a) has structural similarities to plasminogen, Lp(a) can inhibit the action of plasminogen on fibrin and cellular surfaces that are mediated by the lysine binding function of its kringles (Edelberg et al., 1989; Hajjar et al., 1989; Kluft et al., 1989; Miles et al., 1989; Loscalzo et al., 1990). Besides the aforementioned similarities between apo(a) or Lp(a) and plasminogen, the two particles share an additional property. Like Glu-plasminogen (Alkjaersig, 1964; Abiko et al., 1969; Violand et al., 1978; Mangel et

†This work was supported by NIH Grant HL 18577 and by NIH DRR-BRS Shared Instrumentation Grant S10 RR 06579.

* To whom correspondence should be addressed.

‡ Department of Medicine.

§ Department of Pathology.

⊗ Abstract published in *Advance ACS Abstracts,* September 1, 1997.

[1] Abbreviations: Lp(a), lipoprotein(a); apo(a), apolipoprotein(a); apoB, apolipoprotein B; LDL, low-density lipoprotein; t-PA, tissue plasminogen activator; K, kringle; K-IV 1−10, apo(a) kringle domain of subtypes 1−10, all with homology to kringle 4 of plasminogen; LBS, lysine binding site; 4-ABA, 4-aminobutyric acid; 6-AHA, 6-aminohexanoic acid; 8-AOA, 8-aminooctanoic acid; t-AMCHA, *trans*-4-(aminomethyl)cyclohexanecarboxylic acid; N-α-AcLys, N^{α}-acetyl-L-Lysine; N-ϵ-AcLys, N^{ϵ}-acetyl-L-lysine; Glu, glutamic 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_2-EDTA, and 0.01% NaN_3, pH 7.4).

S0006-2960(97)00698-3 CCC: $14.00

al., 1990; Christensen & Molgaard, 1992; Ponting et al., 1992a; Marshall et al., 1994; Markus, 1996), Lp(a) can undergo a conformational change from a compact form to an extended or open structure on binding the lysine analog 6-AHA (Fless et al., 1996). The compact form of Glu-plasminogen appears to be maintained by interactions involving its weak LBS, in particular a link between a lysine residue in the N-terminal peptide and a LBS in K-V, and a second kringle−kringle interaction involving K-III and a LBS in K-IV [reviewed by Markus (1996)]. The conformational change observed with either Glu-plasminogen or Lp(a) is reversible and can be detected by a change in their sedimentation rates. With Lp(a), the magnitude of the decrease in sedimentation velocity caused by the binding of 6-AHA is directly proportional to apo(a) mass (Fless et al., 1996). Analysis of ligand binding indicated that, depending on the phenotype, from 1.4 to 4 molecules of 6-AHA may be bound to Lp(a). Comparative studies with several human Lp(a)s and one rhesus Lp(a) led to the conclusion that weak LBS on K-IV_{5-8} may be involved in maintaining the structure of Lp(a). Binding 6-AHA disrupts these interactions and promotes a change in configuration in which the variable K-IV_2 apo(a) domain extends away from the surface, while the invariable K-IV_{3-9} domains remain close to the lipoprotein surface (Fless et al., 1996). The expanded structure of Lp(a) has a hydrodynamic radius that varies from 168 to 247 Å, and has greatly increased frictional resistance to sedimentation represented by frictional coefficients that range from 1.46 to 2.14 for apo(a)s having from 15 to 32 K-IV domains. The conformational change is entirely due to the apo(a) moiety, since 6-AHA has no effect on the hydrodynamic properties of LDL.

The purpose of the present investigation was to characterize the effect of various lysine analogs, and other amino acids including proline and glutamate, on the conformation of Lp(a). We will show that several ω-amino acids are able to induce a conformational change of Lp(a), that there is a definable relationship between the geometry of the ligand and its ability to induce a conformational change, and that this effect can be modulated by the presence of different salts.

EXPERIMENTAL PROCEDURES

Materials. L-Lysine, L-proline, L-glutamic acid, glycine, N^{α}-acetyl-L-lysine, N^{ϵ}-acetyl-L-lysine, t-AMCHA, 6-AHA, HEPES, and D_2O (99.9% atom D) were obtained from Sigma (St. Louis, MO). Sodium acetate, adipic acid, 4-ABA, and 8-AOA were purchased from Fluka (Ronkonkoma, NY). Sodium phosphate (monobasic) and sodium chloride were obtained from Fisher Scientific (Pittsburgh, PA). Guanidine hydrochloride was obtained from Heico/Whittaker (Delaware Water Gap, PA) and urea from BRL (Gaithersburg, MD). All reagents were the highest quality available.

Preparation of Lipoprotein(a). One subject, whose plasma contained 2 apo(a) polymorphs having either 15 or 27 K-IV domains, at a respective relative abundance of 10 to 1, served as donor for the preparation of Lp(a). He gave informed consent prior to plasmapheresis. Lp(a) (15 K-IV) was isolated from plasma by a combination of lysine−Sepharose chromatography and density gradient centrifugation using previously described methods (Fless et al., 1986; Snyder et al., 1992; Fless & Snyder, 1994). As previously described (Fless et al., 1994), Lp(a) protein was determined by the method of Lowry et al. (1951), as modified by Markwell et al. (1978), using bovine serum albumin as standard. A weighted factor of 1.07 was used to correct the Lowry value to the true protein concentration based on the chromogenicity of apoB (0.926) and apo(a) (1.40).

Analytical Centrifugation. Sedimentation and flotation equilibrium experiments were performed with a Beckman Optima XLA ultracentrifuge interfaced to a Dell Optiplex XMT 590 personal computer, an AN-60 Ti four-place rotor, and analytical cells equipped with six-channel charcoal-filled centerpieces. The molecular weight and partial specific volume of Lp(a) in five different solutions, containing from 0 to 100% D_2O, were determined by equilibrium centrifugation carried out as described previously (Fless et al., 1994; Fless & Santiago, 1997).

Sedimentation velocities of Lp(a) were measured using the An-60 Ti rotor and double-sector cells with aluminum-filled epon centerpieces and quartz windows (Fless et al., 1996). The rotor speed was 40 000 rpm, and the temperature was controlled at 20 °C. Both rotor and cells were preequilibrated to 20 °C before starting the sedimentation velocity runs. The cells were scanned at 8 min intervals; usually 16 data sets were collected after either a 8 or a 16 min delay. Data from sedimentation velocity experiments were analyzed using the XLA-veloc program of the Optima XL-A data analysis software (Beckman, Palo Alto, CA) in conjunction with Origin software (Microcal Software, Northhampton, MA).

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 vibrating density meter cell was controlled to 20 ± 0.01 °C with an external water bath. Solvent viscosity was measured with Cannon-Manning semi-micro calibrated viscometers (Cannon Instrument Co., State College, PA) using a Cannon Instrument Co. constant temperature water bath controlled to 20 ± 0.01 °C.

Lp(a) was dialyzed extensively against 10 mM HEPES, 0.01% Na_2EDTA, and 0.01% NaN_3, pH 7.4, and was then adjusted by dilution to a protein concentration of approximately 0.2−0.3 mg/mL and the desired concentrations of ligand, which were also prepared with the above buffer. For plots of the viscosity-corrected sedimentation coefficient (ηs) vs density or ligand concentration, the ligand concentration ranged from 50 mM to 1 M, or to the limit of solubility for the particular ligand. For experiments designed to determine the binding constant of various ligands to Lp(a), the range of ligand concentration was extended down to 1 mM or less. To prevent the unfolding of Lp(a) at low ionic strength, 100 mM NaCl or 20 mM Glu was added to all solutions of a particular set of ligand. For solution sets containing no ionic strength supplement, ηs was corrected for its downward excursion at low ionic strength by using a standard curve, constructed from a plot of ηs as a function of ionic strength due to variation in the concentration of glutamic acid. The experimental points were adjusted by adding the difference in ηs (at a given ionic strength below 0.05) between the experimental value and the corresponding value determined from a line fitted to the data obtained at concentrations of Glu greater than 50 mM.

Data Analysis. For each ligand examined, plots of ηs vs density were extrapolated to unity. These values were corrected to water at 20 °C using the relation

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

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 ν is the anhydrous lipoprotein partial specific volume determined in D_2O. The effective hydrodynamic radius of Lp(a), R_h, was calculated from the relation:

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

where f is the translational friction coefficient and η is the solution viscosity. The frictional coefficient, f, was determined from the molecular weight and partial specific volume obtained by sedimentation equilibrium in D_2O, 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 preferential hydration, A, in terms of grams of excess water per gram of lipoprotein was calculated from the relation:

$$A = \frac{\rho_h\bar{\nu} - 1}{1 - \rho_h\bar{\nu}_w}$$

where ρ_h is the hydrated density of Lp(a) obtained from the horizontal intercept of ηs vs density plots and $\bar{\nu}_w$ is the partial specific volume of water (Cox & Schumaker, 1961a).

In the determination of binding constants, the hydrodynamic partial specific volume, $\bar{\nu}_h$, was used instead of $\bar{\nu}$; it is the reciprocal of the Lp(a) particle hydrated density, e.g., $\bar{\nu}_h = 1/\rho_h$. This gave a differently corrected sedimentation coefficient, $s'_{20,w}$, which is defined as follows:

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

The binding of ligands to Lp(a) in terms of changes in the sedimentation coefficient was analyzed using the previously derived expression:

$$s = \frac{(s_c - s_e)[L]^n}{K_d + [L]^n} + s_e$$

where s is the sedimentation coefficient, e.g., $s_{20,w}$, s_c and s_e are the corresponding values of the compact and extended forms of Lp(a), [L] is the ligand concentration, and K_d is an apparent dissociation constant (Fless et al., 1996). The latter is defined by the expression:

$$K_d = \frac{[Lp(a)_c][L]^n}{[Lp(a)_e{\cdot}L_n]}$$

where $[Lp(a)_e{\cdot}L_n]$ represents a complex between the extended form of Lp(a) and n molecules of ligand.

Molecular Modeling of Ligands. Conformation-altering ligands of Lp(a) were modeled using the Insight II software package on a Silicon Graphics Interface. The Discover module of this package was used, first, to do an energy minimization of each compound, followed by simulated dynamics. The resulting distance between the amino nitrogen and the carboxyl carbon was measured using the same software module. The distances were plotted against the percent unfolding of the Lp(a) particle as determined in the analysis of the sedimentation data. The resulting curves were then analyzed as a second-order polynomial function, using the curve-fitting routine of the program Kaleidagraph.

Viscometry. Viscosity measurements of Lp(a) were made as described above. The relative viscosity of Lp(a) was obtained from the relation:

$$\eta_{rel} = \frac{\rho_{lp}t_{lp}}{\rho_s t_s}$$

where ρ_{lp} is the density of the Lp(a) solution, ρ_s is the density of the buffer solution, and t_{lp} and t_s are the respective flowout times. The specific viscosity, η_{sp}, is equivalent to $\eta_{rel} - 1$. By plotting the quantity η_{sp}/c as a function of Lp(a) concentration (c), the intrinsic viscosity, $[\eta]$, was obtained at the limit of zero concentration. The density of Lp(a)-containing solutions was calculated from the relation:

$$\rho_{lp} = \rho_s\left(1 - \frac{c\bar{\nu}}{100} + \frac{c}{100\rho_s}\right)$$

where $\bar{\nu}$ is the partial specific volume of Lp(a) and c is the Lp(a) concentration in grams per deciliter (McKie & Brandts, 1972).

RESULTS

The binding of the lysine analog 6-AHA to Lp(a) promotes a profound conformational change of the lipoprotein particle that can be analyzed by measuring and plotting the sedimentation coefficient, corrected for solution viscosity and density, as a function of 6-AHA concentration. In an effort to determine the specificity of this configurational alteration, we exposed Lp(a) to several ω-aminocarboxylic acids, in addition to various amino acids, salts, and other compounds not expected to elicit a change in the molecule.

Molecular Weight and Partial Specific Volume of Lp(a). Lipoproteins, including lipoprotein(a), do not have fixed molecular weights or partial specific volumes, because they are populations of particles with narrow size distributions. For any given individual, these parameters are therefore subject to small variation from one preparation to the next, due to slight differences in the lipid content (and therefore density distribution), reflecting the nutritional status of the donor, and slight methodological differences in Lp(a) isolation. For different preparations of the 15 K-IV Lp(a), the sedimentation coefficient of the compact form was found to vary ±0.5 S. Because this variation made the comparative effects of different ligands on the conformation of Lp(a) difficult to analyze, all results of this study were obtained with one preparation of Lp(a).

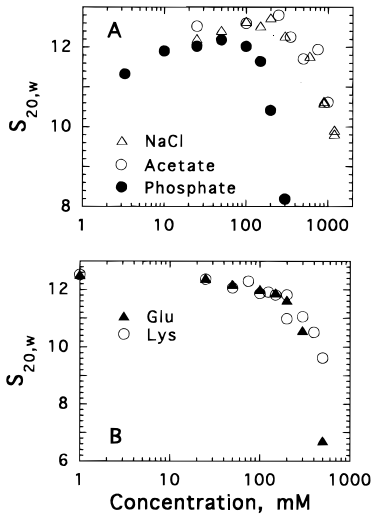

FIGURE 1: Effect of various salts and amino acids on the sedimentation coefficient of Lp(a). (A) Sodium phosphate, filled circles; sodium acetate, open circles; and NaCl, open triangles. (B) Filled triangles, glutamic acid/100 mM NaCl; open circles, lysine/100 mM NaCl. All solutions contained 10 mM HEPES, 0.01% Na_2-EDTA, and 0.01% NaN_3 and had a pH of 7.4.

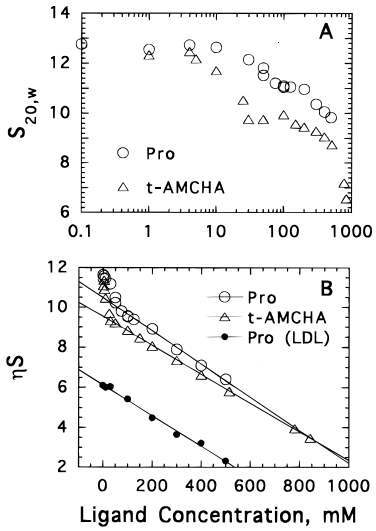

FIGURE 2: Effect of proline and t-AMCHA on the sedimentation coefficient of Lp(a). (A) Plot of the sedimentation coefficient of Lp(a) corrected to standard conditions of water and 20 °C as a function of amino acid concentration. The anhydrous partial specific volume of Lp(a) obtained by sedimentation equilibrium in D_2O was used in the density correction. (B) Plot of the viscosity-corrected sedimentation coefficient as a function of ligand concentration using the same experimental data as in (A). The fact that for both ligands plots of ηs vs ligand concentration are linear above 100 mM indicates that the decrease in $s_{20,w}$ seen at the same concentrations in plot A is not caused by conformational change. LDL which does not undergo a global conformational change when exposed to the ω-aminocarboxylic acid, 6-AHA, is also not affected by proline as shown by the linear ηs vs ligand concentration plot. For Lp(a): open circles, proline; and open triangles, t-AMCHA; for LDL: closed circles, proline.

From equilibrium centrifugation conducted in solutions containing different amounts of D_2O, the anhydrous molecular weight and partial specific volume of the 15 K-IV Lp(a) were calculated to be 3.28×10^6 and 0.9425 mL/g, respectively. D_2O was used instead of NaBr to increase the solution density in order to prevent preferential hydration of Lp(a) (Fless & Santiago, 1997).

Effect of Low Ionic Strength and Preferential Hydration. As observed previously, $s_{20,w}$ values of Lp(a) were greatest around 0.1−0.2 M NaCl and are indicative of the compact form of Lp(a)(Figure 1A)(Fless et al., 1996). With lower concentrations of NaCl, there was a concomitant decrease in the sedimentation coefficient as Lp(a) assumed a more extended conformation that is probably caused by charge repulsion due to low ionic strength. A similar effect was seen with phosphate, although the curve was shifted toward lower concentrations, because sodium phosphate has a higher ionic strength at equivalent concentrations of either NaCl or sodium acetate. In the complete absence of salt, e.g., in water, the $s_{20,w}$ of Lp(a) was found to be 10.58. However, at higher concentrations of either of the three salts, the sedimentation coefficient of Lp(a) decreased again. When the results observed with sodium acetate, chloride, and phosphate were plotted as a function of ionic strength, all three curves superimposed.

All other compounds examined also lowered the sedimentation rate of Lp(a) when tested at higher concentrations. This is illustrated in Figure 1B, where high concentrations of either glutamic acid or lysine, both in 100 mM NaCl, decreased $s_{20,w}$ values in a manner similar to that observed with NaCl or sodium acetate. Although not shown in the figure, the sedimentation coefficient at low concentrations of Glu (without NaCl) decreased in the same manner as a function of ionic strength as the above salts. Inspection of the curves revealed that they are not sigmoidal, and it was difficult to detect an end point because of progressively falling sedimentation rates. In contrast, plots obtained with t-AMCHA, proline, and most ω-aminocarboxylic acids had a more complex shape that is illustrated in Figure 2A. In the presence of increasing concentrations of amino acids, there was a sigmoidal decrease in the sedimentation coefficient that appeared to level off between 30 and 200 mM for t-AMCHA and between 70 and 200 mM for proline. However, with further increases in their concentrations, $s_{20,w}$ values decreased again and had no clear end point. This phenomenon can be explained when preferential hydration of Lp(a) caused by the presence of higher concentrations of salt or amino acids is taken into account. Because preferential hydration decreases the magnitude of the apparent partial specific volume of Lp(a), its neglect leads to a smaller density correction and lower than expected $s_{20,w}$ values.

Viscosity-Corrected Sedimentation Coefficient. In an effort to circumvent this problem, the viscosity-corrected sedimentation coefficient was plotted as a function of solution density and/or ligand concentration. Treating the data in this manner avoided the use of the complicating partial specific volume of Lp(a) and the density correction. Plots of ηs vs density or ligand concentration are usually linear; curvature is introduced only if there is variation in shape, molecular weight, or partial specific volume, or preferential hydration of the macromolecule (Cox & Schumaker, 1961a). For example, the ηs vs concentration plot of LDL exposed to various concentrations of proline is linear, which indicates that LDL does not change conformation when it is treated with this ligand (see Figure 2B). This is in agreement with an earlier finding in which we showed that 6-AHA does not elicit a conformational change in LDL (Fless et al., 1996).

Sedimentation velocity data of Lp(a) in t-AMCHA and proline shown as $s_{20,w}$ vs concentration plots in Figure 2A are compared to ηs vs concentration plots of Figure 2B. It is immediately clear upon an examination of the plots,

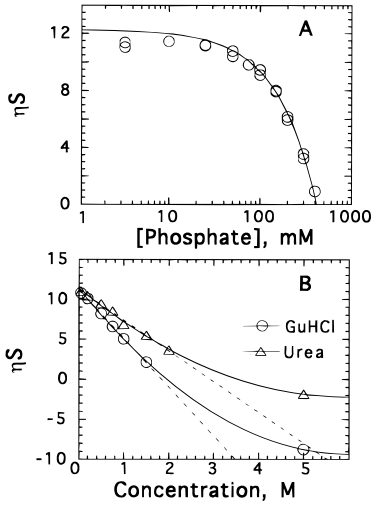

FIGURE 3: Effect of low ionic strength and denaturation on the viscosity-corrected sedimentation coefficient of Lp(a). (A) Data obtained between 50 and 400 mM phosphate were fitted to a least-squares line. The graph clearly shows the downward deviation from a straight line at the lower concentrations of phosphate which is due to unfolding at low ionic strength. A logarithmic scale was used for clearer presentation of the low phosphate data. (B) Unfolding of Lp(a) at high concentrations of urea and guanidine hydrochloride. Dashed lines represent least-squares fits of the linear portion of data points. Solid lines represent polynomial fits through all data points and allow a clear visual portrayal of the denaturation of Lp(a). Open circles, guanidine hydrochloride; open triangles, urea.

especially in the case of t-AMCHA, that the decrease in $s_{20,w}$ at higher ligand concentrations is not caused by a change in conformation but is due to preferential hydration, because the plot for t-AMCHA is linear from 70 to 900 mM. It curves upward only at lower concentrations of t-AMCHA in response to the change in conformation of Lp(a) caused by the binding of ligand. Downward curvature of ηs vs density or concentration plots was observed at low salt concentrations due to unfolding of Lp(a) at low ionic strength (see Figure 3A). The only other deviation from a straight line occurred at concentrations greater than 2 M for urea, or 1.5 M for guanidine hydrochloride, and are due to the denaturation of Lp(a) (Figure 3B).

An obvious advantage of the use of ηs vs density plots is the fact that it allows a clear estimation of the limiting sedimentation coefficient marking the complete unfolding of Lp(a) in the presence of a particular ligand from the intercept of the *y*-axis. Additionally, the horizontal intercepts of ηs vs density or ligand concentration plots give the particle buoyant density and concentration of ligand at which there is zero sedimentation. Knowledge of the buoyant density of Lp(a) and its partial specific volume allows the determination of the preferential hydration, which is actually the difference between the amount of bound water and the amount of bound salt (Cox & Schumaker, 1961b).

Ineffective Compounds. The data obtained with all compounds are grouped into two sets: those that proved ineffective in eliciting a conformational change are presented in Table 1, whereas the results acquired with the conformation-altering ligands are shown in Table 2. With the exception of sodium chloride, acetate, and phosphate, and guanidine hydrochloride, all other compounds were tested in the presence of 100 mM NaCl which was added to prevent unfolding at low ionic strength. The $s_{20,w}$ values calculated from the *y*-intercept of the Sη vs density plots of the ineffective compounds had a mean value of 12.55 ± 0.12 S and are indicative of the compact conformation of Lp(a). The buoyant densities derived from the horizontal axis varied from 1.048 g/mL for lysine to 1.057 g/mL for NaCl, because of differential preferential hydration. There was essentially no preferential hydration in solutions containing from 0 to 100% D_2O (with 100 mM NaBr as ionic strength supplement), because the hydrodynamic partial specific volume (the reciprocal of the buoyant density) was identical to the anhydrous partial specific volume of Lp(a). The latter was determined by sedimentation equilibrium of Lp(a) taken from the same set of D_2O solutions used in the sedimentation velocity experiments. The concentrations required to produce zero sedimentation are also listed and show that for some compounds concentrations as low as 0.4−0.5 M elicited extensive preferential hydration. Overall, preferential hydration of Lp(a) varied from 6.9% in NaCl to 20% in solutions containing lysine.

Perhaps most surprising from this list of compounds was the fact that lysine at concentrations ranging up to 1 M was totally ineffective in inducing a conformational change in Lp(a). Similarly, acetylation of the ϵ-amino group of lysine did not make it an effective conformation-altering ligand. Since lysine as the free base is alkaline, it was used as the hydrochloride at neutral pH. Therefore, solutions of lysine had salt concentrations in amounts that may have been sufficient to inhibit a change in the conformation of Lp(a). Although it was impossible to study lysine in the absence of anions, the fact that 6-AHA, 4-ABA, t-AMCHA, and proline were able to overcome the NaCl induced inhibition of the conformational change indicated that lysine was not an effective ligand. Finally, as already stated above, the denaturants urea and guanidine hydrochloride were also ineffective in altering the configuration of Lp(a) at concentrations less than 2 and 1.5 M, respectively.

Conformation-Altering Ligands. Six additional ligands besides 6-AHA were found to bind to Lp(a) and thereby affect the conformation of this lipoprotein. Of these, five were analogs of 6-AHA and included glycine, 4-ABA, 8-AOA, t-AMCHA, and N-α-AcLys. The only ligand not fitting this category was proline. It was interesting that N-α-AcLys was able to induce a conformational change in Lp(a), because this appears to indicate that a positively charged α-amino group prevents lysine from interacting with Lp(a). Initially, ligands were tested in the presence of 40 mM glycine to prevent unfolding of Lp(a) at low ionic strength. However, this compound was found to be unsuitable, when it was discovered that glycine was able to change the conformation of Lp(a). Two other ionic strength supplements, e.g., 100 mM NaCl and 20 mM Glu, also affected the configuration of Lp(a). Since it proved difficult to find an agent that did not induce or inhibit a conformational change of Lp(a), a third set of experiments was performed without an ionic strength supplement.

The results shown in Table 2 indicate that the ligands varied greatly in their ability to induce a conformational change in Lp(a), and that relatively small concentrations of NaCl or glutamic acid profoundly inhibited the ligand-induced unfolding of Lp(a). The mean sedimentation coefficient of the compact form of Lp(a) obtained from the three sets of experiments was 12.64 ± 0.22 S (n = 14). 6-AHA induced the greatest unfolding of Lp(a) by decreasing

Table 1: Ineffective Compounds

compounds	$s_{20,w}$[a] (S)	hydrated density[a] (g/mL)	concentration[a,b] (M)	hydration[c] [g of H_2O/g of Lp(a)]
D_2O[d]	12.52 ± 0.13	1.061 ± 0.000	−	0
chloride	12.65 ± 0.12	1.057 ± 0.001	1.41 ± 0.02	0.074
acetate	12.64 ± 0.12	1.056 ± 0.001	1.36 ± 0.03	0.087
phosphate	12.47 ± 0.23	1.049 ± 0.001	0.41 ± 0.01	0.228
lysine	12.46 ± 0.06	1.048 ± 0.000	0.85 ± 0.01	0.250
N-ϵ-AcLys	12.51 ± 0.15	1.048 ± 0.001	0.93 ± 0.03	0.244
glutamic	12.41 ± 0.05	1.051 ± 0.000	0.60 ± 0.00	0.187
adipic	12.67 ± 0.09	1.055 ± 0.000	0.52 ± 0.01	0.099
urea	12.74 ± 0.12	1.050 ± 0.000	2.94 ± 0.03	0.201
GuHCl	12.42 ± 0.08	1.054 ± 0.000	1.84 ± 0.02	0.122

[a] Values represent the mean ± standard error of the mean. [b] Concentration of salt, amino acid, or denaturant at which there is zero sedimentation of Lp(a). [c] Preferential hydration was calculated from the hydrated density and partial specific volume of Lp(a) as defined under Experimental Procedures. [d] The sedimentation coefficient of Lp(a) was determined in solutions containing various concentrations of D_2O with 100 mM NaBr as ionic strength supplement.

Table 2: Conformation-Altering Ligands

ligand	salt	$s_{20,w}$[a] (S)	unfolding[b] (%)	density[a,c] (g/mL)	R_h[d] (Å)	concentration[a,e] (M)	hydration[f] [g of H_2O/g of Lp(a)]
glycine	−	11.23 ± 0.09	46.7	1.050 ± 0.001	152	1.62 ± 0.03	0.205
glycine	20 mM Glu	11.95 ± 0.03	22.8	1.055 ± 0.001	143	1.59 ± 0.01	0.104
glycine	100 mM NaCl	12.59 ± 0.11	1.7	1.052 ± 0.001	135	1.53 ± 0.02	0.163
4-ABA	−	9.63 ± 0.13	99.7	1.057 ± 0.002	177	1.96 ± 0.07	0.068
4-ABA	20 mM Glu	10.39 ± 0.15	74.5	1.059 ± 0.002	164	1.87 ± 0.06	0.035
4-ABA	100 mM NaCl	11.05 ± 0.19	52.6	1.058 ± 0.002	154	1.82 ± 0.04	0.051
6-AHA	−	9.62 ± 0.27	100.0	1.048 ± 0.002	177	1.76 ± 0.09	0.252
6-AHA	20 mM Glu	10.41 ± 0.02	73.8	1.049 ± 0.000	164	1.76 ± 0.00	0.228
6-AHA	100 mM NaCl	10.55 ± 0.24	69.2	1.048 ± 0.001	162	1.67 ± 0.04	0.252
8-AOA	−	10.41 ± 0.06	73.8	1.041 ± 0.003	164	1.72 ± 0.11	0.435
8-AOA	20 mM Glu	11.24 ± 0.08	46.4	1.050 ± 0.001	152	2.08 ± 0.06	0.206
8-AOA	100 mM NaCl	12.57 ± 0.18	2.3	1.044 ± 0.002	135	1.77 ± 0.18	0.352
t-AMCHA	−	9.78 ± 0.02	94.7	1.050 ± 0.000	174	1.33 ± 0.00	0.205
t-AMCHA	20 mM Glu	10.06 ± 0.05	85.4	1.055 ± 0.001	169	1.25 ± 0.02	0.104
t-AMCHA	100 mM NaCl	10.49 ± 0.18	71.2	1.047 ± 0.001	163	1.14 ± 0.02	0.276
proline	−	10.41 ± 0.05	73.8	1.045 ± 0.001	164	1.35 ± 0.03	0.328
proline	20 mM Glu	10.75 ± 0.19	62.6	1.053 ± 0.003	159	1.57 ± 0.08	0.142
proline	100 mM NaCl	11.47 ± 0.08	38.7	1.048 ± 0.001	149	1.33 ± 0.03	0.254
N-α-AcLys	−	10.61 ± 0.23	67.2	1.047 ± 0.002	161	0.99 ± 0.04	0.282
N-α-AcLys	20 mM Glu	10.91 ± 0.10	57.3	1.044 ± 0.001	156	0.86 ± 0.01	0.352
N-α-AcLys	100 mM NaCl	12.60 ± 0.06	1.3	1.049 ± 0.000	135	0.96 ± 0.01	0.228

[a] Values represent the mean ± standard error of the mean. [b] Percent unfolding = $(s/s_c - s_e) \times 100$, where s_c and s_e are the sedimentation coefficients of Lp(a) in 100 mM NaCl and saturating amounts of 6-AHA, respectively. [c] Hydrated density was obtained from the horizontal intercept of ηs vs density plots. [d] R_h is the effective hydrodynamic radius of Lp(a) and was calculated as defined under Experimental Procedures. [e] Concentration of ligand at which there is zero sedimentation of Lp(a). [f] Preferential hydration was calculated from the hydrated density and partial specific volume of Lp(a) as defined under Experimental Procedures.

the sedimentation rate from 12.64 to 9.62 S. The conformational changes induced by the other ligands were calculated relative to this change of 3.02 S and are presented in Table 2 as percent unfolding. In the absence of an ionic strength supplement, Lp(a) unfolded to the maximal extent in the presence of 4-ABA, 6-AHA, and t-AMCHA. Somewhat less effective were 8-AOA, proline, and N-α-AcLys which unfolded Lp(a) 67−74%. Glycine, which produced only half of the effect observed with 6-AHA, was least effective. Adding 20 mM glutamic acid to the ligands partially inhibited their ability to unfold Lp(a). Again, 4-ABA, 6-AHA, and t-AMCHA were most effective; however, they unfolded Lp(a) only 74.5, 73.8, and 85.4%, respectively. These were followed by proline (62.6%), N-α-AcLys (57.3%), 8-AOA (46.4%), and glycine (22.8%), which again was the least effective ligand. In the presence of 100 mM NaCl, three ligands, e.g., glycine, 8-AOA, and N-α-AcLys, were totally ineffective in eliciting a conformational change. With 6-AHA and t-AMCHA, only 70% of the maximal conformational change was achieved, followed by 4-ABA (52.6%) and proline (38.7%). Depending on the ligand, hydrated densities ranged from 1.041 g/mL (8-AOA) to 1.059 g/mL (4-ABA/20mM Glu), and the corresponding preferential hydration varied from 30.3 to 3.4%, respectively.

To test the hypothesis that the proline binding site was distinct from the LBS, the concentration of proline was varied from 200 to 750 mM in the presence of saturating amounts of t-AMCHA (200 mM) and 100 mM NaCl, in order to determine if there was additional unfolding of Lp(a) above and beyond that achieved with 200 mM t-AMCHA alone. The $s_{20,w}$ value of 10.57 S measured in the presence of both ligands is almost equal to the one determined in 200 mM t-AMCHA alone (10.49 S), indicating that no additional unfolding took place. This finding provides some evidence suggesting that the two binding sites may be the same, but does not constitute proof, and further experimentation is necessary to clarify this issue.

The effective hydrodynamic radius of Lp(a) was calculated in order to quantify the physical expansion of the molecule in the presence of the conformation-altering ligands. The compact Lp(a) particle had a radius of 135 Å in 100 mM NaCl, which increased to 162 Å in the presence of 6-AHA

Table 3: Binding Parameters of Lp(a)[a]

	20 mM Glu			100 mM NaCl			inhibition of Lp(a) reassembly	
	$K_d \times 10^3$ (M)	n	K_{50} (mM)	$K_d \times 10^3$ (M)	n	K_{50} (mM)	IC^b_{50} (mM)	IC^c_{50} (mM)
glycine	1.06 ± 3.18	1.7 ± 0.7	16.8 ± 2.9	–	–	–	–	–
4-ABA	15.9 ± 15.9	1.6 ± 0.4	97.8 ± 7.6	18.5 ± 41.3	1.8 ± 1.0	106 ± 24	2.1	–
6-AHA	11.9 ± 20.4	1.9 ± 0.6	102 ± 11	22.0 ± 17.9	1.7 ± 0.3	108 ± 3	4.8	11
8-AOA	4.34 ± 8.29	1.5 ± 0.5	24.4 ± 3.4	–	–	–	–	–
t-AMCHA	6.01 ± 5.75	1.3 ± 0.2	23.2 ± 2.2	6.60 ± 8.63	1.7 ± 0.4	51.7 ± 5.6	0.65	–
proline	2.87 ± 4.92	1.7 ± 0.5	29.4 ± 3.1	4.73 ± 5.89	1.4 ± 0.3	23.3 ± 3.2	21	20
N-α-AcLys	61.9 ± 131	1.0 ± 0.6	64.0 ± 9.9	–	–	–	9.7	9.5
lysine	–	–	–	–	–	–	38	11
N-ϵ-AcLys	–	–	–	–	–	–	52	66

[a] The dissociation constant, K_d, was calculated from the binding curves as described under Experimental Procedures. K_{50} values are equivalent to 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), and were obtained by a logit transformation of the binding curves (Fless et al., 1989). All values represent the mean ± standard error of the mean. [b] Values taken from Frank et al. (1995). [c] Values taken from Gabel et al. (1996).

or t-AMCHA. However, when 20 mM Glu was substituted for NaCl, the same ligands increased the radius only a few additional angstroms. The other compounds were much more effective in 20 mM Glu than in 100 mM NaCl, which is indicative of the strong inhibitory effect of NaCl on the conformational change of Lp(a). In the absence of either NaCl or Glu, Lp(a) expanded to 177 Å when exposed to 6-AHA or t-AMCHA, a 42 Å increase. Glycine achieved the smallest expansion (152 Å), which is less than half the magnitude attained with 4-ABA, 6-AHA, and t-AMCHA.

Binding Constants. The titration curves obtained for each ligand were constructed by plotting the viscosity- and density-corrected sedimentation coefficient as a function of ligand concentration. The hydrodynamic partial specific volume, $\bar{\nu}_h$ was used instead of the anhydrous partial specific volume, $\bar{\nu}$, in calculating the density correction. This allowed the generation of sigmoidal curves which proved to be impossible with many ligands when $\bar{\nu}$ was used, because of preferential hydration at higher concentrations of ligand.

In the presence of 20 mM glutamic acid, six additional ligands besides 6-AHA were found to change the conformation of Lp(a), compared to three in 100 mM NaCl. All had dissociation constants that were in the millimolar range, and appeared to bind to two sites. The K_d values were not statistically different from each other. K_{50} values were similar for glycine, 8-AOA, t-AMCHA, and proline, about 2−3-fold higher for N-α-AcLys, and roughly 4-fold higher for 4-ABA and 6-AHA. There was no correlation between a ligand's dissociation constant and its ability to unfold Lp(a) as measured by a decrease in the sedimentation rate. Although the dissociation constants for the ligands 4-ABA, 6-AHA, t-AMCHA, and proline were all lower in 20 mM Glu than in 100 mM NaCl, the difference was not statistically significant (see Table 3). However, it supports the concept that ligand affinities are stronger in 20 mM Glu, because three additional ligands were able to bind to Lp(a) that were totally unable to do so in 100 mM NaCl.

Binding in the presence of 100 mM NaCl probably represents conditions that are more physiological in nature, since the concentrations of both Na^+ and Cl^- ions are above 100 mM in plasma. Proline and t-AMCHA had the highest affinity for Lp(a) followed by 4-ABA and 6-AHA. Again, binding appeared to involve more than one site, with proline showing binding to either one or two sites. The results for 6-AHA in 100 mM NaCl with respect to multivalent binding ($n = 1.7 \pm 0.3$) and millimolar binding constant were

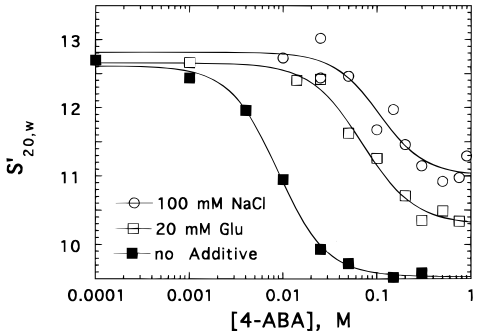

FIGURE 4: Unfolding of Lp(a) with increasing concentrations of 4-ABA as measured by the decrease in the sedimentation coefficient of Lp(a): Effect of 100 mM NaCl and 20 mM glutamic acid (in comparison to no additive) on the conformation of Lp(a). The background buffer contained 10 mM HEPES, 0.01% Na_2EDTA, and 0.01% NaN_3, pH 7.4. The solid lines represent the best fit theoretical curves calculated as described under Experimental Procedures. $s'_{20,w}$ values were calculated using the hydrodynamic partial specific volume of Lp(a) as defined in the text. Solid squares, no additive; open squares, 20 mM glutamic acid; open circles, 100 mM NaCl.

consistent with those obtained previously (Fless et al., 1996). In that study, we examined 5 Lp(a) particles that had between 15 and 32 K-IV domains and determined that the number of bound 6-AHA molecules ranged from 1.4 to 4, and was independent of apo(a) size. K_{50} values were lowest for proline, 2-fold higher for t-AMCHA, and about 4-fold higher for 4-ABA and 6-AHA. As observed with ligands in 20 mM Glu, the affinity of ligands for Lp(a) did not go hand-in-hand with greater conformational changes. The tightest binding ligand, e.g., proline, induced the smallest conformational change, whereas 6-AHA, which bound weakest, produced a change that was almost 2-fold larger.

Ligand affinity for Lp(a) was highest when no additional salt was added to the ligand-containing solution. However, at low ligand concentrations and therefore low ionic strength, unshielded charge repulsion also contributed to unfolding of the Lp(a) protein moiety. Therefore it was impossible to obtain accurate binding constants. We attempted to construct binding curves by correcting for unfolding at ionic strengths below 0.05 through the use of a standard curve constructed from plots of ηs vs ionic strength at different concentrations of glutamic acid. Such a binding curve is illustrated in Figure 4 for the ligand 4-ABA. Weaker binding of 4-ABA in the presence of both 20 mM Glu and 100 mM NaCl is clearly shown by the displacement of their binding curves to higher concentrations. The high affinity for 4-ABA in the absence

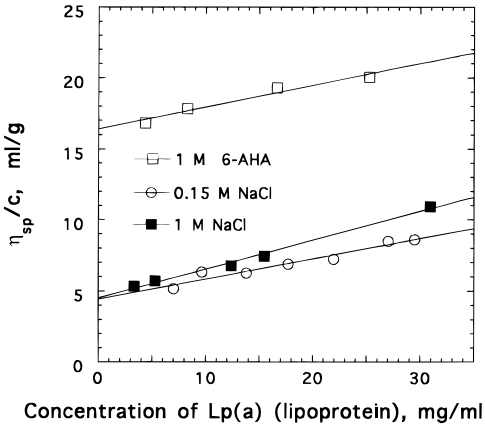

FIGURE 5: Effect of 6-AHA on the intrinsic viscosity of Lp(a). The intrinsic viscosity of Lp(a) was obtained by extrapolating η_{sp}/c to infinite dilution, where η_{sp} is the specific viscosity. Open squares, 1 M 6-AHA; filled squares, 1 M NaCl; open circles, HBS.

of salt, or conversely the strong inhibitory effect of NaCl on the conformational change, is readily apparent by the observation that at 50 mM 4-ABA and 100 mM NaCl there is almost no reduction in the sedimentation coefficient, indicating that Lp(a) is compact. In contrast, about 40% of the conformational change has taken place in 20 mM Glu, compared to 100% unfolding when no salt was added to the ligand. This analysis allowed the ordering of the ligands according to their K_{50} values or decreasing affinity as follows: t-AMCHA (4.9 mM), proline (5.7 mM), glycine (6.7 mM), 8-AOA (9.2 mM), 4-ABA (10 mM), N-α-AcLys (12 mM), and 6-AHA (16 mM). These are approximate values since the analysis required the untested assumption that the binding of ligand by Lp(a) was not affected by the unfolding of Lp(a) at low ionic strength.

Viscosity of Lp(a). In order to verify the conformational change detected in Lp(a) by a decrease in its sedimentation coefficient, a different physical method was chosen. Measurement of the viscosity of Lp(a) in the presence and absence of 1 M 6-AHA gave independent confirmation that this ligand does indeed induce a conformational change in Lp(a) (see Figure 5). The intrinsic viscosity of Lp(a) in HBS was 4.43 ± 0.30 mL/g, compared to 16.4 ± 0.4 mL/g in 1 M 6-AHA. To rule out that the unfolding was caused by the high ionic strength of 1 M 6-AHA, the intrinsic viscosity of Lp(a) was also determined in 1 M NaCl and found to be 4.50 ± 0.18 mL/g, a value not significantly different from the one determined in HBS.

DISCUSSION

Previous studies have shown that lipoprotein(a) undergoes a dramatic reversible ligand-induced conformational change, which can be modeled as a two-state process in which the binding of 6-AHA elicits a transition from a compact to an extended form of the Lp(a) particle (Fless et al., 1996). The present study extended these observations to a series of ω-aminocarboxylic acids of different chain length, and other, more distant analogs of 6-AHA, such as proline and N-α-AcLys. As detailed under Results, some but not all of these compounds induce a conformational change in Lp(a) as measured by a decrease in the sedimentation rate. Compounds containing two carboxyl groups but no amino groups (e.g., adipic acid) were ineffective in changing the conformation. Lysine, which has a relatively weak effect on the

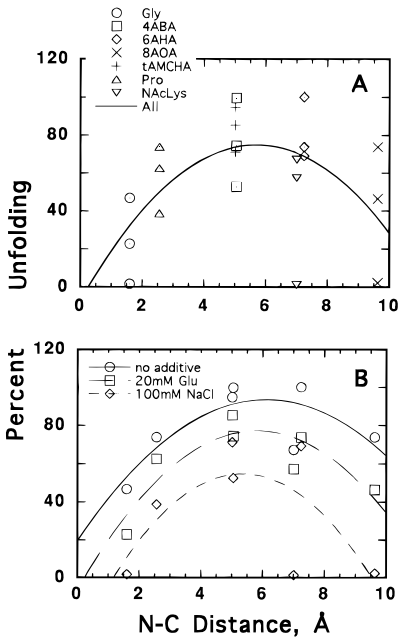

FIGURE 6: Relationship between the percent unfolding of Lp(a) and the distance between the amine nitrogen and carboxyl carbon (N−C distance) of conformation-altering ligands. (A) Relationship between N−C distance and percent unfolding for seven compounds, each under three different solvent conditions, is shown in the legend. The second-order polynomial, $Y = -7.48 + 28.86X - 2.53X^2$ ($R = 0.57$), was fitted using all 21 data points. (B) Effect of solvent composition on this same relationship. The equations of the polynomials are the following: for the low-salt solvent (e.g., no added salt), $Y = -35.13 + 33.59X - 3.14X^2$ ($R = 0.70$); for 20 mM Glu, $Y = -6.77 + 28.86X - 2.48X^2$ ($R = 0.87$); and for 100 mM NaCl, $Y = 19.47 + 24.14X - 1.97X^2$ ($R = 0.79$).

conformation of plasminogen (Violand et al., 1978), could not induce a conformational change in Lp(a). N-ε-AcLys, unlike N-α-AcLys, was also ineffective in eliciting a change, thus indicating the importance of the ε-amino group and the likelihood that the positively charged α-amino group of lysine interferes with efficient binding by the LBS.

The observation that several related compounds were able to alter Lp(a) conformation allowed us to formulate a hypothesis as to the cause of the conformational change. Figure 6 shows the relationship between the distance separating the amino and carboxyl groups of conformation-altering ligands and percent unfolding. The data shown in the figure suggest that there is an optimal distance for inducing this conformational change, which is close to 6 Å. Both shorter (e.g., glycine) and longer (e.g., 8-AOA) ligands were less effective than compounds of the optimal length. The figure further suggests that the effective 6-AHA analogs were able to change the conformation of Lp(a) by competing with stabilizing interactions between lysine binding sites of apo(a) and lysine side chains within apoB and/or apo(a). In this connection, it is noteworthy that the linear dimensions of the apo(a) K-IV_{37} LBS are defined by a 6.6 Å separation of the cationic and anionic centers (Mikol et al., 1996). It is possible that compounds like 6-AHA bind to such a center, and thereby disrupt stabilizing interactions between kringles and amino acid side chains of apoB or apo(a), and/or between kringles of apo(a).

Figure 6 demonstrates also that analogous changes were observed under three solvent conditions: 100 mM NaCl, 20 mM glutamate, and no added salt. The three curves appear to have similar shapes, and are only displaced along the

y-axis. Thus, a given ω-amino acid or analog had an ordained efficacy in altering the conformation of apo(a), but in all cases was most effective in the absence of added salt, followed by 20 mM glutamate, followed by 100 mM NaCl. This concentration of sodium chloride was more effective at stabilizing the compact form than was 20 mM Glu, despite the fact that these two media were of nearly identical ionic strength. It has been shown that the kringles of plasminogen contain an anion binding site (Wu et al., 1994), which, when occupied by chloride or other anions, sterically hinders access of 6-AHA to the lysine binding site, and induces the compact conformation (Chibber & Castellino, 1986; Urano et al., 1987, 1988). The effect of halogen ions can be so pronounced that I^- can completely prevent the expansion of Glu-plasminogen in the presence of 6-AHA (Markus, 1996). Our current and previous data (Fless & Santiago, 1997) are also consistent with a stabilizing role for Cl^- ions and perhaps glutamate to a lesser extent. The fact that other proteins (plasminogen and tissue plasminogen activator) with LBS have Cl^- binding sites makes it likely that apo(a) also has binding site(s) for Cl^- (De Vos et al., 1992; Wu et al., 1994). The Cl^- found close to the cationic center of the LBS may compete with the binding of ω-aminocarboxylic acids by the LBS, as suggested by Menhart et al. (1995). In our experiments, however, we used saturating concentrations of ligands, suggesting that that the effect of Cl^- may be 2-fold: (1) steric hindrance of ligand binding to the LBS; and (2) stabilization of the compact form of Lp(a) even in the presence of ligand binding to the LBS. In support of the latter of these two effects, Cl^- ions located in crystals of tPA-K-II have been shown to contribute to the interaction of neighboring kringles (De Vos et al., 1992). Padmanahban et al. (1994) hypothesized that such kringle−kringle interactions in apo(a) might stabilize multikringle structures containing screw-axis symmetry. Because such arrangements are satisfied only by long interkringle peptides which are prevalent in apo(a), the existence of such long-range structure appears even more likely in Lp(a) than in plasminogen.

Measurement of the conformational change was difficult, because Lp(a) is prone to preferential hydration in the presence of moderate amounts of salts and ligands. If this hydration is not taken into account, then $s_{20,w}$ values calculated with the anhydrous partial specific volume will be too low, and the decrease that is due to hydration will be added to the decrease caused by a change in conformation. For example, with Lp(a) in 500 mM 6-AHA, 100 mM NaCl, hydration decreased the sedimentation coefficient by 12.5%. Because the $\bar{\nu}$ of Lp(a) is close to 1, the error is relatively large. With plasminogen, the corresponding difference is quite diminished, since it has a much smaller $\bar{\nu}$ (0.72 cm^3/g in the compact form and 0.75 cm^3/g in the expanded form) (Mangel et al., 1990). Thus, with a similar hydration, the difference in the sedimentation coefficient is only 1.8−2.0%, and therefore neglecting the effect of hydration on the $\bar{\nu}$ of plasminogen is much less serious. This problem was largely overcome with the viscosity-corrected sedimentation coefficient that allowed an unambiguous determination whether or not a ligand had the capacity to induce a conformational change in Lp(a). Similarly, the use of $s'_{20,w}$ values calculated with the hydrodynamic $\bar{\nu}_h$, instead of the anhydrous $\bar{\nu}$, permitted the evaluation of binding constants without having to consider the effect of hydration on the sedimentation coefficient. To seek reassurance that the conformational change detected by the decrease in the sedimentation coefficient was real, viscometry was chosen as a means to independently confirm the change in Lp(a) structure. This was in part because the $\bar{\nu}$ of Lp(a) is a much smaller factor in determining the intrinsic viscosity than the sedimentation coefficient of Lp(a). As the results presented in Figure 5 show, the large increase in the intrinsic viscosity of Lp(a), from 4.43 mL/g in HBS to 16.4 mL/g in the presence of 1 M 6-AHA, confirms the fact that the great expansion of the Lp(a) particle is caused by the binding of this ligand. This is a ligand-specific expansion, that cannot be duplicated by a similar concentration of NaCl.

In considering the possible physiological significance of these data, one is struck by the similarity in the physical forces controlling the conformation of Lp(a), and those involved in the assembly of Lp(a) from apo(a) and LDL. Lp(a) assembly is believed to occur by a two-stage mechanism in which apo(a) binds initially through noncovalent interactions to apoB of LDL, followed by the creation of a disulfide bond between Cys 4326 of apoB and Cys 4057 of apo(a)-K-IV_9 (McLean et al., 1987; Brunner et al., 1993; Koschinsky et al., 1993; Callow & Rubin, 1995; McCormick et al., 1995). The noncovalent interactions between LBS of apo(a) and lysines of apoB are probably mediated by K-IV_{5-8} (Frank et al., 1994, 1995; Edelstein et al., 1995; Ernst et al., 1995; Trieu & McConathy, 1995; Gabel et al., 1996; Klezovitch et al., 1996). Our studies, showing ligand-induced disruption of the compact form of Lp(a), and the stabilization of the compact structure by Cl^-, suggest that Lp(a) assembly occurs through at least the following three topological[2] stages: (1) the binding of LBS to select lysine residues in apoB; (2) the stabilization of these interactions by Cl^- at physiological concentrations in the blood; and (3) the further stabilization of these interactions through the formation of a covalent (disulfide) bond.

In general, the IC_{50} values for reassembly were much lower (10−80-fold) than the K_{50} values we observed for the conformational changes (see Table 3). This is expected, since the assembled particle is stabilized by additional forces—a disulfide bond, and perhaps hydrophobic interactions—which are not yet present when the components are in the process of reassembling. The native form of Glu-plasminogen is a compact structure with a right-handed spiral, that is stabilized by an interaction between K-V and a site in the N-terminal peptide, and by a second interaction, which is 6-AHA sensitive, between K-IV and a region in K-III (possibly -Cys_{256}-Leu-Lys-Gly-Thr-) (Ponting et al., 1992b; Marshall et al., 1994; Markus, 1996). In plasminogen, benzamidine induces a conformation which is intermediate between the compact and extended forms (Marshall et al., 1994). It is likely that the two-state model described for Lp(a) is an oversimplification, and that in apo(a), as with plasminogen, multiple conformational states will be found. Indeed, this is one possible interpretation of the data shown in Figure 6, in which there appears to be a gradation in the percentage of unfolding induced by the various ligands. Although Lp(a) and plasminogen are obviously different in many ways, it seems likely that additional stabilizing interactions will be found for Lp(a) which have analogies with those of plasminogen. The exact sites of the lysines

[2] The word "topological" is meant to denote, also, that these three stages may be either synchronous or asynchronous.

involved in these interactions remain to be determined. Nevertheless, the studies presented in this paper point to the importance of the 6-AHA-sensitive, weak LBS, together with Cl^-, in stabilizing the native, compact form of Lp(a), and suggest a role for these same forces in Lp(a) assembly.

REFERENCES

Abiko, Y., Iwamoto, M., & Tomikawa, M. (1969) *Biochim. Biophys. Acta 185*, 424−431.

Alkjaersig, N. (1964) *Biochem. J. 93*, 171−182.

Brunner, C., Kraft, H. G., Utermann, G., & Muller, H. J. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 11643−11647.

Callow, M. J., & Rubin, E. M. (1995) *J. Biol. Chem. 270*, 23914−23917.

Chibber, B. A. K., & Castellino, F. J. (1986) *J. Biol. Chem. 261*, 5289−5295.

Christensen, U., & Molgaard, L. (1992) *Biochem. J. 285*, 419−425.

Cox, D. J., & Schumaker, V. N. (1961a) *J. Am. Chem. Soc. 83*, 2433−2438.

Cox, D. J., & Schumaker, V. N. (1961b) *J. Am. Chem. Soc. 83*, 2445−2448.

De Vos, A. M., Ultsch, M. H., Kelley, R. F., Padmanabhan, K., Tulinsky, A., Westbrook, M. L., & Kossiakoff, A. A. (1992) *Biochemistry 31*, 270−279.

Edelberg, J., Gonzalez-Gronow, M., & Pizzo, S. (1989) *Biochemistry 28*, 2370−2374.

Edelstein, C., Mandala, M., Pfaffinger, D., & Scanu, A. M. (1995) *Biochemistry 34*, 16483−16492.

Ernst, A., Helmhold, M., Brunner, C., Pethoschramm, A., Armstrong, V. W., & Muller, H. J. (1995) *J. Biol. Chem. 270*, 6227−6234.

Fless, G. M., & Snyder, M. L. (1994) *Chem. Phys. Lipids 67/68*, 69−79.

Fless, G. M., & Santiago, J. Y. (1997) *Biochemistry 36*, 233−238.

Fless, G. M., Rolih, C. A., & Scanu, A. M. (1984) *J. Biol. Chem. 259*, 11470−11478.

Fless, G. M., ZumMallen, M. E., & Scanu, A. M. (1985) *J. Lipid Res. 26*, 1224−1229.

Fless, G. M., ZumMallen, M. E., & Scanu, A. M. (1986) *J. Biol. Chem. 261*, 8712−8718.

Fless, G. M., Snyder, M. L., & Scanu, A. M. (1989) *J. Lipid Res. 30*, 651−662.

Fless, G. M., Snyder, M. L., Furbee, J. W., Jr., Garcia-Hedo, M.-T., & Mora, R. (1994) *Biochemistry 33*, 13492−13501.

Fless, G. M., Furbee, J., Snyder, M. L., & Meredith, S. C. (1996) *Biochemistry 35*, 2289−2298.

Frank, S., Durovic, S., & Kostner, G. M. (1994) *Biochem. J. 304*, 27−30.

Frank, S., Durovic, S., Kostner, K., & Kostner, G. M. (1995) *Arterioscler. Thromb. Vasc. Biol. 15*, 1774−1780.

Gabel, B. R., May, L. F., Marcovina, S. M., & Koschinsky, M. L. (1996) *Arterioscler. Thromb. Vasc. Biol. 16*, 1559−1567.

Hajjar, K., Gavish, D., Breslow, J., & Nachman, R. (1989) *Nature 339*, 303−305.

Hoover-Plow, J. L., Boonmark, N., Skocir, P., Lawn, R., & Plow, E. F. (1996) *Arterioscler. Thromb. Vasc. Biol. 16*, 656−664.

Klezovitch, O., Edelstein, C., & Scanu, A. M. (1996) *J. Clin. Invest. 98*, 185−191.

Kluft, C., Jie, A. F., Los, P., de Wit, E., & Havekes, L. (1989) *Biochem. Biophys. Res. Commun. 161*, 427−433.

Koschinsky, M. L., Beisiegel, U., Henne-Bruns, D., Eaton, D. L., & Lawn, R. M. (1990) *Biochemistry 29*, 640−644.

Koschinsky, M. L., Cote, G. P., Gabel, B., & Vanderhoek, Y. Y. (1993) *J. Biol. Chem. 268*, 19819−19825.

Lackner, C., Cohen, J. C., & Hobbs, H. H. (1993) *Hum. Mol. Genet. 2*, 933−940.

Lograsso, P. V., Cornellhennon, S., & Boettcher, B. R. (1994) *J. Biol. Chem. 269*, 21820−21827.

Loscalzo, J., Weinfeld, M., Fless, G. M., & Scanu, A. M. (1990) *Arteriosclerosis 10*, 240−245.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265−275.

Mangel, W. F., Lin, B., & Ramakrishnan, V. (1990) *Science 248*, 69−73.

Markus, G. (1996) *Fibrinolysis 10*, 75−85.

Markwell, M. A. K., Haas, S. M., Bieber, L. L., & Tolbert, N. E. (1978) *Anal. Biochem. 87*, 206−210.

Marshall, J. M., Brown, A. J., & Ponting, C. P. (1994) *Biochemistry 33*, 3599−3606.

McCormick, S. P. A., No, J. K., Taylor, S., Flynn, L. M., Hammer, R. E., & Young, S. G. (1995) *Proc. Natl. Acad. Sci. U.S.A. 92*, 10147−10151.

McKie, J. E., & Brandts, J. F. (1972) *Methods Enzymol. 26*, 257−288.

McLean, J., Tomlinson, J., Kuang, W., Eaton, D., Chen, E., Fless, G., Scanu, A., & Lawn, R. (1987) *Nature 300*, 132−137.

Menhart, N., Hoover, G. J., McCance, S. G., & Castellino, F. J. (1995) *Biochemistry 34*, 1482−1488.

Mikol, V., Lograsso, P. V., & Boettcher, B. R. (1996) *J. Mol. Biol. 256*, 751−761.

Miles, L., Fless, G., Levin, E., Scanu, A., & Plow, E. (1989) *Nature 339*, 301−303.

Morrisett, J. D., Gaubatz, J. W., Knapp, R. D., & Guevara, J. G. J. (1990) in *Lipoprotein(a)* (Scanu, A. M., Ed.) pp 53−74, Academic Press, San Diego, CA.

Padmanabhan, K., Wu, T. P., Ravichandran, K. G., & Tulinsky, A. (1994) *Protein Sci. 3*, 898−910.

Ponting, C. P., Holland, S. K., Cederholm-Williams, S. A., Marshall, J. M., Brown, A. J., Spraggon, G., & Blake, C. C. F. (1992a) *Biochim. Biophys. Acta 1159*, 155−161.

Ponting, C. P., Marshall, J. M., & Cederholm-Williams, S. A. (1992b) *Blood Coagulation Fibrinolysis 3*, 605−614.

Prassl, R., Schuster, B., Abuja, P. M., Zechner, M., Kostner, G. M., & Laggner, P. (1995) *Biochemistry 34*, 3795−3801.

Scanu, A. M., Miles, L. A., Fless, G. M., Pfaffinger, D., Eisenbart, J. E., Jackson, E., Hoover-Plow, J. L., Brunck, T., & Plow, E. F. (1993) *J. Clin. Invest. 91*, 283−291.

Scanu, A. M., Pfaffinger, D., Lee, J. C., & Hinman, J. (1994) *Biochim. Biophys. Acta 1227*, 41−45.

Snyder, M. L., Polacek, D., Scanu, A. M., & Fless, G. M. (1992) *J. Biol. Chem. 267*, 339−346.

Trieu, V. N., & McConathy, W. J. (1995) *J. Biol. Chem. 270*, 15471−15474.

Urano, T., Chibber, B. A. K., & Castellino, F. J. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 4031−4034.

Urano, T., de Serrano, V. S., Gaffney, P. J., & Castellino, F. J. (1988) *Biochemistry 27*, 6522−6528.

Vanderhoek, Y. Y., Wittekoek, M. E., Beisiegel, U., Kastelein, J. J. P., & Koschinsky, M. L. (1993) *Hum. Mol. Genet. 2*, 361−366.

Violand, B. N., Byrne, R., & Castellino, F. J. (1978) *J. Biol. Chem. 253*, 5395−5401.

Wu, T. P., Padmanabhan, K. P., & Tulinsky, A. (1994) *Blood Coagulation Fibrinolysis 5*, 157−166.

BI9706982