Molecular Weight Determination of Lipoprotein(*a*) [Lp(*a*)] in Solutions Containing either NaBr or D_2O: Relevance to the Number of Apolipoprotein(*a*) Subunits in Lp(*a*)†

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ABSTRACT: Molecular weight determination of low-density lipoprotein (LDL) is usually performed in solutions containing high concentrations of salt (up to 13.4 M NaBr) by sedimentation velocity and diffusion experiments, because it does not preferentially bind salt or water. Considering that lipoprotein(*a*) [Lp(*a*)] is structurally similar to LDL, differing only by the presence of Apo(*a*), the molecular weight, *M*, of Lp(*a*) has also been measured in solutions containing high concentrations of NaBr. We questioned the suitability of this practice by comparing the apparent molecular weight, M_{app}, and partial volume, ϕ', of Lp(*a*) determined by sedimentation and flotation equilibrium in a three-component system containing NaBr with the analogous parameters, *M* and partial specific volume, $\bar{\nu}$, determined in a two-component system containing D_2O. LDL served as a control. In agreement with previous findings obtained with different methods, our results indicate no significant differences in *M* and $\bar{\nu}$ of four different LDL samples and apparently no significant preferential binding of solvent components. In contrast, values of M_{app} and ϕ' of Lp(*a*) evaluated in NaBr are significantly greater than *M* and $\bar{\nu}$. Preferential binding of solvent components appeared to be a function of Apo(*a*) mass or the number of kringle IV domains, as expressed by increasing percentage differences between the two sets of parameters, ranging from 4 to 13% in *M* and 0.2 to 0.5% in $\bar{\nu}$ of Lp(*a*) species having Apo(*a*) with 15−27 kringle IV domains. Furthermore, our results indicate that the variable Apo(*a*) kringle IV domains are more involved in this process than the constant domain of Apo(*a*). These findings indicate that the Lp(*a*) molecular weight should be determined in D_2O and that high concentrations of NaBr should be avoided as their use would lead to overestimated molecular weights and partial specific volumes. Application of this method to the question of how much Apo(*a*) is released upon the reduction of Lp(*a*) led to the conclusion that Lp(*a*) contains only one Apo(*a*) molecule.

Molecular weight determination of low-density lipoprotein (LDL)[1] is often carried out in solutions containing large amounts of salt (Adams & Schumaker, 1969a,b; Lindgren et al., 1969; Fisher et al., 1971; Schumaker, 1973; Nelson et al., 1974; Fless et al., 1976; Fless & Scanu, 1979). The reason for this unusual procedure is the fact that the partial specific volume of LDL is only slightly less than that of water, therefore causing its buoyancy factor $(1 - \bar{\nu}\rho)$ to approach 0. Since the term appears in the denominator of the molecular weight equation, any small error in the magnitude of the partial specific volume would result in a large error in the molecular weight of LDL. The addition of salt therefore affords two advantages. First, it reduces the error in the molecular weight of LDL by increasing the buoyancy factor up to 10-fold, and second, it decreases the duration of the sedimentation velocity run (Adams & Schumaker, 1969b). The parameters of choice used in determining the molecular weight have been the sedimentation coefficient in combination with either the frictional (Adams & Schumaker, 1969b; Lindgren et al., 1969) or diffusion coefficient (Fisher et al., 1971). Additionally, the mass of LDL and its partial specific volume have been derived simultaneously from sedimentation and flotation equilibrium in solutions containing different concentrations of salt (Nelson et al., 1974; Fless et al., 1976; Fless & Scanu, 1979). The practice of measuring the molecular weight of LDL in solutions containing large amounts of salt was made possible only by the observation that LDL neither selectively binds salt nor is preferentially hydrated in such a milieu. Thus, diffusion coefficients of LDL were identical in solutions containing either 0.2 M KCl or 1.4 M KBr (Fisher et al., 1971). Similarly, the mass of LDL derived from sedimentation velocity runs, performed over a concentration range of NaBr of 0.15−13.4 M, was constant (Adams & Schumaker, 1969a).

Because of a structural makeup that is akin to that of LDL, similar assumptions have been made in determining the molecular weight of Lp(*a*) by equilibrium centrifugation in solutions containing different concentrations of salt (Fless et al., 1984, 1986, 1994). Data derived from these studies led us to propose a model for the subunit structure of Lp(*a*) in which two Apo(*a*) molecules are disulfide-linked to one molecule of ApoB (Fless et al., 1986, 1994). However, this

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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*); K, kringle; K-IV 1−10, Apo(*a*) kringle domain of subtype 1−10, all with homology to kringle 4 of plasminogen; Pg, plasminogen; tPA, tissue plasminogen activator; Na_2EDTA, sodium salt of ethylenediaminetetraacetic acid.

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model has come into question because of recent reports indicating that the molar ratio of Apo(*a*) to ApoB is actually 1 (Callow & Rubin, 1995; McCormick et al., 1995; Albers et al., 1996). The most likely way to reconcile the two divergent models is to investigate whether the molecular weight of Lp(*a*) was overestimated in our studies, particularly in light of the fact that Lp(*a*) mass was the most important factor in calculating the molar ratio of Apo(*a*) to ApoB. Incorrect molecular weights for Lp(*a*) could result if the assumption, i.e., that Lp(*a*) does not preferentially bind salt or water, is invalid, thereby resulting in an erroneous ratio.

To test this hypothesis, we measured the molecular weight and partial specific volume of Lp(*a*) in mixtures of H_2O/D_2O as outlined by Edelstein and Schachman (1967), because these solutions can tacitly be considered two-component systems, with H_2O and buffer salts representing component one and Lp(*a*) component two. The same parameters were also measured in solutions containing NaBr in order to test the contribution of the third component, e.g., NaBr. Under all conditions tested, LDL served as the control.

EXPERIMENTAL PROCEDURES

Preparation of Lipoproteins. Autologous Lp(*a*) and LDL were isolated from the plasma of subjects that gave informed consent prior to plasmapheresis or collection of blood. A procedure employing a combination of lysine-Sepharose and density gradient centrifugation was used essentially as described previously (Fless et al., 1984; Snyder et al., 1992; Fless & Snyder, 1994). Lp(*a*−) was prepared from Lp(*a*) by reduction with 50 mM dithiothreitol, followed by alkylation with 150 mM iodoacetic acid as described previously (Fless et al., 1994).

D_2O Exchange. Lipoproteins at a protein concentration of 3 mg/mL or higher were passed over a Sephadex G25 column (1.5 × 15 cm) equilibrated with buffered D_2O (99.9 at. % D, Sigma, St. Louis, MO). The column was monitored at 280 nm with an Altex 150 UV monitor that was coupled to a BD 41 Kipp-Zonen recorder. The flow rate of 10 mL/h was maintained with an ISCO WIZ pump. D_2O was buffered with 10 mM phosphate (pH 7.4) containing 100 mM NaBr and 0.01% NaN_3. The pH of the solution was adjusted with 6 M NaOH. The buffering process decreased the mole percent of D of the D_2O solution by approximately 0.13% and increased the density of D_2O from 1.105 to 1.1142 g/mL. Because this increase is similar to that observed between the density of H_2O (0.9982 g/mL) and 10 mM phosphate containing 100 mM NaBr and 0.01% NaN_3 (1.0076 g/mL), it confirms the fact that the buffered D_2O solution is essentially 100% D_2O. Aliquots of these lipoprotein solutions (0.5 mL) were diluted gravimetrically with 10 mM phosphate, 100 mM NaBr, and 0.01% NaN_3, to make solutions containing lower percentages of D_2O. They were then dialyzed in amber-colored 15 mL vials that were filled to the top with the appropriate nitrogen-saturated D_2O solution to allow no air space. To ensure optimal hydrogen exchange with deuterium, dialysis of the lipoprotein samples was performed for 3−4 days, followed by at least another 3 days in the analytical centrifuge. The percentage of D_2O was estimated from the density of the dialysate. Solvent densities were measured with a DMA-02-C precision density meter (Mettler/Paar) as described previously (Fless et al., 1994).

Equilibrium Ultracentrifugation. 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. Equilibrium ultracentrifugation of Lp(*a*) and LDL at 20 °C, in four or five different solutions of D_2O or NaBr, was carried out as described previously (Fless et al., 1994). Rotor speeds varied between 3000 and 5000 rpm. When equilibrium was reached, usually after 3 days of centrifugation, the rotor was accelerated to 40 000 rpm, held there for 2 h, and returned to the original speed in order to initiate a baseline scan.

Data were analyzed using the Optima XL-A data analysis software (Beckman, Palo Alto, CA) in conjunction with Origin software (Microcal Software, Northhampton, MA). Assuming a single ideal solute, values of $M(1 - \bar{\nu}\rho)$ were calculated with the "ideal 1 model" using a baseline offset determined from the baseline run.

For an ideal two-component system, the sedimentation equilibrium equation can be described by

$$M(1 - \bar{\nu}\rho) = \frac{2RT}{\omega^2}\frac{\mathrm{d}(\ln c)}{\mathrm{d}r^2}$$

where M is the weight average molecular weight, $\bar{\nu}$ is the partial specific volume, ρ is the solution density, c is the concentration of the solute, ω is the angular velocity, R is the universal gas constant, T is the absolute temperature, and r is the radial distance from the center of rotation. Solving for ρ, the equation can be rearranged to yield

$$\rho = \frac{1}{\bar{\nu}} - \frac{1}{M\bar{\nu}}\frac{2RT}{\omega^2}\frac{\mathrm{d}(\ln c)}{\mathrm{d}r^2}$$

By plotting ρ against $(2RT/\omega^2)[d(\ln c)/dr^2]$ or the equivalent expression $M(1 - \bar{\nu}\rho)$, e.g., the M-Buoyant value, both the molecular weight and partial specific volume are obtained simultaneously. In case there are preferential interactions between the macromolecule and the third component, e.g., NaBr, incorrect values for the molecular weight and partial specific volume will be attained which may be labeled M_{app} and $\bar{\nu}_{app}$. Although Edelstein and Schachman (1967) have shown that correct values of M and $\bar{\nu}$ can be determined in D_2O, both have to be adjusted as a result of deuterium exchange. In D_2O, the molecular weight takes the value kM and the partial specific volume becomes $\bar{\nu}/k$, where k is the ratio of the molecular weight of the macromolecule in the deuterated solvent to that in H_2O (Edelstein & Schachman, 1967). For solutions with a lower percentage of D_2O, k is reduced proportionally.

Estimation of k. The parameter k for the whole lipoprotein molecule, at pH 7.0, was obtained from the composition of several LDL and Lp(*a*) preparations analyzed previously (Fless et al., 1994) and is given in Table 1. For the lipoprotein components ApoB and Apo(*a*) protein, k was determined from their respective amino acid sequences (Knott et al., 1986; McLean et al., 1987); the value for ApoB was 1.0167 and 1.0173 for the 15, 18, and 27 K-IV Apo(*a*) polymorphs. The three Apo(*a*)s had virtually identical k parameters that changed only in the fifth place after the decimal in response to the K-IV number. Because only the carbohydrate content (23% by weight) and composition of

Table 1: Determination of *k*, the Ratio of the Deuterated to Nondeuterated Molecular Weight of Lp(*a*) and LDL

	exchangeable hydrogens [g/(100 g)]							
	component	15 K-IV Lp(*a*)	component	18 K-IV Lp(*a*)	component	27 K-IV Lp(*a*)	component	LDL
protein	1.69 (25.4)[a]	0.429	1.69 (28.5)	0.482	1.70 (36.2)	0.615	1.67 (18.8)[b]	0.313
carbohydrate	1.63 (7.7)	0.126	1.63 (8.7)	0.142	1.64 (10.9)	0.179	1.58 (1.4)	0.022
phospholipid	0.11 (18.6)	0.020	0.11 (17.4)	0.019	0.11 (15.4)	0.017	0.10 (22.5)	0.023
free cholesterol	0.26 (6.2)	0.016	0.26 (5.6)	0.015	0.26 (5.5)	0.014	0.26 (7.0)	0.018
total		0.591		0.658		0.825		0.376
k		1.0059		1.0066		1.0083		1.0038

[a] Values in parentheses represent percent by weight of the component in the lipoprotein and were taken from Fless et al. (1994). [b] Values represent the mean composition of three different LDL samples (Fless et al., 1994).

Apo(*a*) are known (Fless et al., 1994), and there is no knowledge of the structure of its oligosaccharide units, several assumptions had to be made in order to estimate the number of exchangeable hydrogens of the carbohydrate moiety. It was assumed that all N-linked and O-linked oligosaccharide units have sialic acid as the terminal sugar, with the remaining sugars being internal. By assuming that there are no multiply substituted sugars, the number of exchangeable hydrogens would only be slightly higher than the theoretical number. The value of *k* for Apo(*a*) carbohydrate was found to be 1.0165. For ApoB carbohydrate (7% by weight), *k* was 1.0158, as calculated from the major structures of the high-mannose and complex type oligosaccharides found on ApoB by Taniguchi et al. (1989).

In calculating *k* for Lp(*a*) protein, it was assumed that the protein moiety consisted of one molecule of Apo(*a*) and one molecule of ApoB. Similarly, *k* for the Lp(*a*) carbohydrate was estimated from the portions coming from Apo(*a*) and ApoB. Phospholipid *k* values were calculated from the Lp(*a*) and LDL phospholipid compositions determined by Sommer et al. (1992). The *k* values for both Lp(*a*) and LDL shown in Table 1 are substantially less than that for protein alone, because a large part of these lipoproteins, consisting of triglyceride, cholesteryl ester, and phosphatidylcholine, has no exchangeable hydrogens. The parameter *k* for LDL (1.0038) is slightly higher than the one (1.0034) reported by Fisher et al. (1971), probably because our *k* value for ApoB is higher. We calculated *k* from the amino acid sequence of ApoB, which was not available at the time their work was performed (Fisher et al., 1971). The ratio *k* increased with the number of K-IV domains in Apo(*a*) and is shown in Figure 1, together with the mean ratio obtained from three different LDL preparations. It obeys a second-order relationship (given in the legend to Figure 1), from which *k* values for different Lp(*a*) phenotypes can be calculated.

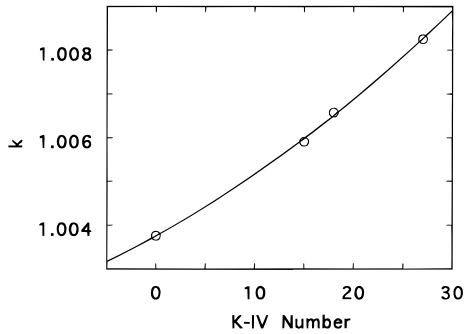

FIGURE 1: Ratio of the molecular weights of Lp(*a*) in deuterated to nondeuterated solvent (*k*) as a function of Apo(*a*) mass expressed in number of K-IV domains. The point at 0 K-IV domains represents LDL. The plot follows a second-order relationship [$y = 1.003\,76 + 1.246 \times 10^{-4} \times x + 1.568 \times 10^{-6} \times x^2$] from which *k* values for different Lp(*a*) phenotypes can be calculated.

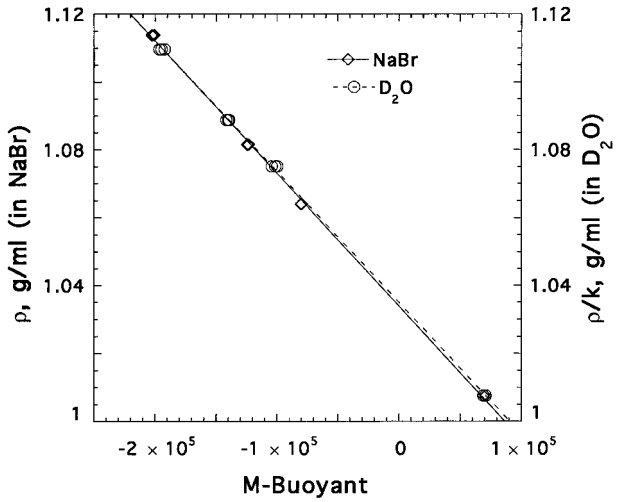

FIGURE 2: Representative plot for the simultaneous determination of the molecular weight and partial specific volume of LDL (donor KB). LDL was dialyzed against four different concentrations each of D_2O and NaBr and subjected in triplicate to either sedimentation or flotation equilibrium. In NaBr, M-Buoyant is equivalent to $M_{app}(1 - \phi'\rho)$ and is plotted against density (ρ), whereas in D_2O, M-Buoyant is equivalent to $(1/k)M(1 - \bar{\nu}\rho)$ and is plotted against ρ/k. The lines represent the least-square fit through the data points. Depending on the solvent, the *y* intercepts yield either $1/\bar{\nu}$ (D_2O) or $1/\phi'$ (NaBr) and the slopes give either $1/(M\bar{\nu})$ (D_2O) or $1/(M_{app}\phi')$ (NaBr).

RESULTS

Four different preparations of LDL were examined by equilibrium centrifugation for differences in their molecular weights as determined in solutions containing varying concentrations of either NaBr or D_2O. Such differences would provide evidence that LDL undergoes preferential binding of either water or the third component, NaBr. A representative molecular weight analysis of one LDL preparation, in which solvent density is plotted as a function of the M-Buoyant value, is shown in Figure 2. In the presence of NaBr, this is a graph of solvent density (ρ) against the term $M(1 - \phi'\rho)$; however, in D_2O, it is ρ/k vs $(1/k)M(1 - \bar{\nu}\rho)$. The slope of the plot in D_2O is equivalent to $1/(M\bar{\nu})$

Table 2: Molecular Weight and Partial Specific Volume of LDL and Lp(*a*) Determined in Varying Concentrations of NaBr and D_2O[a]

	subject	K-IV number	M_{app} with NaBr $\times 10^{-6}$	M with $D_2O \times 10^{-6}$	$M_{NaBr} - M_{D_2O} \times 10^{-5}$	ϕ' w2ith NaBr (mL/g)	$\bar{\nu}$ with D_2O (mL/g)
LDL	KB	0	2.64 ± 0.03	2.59 ± 0.02	0.5 ± 0.5	0.9672 ± 0.0005	0.9663 ± 0.0004
LDL 1[b]	PT	0	3.20 ± 0.02	3.15 ± 0.03	0.5 ± 0.5	0.9759 ± 0.0004	0.9760 ± 0.0005
LDL 2[b]	PT	0	2.49 ± 0.02	2.49 ± 0.04	0 ± 0.6	0.9623 ± 0.0003	0.9619 ± 0.0006
LDL	JJ	0	2.57 ± 0.02	2.55 ± 0.05	0.2 ± 0.7	0.9625 ± 0.0003	0.9623 ± 0.0007
Lp(*a*−)	BK	0	2.86 ± 0.06	2.84 ± 0.03	0.2 ± 0.9	0.9702 ± 0.0008	0.9707 ± 0.0004
Lp(*a*) 1a[c]	KB	15	3.25* ± 0.04	3.12 ± 0.04	1.3 ± 0.8	0.9441** ± 0.0004	0.9423 ± 0.0004
Lp(*a*) 1b[c]	KB	15	3.57*** ± 0.02	3.39 ± 0.03	1.8 ± 0.5	0.9442*** ± 0.0002	0.9419 ± 0.0003
Lp(*a*)	BK	18	3.51*** ± 0.03	3.27 ± 0.02	2.4 ± 0.5	0.9362*** ± 0.0003	0.9328 ± 0.0003
Lp(*a*)	PT	20	3.19*** ± 0.02	2.93 ± 0.03	2.6 ± 0.5	0.9321*** ± 0.0002	0.9279 ± 0.0003
Lp(*a*) 2	KB	27	3.66*** ± 0.04	3.24 ± 0.02	4.2 ± 0.6	0.9257*** ± 0.0006	0.9205 ± 0.0003

[a] Values of LDL, Lp(*a*), and Lp(*a*−) are of triplicate samples determined at four or five different densities and represent the mean ± SEM. Parameters evaluated in NaBr vs D_2O are significantly different at $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***) by a two-tailed Student's *t* test. [b] Two different fractions of LDL from the same preparation. [c] Two different preparations of Lp(*a*) with the same Apo(*a*) polymorph.

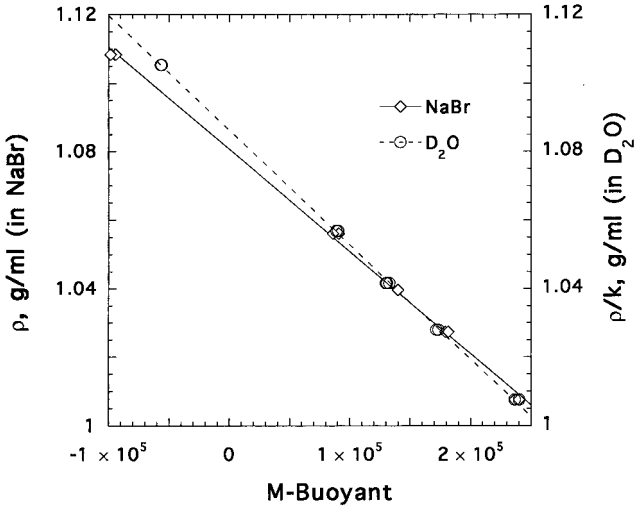

FIGURE 3: Representative plot for the simultaneous determination of the molecular weight and partial specific volume of Lp(*a*) containing a 27 K-IV Apo(*a*) polymorph [Lp(*a*) 2 from donor KB]. Lp(*a*) was dialyzed against five different concentrations each of D_2O and NaBr and subjected in triplicate to either sedimentation or flotation equilibrium. For further details, see the legend to Figure 2.

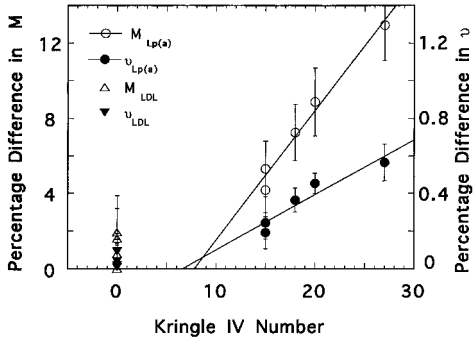

FIGURE 4: Effect of Apo(*a*) mass, expressed in the number of K-IVs, on the difference in the molecular weight or partial specific volume of Lp(*a*) between NaBr and D_2O. Data points at 0 K-IV are those obtained with four different LDL preparations. The *x* intercept for the molecular weight difference is 7.8 ± 1.1 and 6.6 ± 2.2 for the partial specific volume difference.

and *y*-intercept is $1/\bar{\nu}$, whereas in NaBr, ϕ' replaces $\bar{\nu}$ in these expressions. As can be seen in the figure, the least-square lines fitted through the data points obtained in either solvent virtually superimpose and yield, within the experimental error of the method, nearly identical molecular weights and partial specific volumes. The other three LDL preparations yielded similar results which are summarized in Table 2. Overall, molecular weights differed by less than 2% and partial specific volumes by less than 0.09%. These observations confirm the conclusion of Fisher et al. (1971) and Adams and Schumaker (1969a,b) from measurements of the sedimentation and diffusion coefficients that LDL is not subject to preferential hydration in salt solutions.

Although Lp(*a*) is similar in structure to LDL, differing mainly by the presence of the glycoprotein Apo(*a*) which is disulfide linked to ApoB, its behavior in solutions containing NaBr is different from that of LDL. This is evident from an examination of Figure 3, which is a molecular weight and partial specific volume analysis of an Lp(*a*) sample and is analogous to Figure 2. In this case, the least-square lines fitted to the data obtained in either NaBr or D_2O have clearly divergent slopes and different intercepts. The data obtained with five preparations of Lp(*a*) having four different Apo(*a*) polymorphs are summarized in Table 2. Apparent molecular weights of Lp(*a*) determined in NaBr are significantly higher from *M* measured in D_2O. The differences are much higher than those obtained with LDL, ranging from 4.2 to 13.0%. Similarly, the apparent partial specific volumes of Lp(*a*) determined in NaBr are significantly higher (0.19−0.56%) than the ones measured in D_2O.

Upon closer inspection, it appeared that the effect of NaBr on the apparent molecular weight and partial specific volume of Lp(*a*) was directly proportional to the size or number of K-IVs of the Apo(*a*) polymorph. This observation was substantiated when the differences in molecular weight or partial specific volumes of Lp(*a*) between NaBr and D_2O were plotted against K-IV number (see Figure 4). Both parameters exhibited a linear relationship that is evident from the least-square lines fitted to the data points obtained with Lp(*a*). Interestingly, the lines intersect the *x*-axis at 7.8 ± 1.1 and 6.6 ± 2.2 K-IV domains for the molecular weight difference and partial specific volume difference, respectively, and not at the origin or the LDL data points.

We also examined one preparation of Lp(*a*−), which is the remnant lipoprotein particle produced after the reduction of Lp(*a*) and the removal of Apo(*a*) by rate zonal density centrifugation. Because Lp(*a*−) is essentially an LDL particle, it was interesting to determine if it, like LDL, did not preferentially bind water or salt components in the presence of high concentrations of NaBr. The results shown in Table 2 validate this assumption since both molecular weight and partial specific volume determined in either

Table 3: Number of Apo(*a*) Molecules per Lp(*a*) Particle[a]

	D_2O	NaBr
$M_{Lp(a)}$	$(3.27 \pm 0.02) \times 10^6$	$(3.51 \pm 0.03) \times 10^6$
$M_{Lp(a-)}$	$(2.84 \pm 0.03) \times 10^6$	$(2.86 \pm 0.06) \times 10^6$
difference	$(4.30 \pm 0.5) \times 10^5$	$(6.50 \pm 0.9) \times 10^5$
$M_{Apo(a)}$[b]	3.41 ± 0.14	3.41 ± 0.14
number of apo(*a*)s lost from Lp(*a*)	1.26 ± 0.20	1.91 ± 0.34

[a] The Lp(*a*) (BK) analyzed contained an 18 K-IV Apo(*a*) and was the starting material for the preparation of Lp(*a*−). [b] Data taken from Fless et al. (1994).

solvent did not differ significantly from each other. Second, the molecular weight of Lp(*a*−) was of interest because it allows the calculation of the number of Apo(*a*) molecules attached to the Lp(*a*) particle. Since Apo(*a*) is the only component lost from Lp(*a*) upon reduction and centrifugation (Fless et al., 1986), the number of Apo(*a*) molecules can be computed from the difference in mass between Lp(*a*) and Lp(*a*−). As shown in Table 3, the difference in the molecular weight of autologous Lp(*a*) and Lp(*a*−) is equivalent to 1.26 mol of Apo(*a*). This value derived from the Lp(*a*) weight estimates performed in D_2O indicates that there is only one Apo(*a*) molecule per Lp(*a*). In contrast, use of the overestimated molecular weight of Lp(*a*) in the presence of NaBr leads to the erroneous conclusion that there are two molecules of Apo(*a*) per Lp(*a*) particle (see Table 3).

DISCUSSION

The primary purpose of this investigation was to determine whether molecular weights of Lp(*a*) obtained by sedimentation or flotation equilibrium in solutions containing high concentrations of the salt NaBr are equivalent to those measured in D_2O and, if not, to relate this difference to the molar ratio of Apo(*a*) to ApoB in Lp(*a*). The practice of molecular weight determination in high-salt solutions has been the accepted method of choice for LDL but was never tested for its suitability in obtaining the mass of Lp(*a*). Our strategy was to measure this parameter for Lp(*a*) in solutions containing varying concentrations of D_2O and to compare these results with those obtained in NaBr. Because the chemical and physical properties of D_2O are so similar to those of H_2O, H_2O/D_2O solutions are considered one-component systems. Although the D_2O mixtures contained 10 mM phosphate and 100 mM NaBr, which represent the third component, these salt and buffer concentrations are so low that the buffered H_2O/D_2O mixtures with low concentrations of lipoproteins can still be considered two-component systems (Edelstein & Schachman, 1967). Thus, correct molecular weights and partial specific volumes of either Lp(*a*) and LDL can be attained, provided proper adjustments are made to account for deuterium exchange. However, when molecular weight determinations are made in solutions containing high concentrations of NaBr, incorrect apparent molecular weights and partial specific volumes could result if the buoyancy factor $(1 - \bar{\nu}\rho)$ is not corrected for preferentially bound salt and water. In this case, the buoyancy factor is actually an analogous operational factor $(1 - \phi'\rho)$, where the apparent volume ϕ' includes contributions from the lipoprotein and all other bound solvent components (Casassa & Eisenberg, 1964; Reynolds & McCaslin, 1985).

In the case of LDL, values of M and $\bar{\nu}$ obtained from equilibrium runs conducted in D_2O did not differ significantly in magnitude from M_{app} and ϕ' determined in NaBr. This finding confirms the classic studies of Fisher et al. (1971) and Adams and Schumaker (1969a,b), who concluded that LDL was not preferentially hydrated in high-salt solutions and that $\bar{\nu}$ did not have to be corrected for bound solvent components. These authors determined the LDL molecular weight from the sedimentation and diffusion coefficients. We have used sedimentation and flotation equilibrium instead, primarily because both molecular weight and partial specific volume are attained simultaneously. Knowing that Lp(*a*) can undergo substantial conformational changes that could affect both the sedimentation and diffusion coefficients (Fless et al., 1996), it is advantageous to use the equilibrium methods, since they are not influenced by conformational changes. Finally, because of the low speeds employed, it is probably also not affected by pressure effects, which become significant at the higher speeds used in measuring the sedimentation coefficient (Kahlon et al., 1982; Schumaker et al., 1994).

Our results indicate that Lp(*a*), unlike LDL or Lp(*a*−), when present in a multicomponent system containing NaBr, appears to undergo preferential interaction with the solvent components, because values of M and $\bar{\nu}$ are significantly different from M_{app} and ϕ' determined in NaBr (see Table 2 and Figure 4). The magnitude of this effect, as expressed by increases in the value of both M_{app} and ϕ' over M and $\bar{\nu}$, is related to the mass of Apo(*a*) or the number of K-IVs. The lines generated by the percentage differences in either M or $\bar{\nu}$ intersect the abscissa at approximately 7−8 K-IV domains, thereby showing that the interaction of Apo(*a*) structural domains with solvent components is nonuniform. Hence, the variable portion of Apo(*a*), consisting of multiple K-IV_2s, may be more important in preferential hydration and binding of NaBr than the invariable domain of Apo(*a*). Binding of salt components by kringles is not unprecedented; for example, both the unit cell of the K-I_{Pg} (Wu et al., 1994) and the K-II_{tPA} domain (Devos et al., 1992) contained Cl−, an anion important in the activation of plasminogen (Menhart et al., 1995).

The second aim of this study was to determine how any observed differences in molecular weight would affect the molar ratio of Apo(*a*) to ApoB in Lp(*a*). In a previous report, we concluded, from methods that relied heavily on the molecular weight of Lp(*a*) measured in the presence of NaBr, that there were two subunits of Apo(*a*) per Lp(*a*) particle (Fless et al., 1994). One method in particular was based on the difference in the molecular weight between Lp(*a*) and the Apo(*a*)-free Lp(*a*−) particle. Division of this difference by the mass of Apo(*a*) gave an average of two Apo(*a*) molecules lost from Lp(*a*) upon its reduction and carboxymethylation. We repeated this experiment with Lp(*a*) and Lp(*a*−) prepared from the same donor but determined their molecular weights in both D_2O and NaBr. As the results presented in Table 3 show, if the overestimated values obtained in NaBr are employed, one is forced to conclude that 1.91 mol are lost upon the reduction of Lp(*a*). On the other hand, only 1.26 mol of Apo(*a*) are lost when the correct molecular weights determined in D_2O are used. Therefore, for this particular case, the correct molar ratio of Apo(*a*) to Apo(B) is 1:1. This ratio probably applies to other Lp(*a*) particles as well, in light of the substantial Lp(*a*) mass

differences found between the two solvents that range from 4 to 13% for Lp(*a*) with Apo(*a*) polymorphs having from 15 to 27 K-IV domains, respectively. They are equivalent to 54, 70, 69, and 86% of the mass of Apo(*a*) polymorphs having 15, 18, 20, and 27 K-IV domains, respectively. By taking these differences into account, the previously determined molar ratio of 2 would reduce to 1.16−1.46 mol of Apo(*a*) per Lp(*a*) molecule. Thus, our present findings, obtained from physicochemical measurements, are in accord with the evaluation of Lp(*a*) protein amino acid analysis using the amino acid sequences of Apo(*a*) and ApoB (Albers et al., 1996), concluding that there is only one Apo(*a*) attached to ApoB of Lp(*a*). Albers and co-workers proposed that a methodological error in measuring protein mass was a contributing factor that could account for the higher molar ratio. Since molecular weight determination as used in the present study does not require knowledge of the Lp(a) protein concentration, the higher ratio can simply be explained by overestimation of Lp(*a*) weight due to preferential binding and hydration in NaBr. Our results are also in agreement with studies that demonstrated by site-specific mutagenesis that the terminal cysteine of ApoB (Cys 4326) is necessary for the linkage of Apo(*a*) to ApoB (Callow & Rubin, 1995; McCormick et al., 1995). Because mutated ApoB lost its ability to form Lp(*a*) in a transgenic mice model, the authors concluded that only one Apo(*a*) is attached to ApoB.

Finally, our findings are of importance also to the practice of determining the buoyant densities of Lp(*a*) by density gradient centrifugation in NaBr. The results show that partial specific volumes of Lp(*a*) are overestimated in NaBr. Since the reciprocal of the partial specific volume is equivalent to the buoyant density of Lp(*a*), the latter is underestimated in NaBr. For the 15 K-IV Lp(*a*) particle this translates to a density of 1.062 instead of 1.059 g/mL, and for the 27 K-IV Lp(*a*) particle, the density is 1.086 rather than 1.080 g/mL.

In conclusion, we have shown that molecular weight determinations of Lp(*a*) should be carried out in solutions with different densities where the increase in density is achieved with D_2O rather than with NaBr. Unlike LDL, which apparently does not undergo preferential hydration or binding of salt, Lp(*a*) is subject to these interactions in the presence of high concentrations of NaBr. Failure to account for binding of the solvent components in this multicomponent system will lead to overestimated molecular weights and partial specific volumes of Lp(*a*) and underestimated buoyant densities. From mass estimates of Lp(*a*) and Lp(*a*−) conducted in D_2O, we conclude that there is one molecule of Apo(*a*) linked to ApoB of Lp(*a*).

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