MOLECULAR PARAMETERS OF Lp(a)-LIPOPROTEIN BY LIGHT SCATTERING

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1. Introduction

The Lp(a) lipoprotein is thought to be an additional risk factor for coronary heart disease [1–3]. Papers published up to now have been concerned with its electrophoretical mobility [4,5], its chemical composition [6,7] and its physiological role [1–3]. Concerning the size of the particle, only disagreeing information has been reported [8]. Serum concentration of that lipoprotein differs from man to man, and Harvie and Schultz were the first to demonstrate that Lp(a) is expressed by a quantitative genetic trait [9,10]. A diet enriched by cholesterol did not influence the serum Lp(a) level, while LDL increased markedly [11,12]. As pointed out [13,14] Lp(a) reacts very sensitively on addition of Ca^{2+}.

The aim of this work was to provide for this lipoprotein the values for molecular weight ($\bar{M}_w$) diffusion coefficient (D), radius of gyration ($\bar{s}_z$) and hydrodynamic radius (a) by means of both classical and quasielastic light scattering.

2. Materials and methods

2.1. *Isolation of the Lp(a) lipoprotein*

Lp(a) was isolated from pooled plasma of highly Lp(a) positive donors and tested by radial immunodiffusion [7]. To inhibit enzymatic activity associated with Lp(a), EDTA (1 mg/ml) was added to both fresh plasma and all solutions used during the preparation [15]. To prevent contamination by microorganisms, 1 mg/ml NaN_3 and 0.1 mg/ml Chloramphenicol was kept in the buffers used. The VLDL, LDL and the Lp(a) lipoproteins were precipitated by sodium phosphotungstate and $MgCl_2$ as in [16]. After centrifugation, the precipitate was separated, mixed with solid sodium citrate and dialysed against 0.15 mol/l NaCl solution. The density of the lipoprotein solution was adjusted to 1050 g/cm^3 by adding solid NaBr. After spinning in the preparative ultracentrifuge (Beckman L II) at 120 000 × g and 16°C for 20 h, the lower third of the tubes contained most of the Lp(a) lipoprotein, while the LDL and VLDL floated. The material of the lower third was further purified by flotation at 1090 g/cm^3 with 140 000 × g at 16°C for 20 h. Pure Lp(a) was obtained after chromatography of these fractions on a column filled with Biogel A 5 M (200–400 mesh) (Biorad) in 1.6 mol/l NaBr buffered with 0.1 mol/l Tris–HCl (pH 7.5). To test the purity of the lipoprotein fractions, the methods of immunodiffusion [17], immunoelectrophoresis [18], lipid-electrophoresis [19], quantitative immunoelectrophoresis [20] and disc-electrophoresis [21] were used. Before the measurements, the material was checked for aggregates by sedimentation or flotation runs in the analytical ultracentrifuge (Beckman Model E).

2.2. *Preparation of samples*

Before measuring, Lp(a) was dialysed against buffer (Tris–HCl (pH 7.5), 0.1 mol/l, 1.6 mol/l NaBr). The Lp(a) stock solution was centrifuged (Sorvall RC-5, swingout rotor) at 5000 rev./min for 30 min. To eliminate visible dust particles, the buffer was prefiltered twice (Nuclepore polycarbonate filters, 0.6 and 0.2 μm) and centrifuged at 100 000 × g for 10 h (Beckman 60 Ti rotor); the middle fraction of the tube being used. The Rayleigh factor of the cleaned buffer was 2.3×10^{-6} cm^{-1} (Rayleigh factor of water

is 1.10^{-6} cm^{-1}). A smaller value was not obtainable, since due to its high density (1134 ± 0.0015 g/cm^3) the solution could not be clarified completely by centrifuging. With automatic pipettes, stock solution and buffer were brought directly into the light scattering cell, which had been cleaned in chromo-sulphuric acid and made dust free in a distilling device (Dr Dinkelacker and Co, Mainz) under reflux with acetone. For measuring with the Chromatix KMX-6 instrument, samples were prepared in a syringe and filtered directly into the cell (Sartorius cellulose-acetate, 0.2 μm).

2.3. *Light scattering methods*

2.3.1. Classical light scattering

Classical light scattering measurements were performed with a FICA 50 instrument (SOFICA). With that instrument, Rayleigh factors $R(\theta)$ are measured by comparing the intensity of the sample with that of a solvent (benzene) of known Rayleigh factor. The angular range was 30–150° in steps of 15° (automatic mode) or in steps of 5° (manual mode). The wavelength used was 546 nm. Constant temperature of 21 ± 0.5°C was maintained by means of an external thermostat (Haake FE). The refractive index n was measured with a Pulfrich refractometer (Zeiss) and the specific refractive index increment dn/dc was determined by using a differential refractometer (Brice Phoenix, Model BP-2000-V). Results were n = 1.356 and dn/dc = 0.228 ml/g (λ = 546 nm, T = 21 ± 0.5°C).

2.3.2. Quasielastic light scattering

Quasielastic light scattering was measured in a Chromatix KMX-6 laser low-angle light scattering photometer plus digital correlator, model 64. Because of the Brownian motion or diffusion of the scattering macromolecules, the scattered light is Doppler shifted. These spectral shifts permit the determination of the z-average translational diffusion coefficient D of the macromolecules [22,23]. The output of the photomultiplier contains a frequency component corresponding to the Doppler shift in form of a time-averaged intensity. The autocorrelation function $C(t)$ (time domain) of the detector output is the Fourier transform of the power spectrum (frequency domain). It is an exponential function of the general form:

$$C(t) = e^{-t/\tau}$$

with $\tau = 1/2K^2D$ (homodyne experiment)

and $K = (4\pi n/\lambda)\sin(\theta/2)$ [24]

For Lp(a) we obtained $K = 1.124 \times 10^4$ cm^{-1} (scattering angle $\theta = 4.8°$). As rheological investigations suggest, Lp(a) should be strictly spherical [14]. So the measured diffusion coefficient D can be related to the hydrodynamic radius a of the scattering particle by the Stokes-Einstein equation:

$$D = kT/6\pi\eta a$$

The hydrodynamic radius a for spheres is related to the radius of gyration by:

$$\overline{s}_z = a\sqrt{3/5}$$

The evaluation of $C(t)$ was performed on an oscilloscope. 64 channels of the digital correlator yield 64 analog points on the screen of the oscilloscope; 56 points define the exponential function, the last 8 points giving the base line.

3. Results and discussion

3.1. *Classical light scattering*

In the Guinier plot log (R/Kc) versus h^2 ($h = (4\pi n/\lambda)\sin(\theta/2)$), we note an exponential increase at smaller angles (fig.1,2). This can be interpreted by the

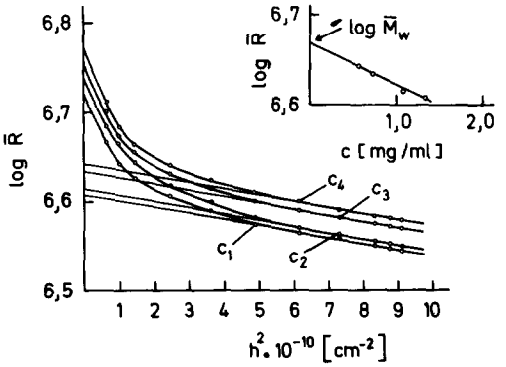

Fig.1. Guinier plot of Lp(a): c_1 = 1.3295 mg/ml; c_2 = 1.0669 mg/ml; c_3 = 0.7246 mg/ml; c_4 = 0.5649 mg/ml.

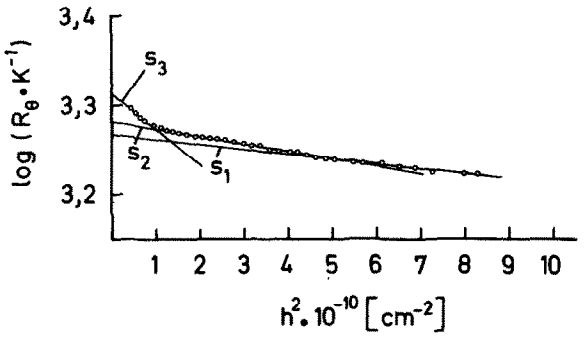

Fig.2. Guinier plot of Lp(a) without knowledge of concentration of the sample. s_1, s_2 and s_3 give the approximation for the slopes.

presence of aggregates, which could falsify both the $\bar{M}_w$ and the $\bar{s}_z$. Therefore, only the linear range (60–150°) was extrapolated to zero angle and zero concentration. In this way $\bar{M}_w$ was obtained from the intercept and $\bar{s}_z$ from the slope. Since $\bar{s}_z$ depends mainly on the refractive index n, it was possible to determine $\bar{s}_z$ from the slope without knowledge of the concentration (fig.2). This is important, since the exact determination of small Lp(a) concentrations has met with great difficulties. Figure 1 and 2 suggest, that ~1–5% of aggregated particles were present in the solutions (steep rise at small angle). On the basis of unpublished measurements on LDL, we may assume a degree of hydration of 5–10% for Lp(a). Therefore, our $\bar{M}_w$ and $\bar{s}_z$ values could be too high by ~6–15%. The final results are listed in table 1. The molecular weight differs somewhat from those obtained by other methods. By electron microscopy $\bar{M}_w$ 5.6 × 10^6 was obtained [25], and $\bar{M}_w$ 4.8 × 10^6, estimated from gel filtration-experiments, and $\bar{M}_w$ 5.2 × 10^6 from sedimentation equilibrium runs, respectively, was reported [8].

Table 1
Results from classical light scattering

$\bar{M}_w \times 10^6$	$\bar{s}_z$ (nm)	
4.66	14.7	(fig.1)
	13.4 (s_1)	
	15.8 (s_2)	(fig.2)
	50.5 (s_3)	

3.2. *Quasielastic light scattering*

Solutions were filled directly into the cell with a syringe equipped with Sartorius celluloseacetate filters 0.2 μm. To check the influence of the concentration, the measured values of $\bar{s}_z$ were plotted against the Rayleigh ratio $R(\theta)$ (logarithmic scale) of the solution, which is proportional to the concentration. A horizontal line was obtained, which gives the z-average of the hydrated radius of gyration $\bar{s}_z$ (fig.3). Our results with several Lp(a) samples are listed in table 2. As one can see, the solutions became unstable

Table 2
Results from quasielastic light scattering

Sample	Decay constant $\tau = 1/2K^2D$ (s)	Diffusion constant $D \times 10^{-8}$ ($cm^2 \cdot s^{-1}$)	Stokes radius a (nm)	Radius of gyration $\bar{s}_z$ (nm)
Fresh solutions				
1	0.0343 ± 0.0035	11.54 ± 1.15	18.4 ± 1.90	14.2 ± 1.4
2	0.0332 ± 0.0020	11.92 ± 0.72	17.8 ± 1.10	13.8 ± 0.8
3	0.0332 ± 0.0020	11.92 ± 0.72	17.8 ± 1.10	13.8 ± 0.8
3 (after 12 h)	0.0332 ± 0.0012	11.92 ± 0.43	17.8 ± 1.10	13.8 ± 0.5
3 (diluted)	0.0330 ± 0.0016	11.99 ± 0.58	17.7 ± 0.85	13.7 ± 0.66
Average:	0.03338 ± 0.002	11.86 ± 0.72	17.9 ± 0.83	13.86 ± 0.83
Aged solutions				
4	0.0403 ± 0.0014	9.82 ± 0.33	21.6 ± 0.73	16.7 ± 0.57
4 (after 16 h)	0.0407 ± 0.0010	9.72 ± 0.23	21.8 ± 0.52	16.9 ± 0.40
4 (after 84 h)	0.0459 ± 0.0018	8.62 ± 0.35	24.6 ± 1.00	19.1 ± 0.76

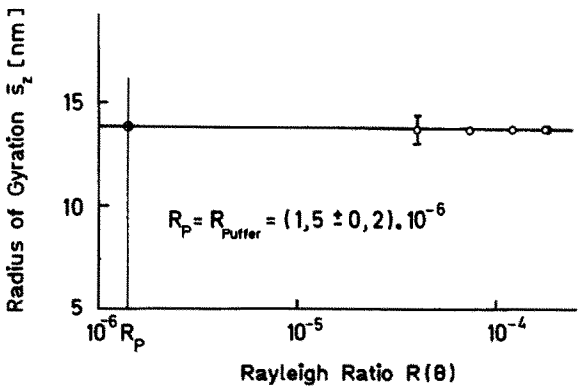

Fig.3. Radius of gyration $\bar{s}_z$ (13.8 nm) in dependence of the Rayleigh ratio.

after 16 days and aggregation followed. Further experiments on size, shape and structure of the aggregates formed in Lp(a) solutions are in progress.

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References

[1] Berg, K., Dahlen, G. and Frick, M. H. (1974) Clin. Genet. 6, 230–235.
[2] Dahlen, G., Berg, K., Gillnäs, T. and Ericson, C. (1975) Clin. Genet. 7, 334–341.
[3] Dahlen, G., Frick, M. H., Berg, K., Valle, M. and Wiljasalo, M. (1975) Clin. Genet. 8, 183–189.
[4] Vogelberg, K. H., Utermann, G. and Gries, F. H. (1973) Z. Klin. Chem. Klin. Biochem. 11, 291–296.
[5] Moulin, S., Fruchart, J. C., Dewailly, P. and Sezille, G. (1979) Clin. Chim. Acta 91, 159–163.
[6] Ehnholm, C., Garoff, H., Renkonen, O. and Simons, K. (1972) Biochem. 11, 3229–3232.
[7] Jürgens, G. and Kostner, G. M. (1975) Immunogenet. 1, 560–569.
[8] Kostner, G. M. (1976) in: Low Density Lipoproteins (Day, C. E. and Levy, R. S. eds) pp. 229–269, Plenum Press, Oxford.
[9] Harvie, N. R. and Schultz, J. S. (1970) Proc. Natl. Acad. Sci. USA 66, 99–103.
[10] Albers, J. J., Wahl, P. and Hazzard, W. R. (1974) Biochem. Genet. 11, 475–486.
[11] Krempler, F., Kostner, G. M., Bolzano, K. and Sandhofer, F. (1978) Atheroscler. 30, 57–65.
[12] Albers, J. J., Adolphson, J. L. and Hazzard, W. R. (1977) J. Lipid Res. 18, 331–338.
[13] Dahlen, G., Ericson, C. and Berg, K. (1978) Clin. Genet. 14, 115–124.
[14] Schurz, J., Niedermayer, H., Jürgens, G., Kostner, G. M. and Holasek, A. (1979) in preparation.
[15] Jürgens, G., Marth, E., Kostner, G. M. and Holasek, A. (1977) Artery 3, 13–26.
[16] Burstein, M. and Morfin, R. (1969) Life Sci. 8, 345–348.
[17] Crowle, A. J. (1961) Immunodiffusion, Academic Press, New York.
[18] Grabar, P. and Burtin, R. (1974) Immunoelectrophoretic Analysis, Elsevier, Amsterdam, New York.
[19] Kostner, G. M. and Holasek, A. (1972) Biochemistry 11, 1217–1223.
[20] Kostner, G. M. and Holasek, A. (1972) Anal. Biochem. 46, 668–683.
[21] Maurer, H. R. (1968) Disk Electrophoresis, Walter de Gruyter Berlin.
[22] Berne, B. J. and Pecora, R. (1976) Dynamic Light Scattering, Wiley Interscience, New York.
[23] Chu, B. (1974) Laser Light Scattering, Academic Press, New York.
[24] Chromatix KMX-6 Application Note LS 8, Chromatix, 560 Oakmead Parkway, Sunnyvale, CA 94086, USA.
[25] Simons, K., Ehnholm, C., Renkonen, O. and Bloth, B. (1970) Acta Pathol. Microbiol. Scand. sect. B 78, 459–466.