FLUORESCENT PROBE ASSAY OF BLOOD PLASMA LIPOPROTEINS

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Assay of blood plasma lipoproteins (LP) is an essential stage in the diagnosis of dyslipoproteinemia, cholestasis, pyelonephritis, and other diseases. After preparative isolation of each of the classes of LP, their quantitative estimation is carried out by chemical methods.

Chemical methods could be replaced by simpler and more sensitive fluorescence methods — for example, the protein content could be determined from its intrinsic fluorescence. However, in atherosclerosis the content and qualitative composition of protein in LP are known to be changed, whereas the relative content of phospholipids changes only negligibly [7, 12, 13]. It has therefore been suggested that the LP concentration be determined on the basis of their phospholipid content [15].

For the quantitative determination of LP the writers have tested the use of the fluorescent probe 1-anilinonaphthalene-8-sulfonate (ANS), the intensity of fluorescence of which rises in the presence of phospholipids [1, 11]. ANS also can interact with LP, mainly, it is considered, with the lipid moiety [4, 5]. The aim was to find the quantitative relations between fluorescence of ANS and concentrations of LP of different classes and to use these data to measure microquantities of LP.

EXPERIMENTAL METHOD

Very low density LP (VLDLP; $d < 1.006$ g/ml), low density LP (LDLP; $1.006 < d < 1.063$ g/ml), and high density LP (HDLP; $d > 1.063$ g/ml) were isolated preparatively by flotation with ultracentrifugation as described by Havel et al. [6]. Human, pig (*Sus domesticus*), Chinchilla rabbit (*Oryctolagus cuniculus*), rat (*Rattus norvegicus*), and guinea pig (*Cavia cobaya*) blood plasma was used for isolation. The purity of the isolated LP preparations was verified by comparing their chemical composition with data given in the literature [3, 6, 8, 10]. For this purpose, total cholesterol and triglycerides were estimated with the AAII Autoanalyzer (Technicon) and protein by Lowry's method [9]. Phospholipids were determined by the method of Swannborg and Svennerholm [14].

Hypercholesteremia was produced in rabbits by feeding them with cholesterol in a dose of 0.2 g/kg body weight daily for 1 week to 4 months.

An aqueous solution of 1 mM ANS (from Serva) was added to a solution of LP in 1 M NaCl and 0.01 M Tris-HCl to a final concentration of 50 μM (optical density of 50 μM ANS in buffer solution at 350 nm is 0.3 cm^{-1}). Fluorescence was measured on the Hitachi MPF-2a spectrofluorometer at 480 nm (excitation wavelength 365 nm) in cylindrical cuvettes 5.5 mm in diameter. The spectral width of the excitation and fluorescence slits was 10 and 4 nm respectively.

The accuracy of measurement of the phospholipid content, calculated by means of the coefficient of variation, was within limits of 3%.

EXPERIMENTAL RESULTS

Fluorescence and excitation spectra of ANS in solution of LP are illustrated in Fig. 1. Dependence of fluorescence of ANS in a solution of LP (F), reduced to the intensity of fluo-

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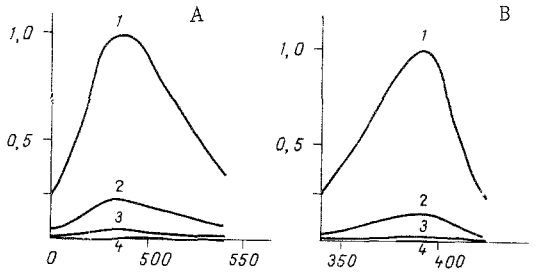

Fig. 1. Fluorescence (A) and fluorescence excitation (B) spectra of ANS in hog LP. 1) HDLP, 2) LDLP, 3) VLDLP, 4) ANS without LP. Phospholipid concentration in each LP $2.25 \cdot 10^{-2}$ mg/ml. Abscissa, wavelength (in nm); ordinate, intensity of fluorescence (in relative units).

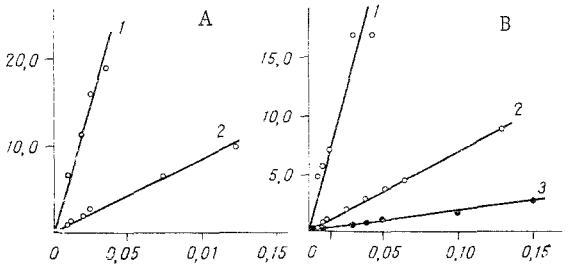

Fig. 2. Changes in intensity of fluorescence of ANS in human (A) and hog (B) LP as a function of phospholipid concentration in LP. 1) HDLP, 2) LDLP, 3) VLDLP. Abscissa, concentration of phospholipids in LP (in mg/ml); ordinate, intensity of fluorescence of ANS in LP, divided by intensity of fluorescence of ANS in 35% ethanol solution.

rescence of 50 μM ANS in a 35% solution of ethanol on the concentration of phospholipids (C) of each class of LP was described by a curve with a linear initial segment:

$$F = R \cdot C,$$

where R is the coefficient of proportionality for an instrument of the same sensitivity.

Each class of LP was characterized by its own value of R. The mean values of R calculated by regression analysis for 5-7 preparations of each type are given in Table 1. They show that LP of the same class, isolated from the blood of different animals, have similar values of R; human and rat HDLP, moreover, have R values 1.4 times greater than HDLP of other animals. HDLP have the highest R values, VLDLP the lowest (Fig. 2).

Values of R obtained for rabbits with experimental hypercholesteremia are given in Table 2. They show that the values of R in the control and experimental groups were virtually the same ($P < 0.001$). To determine the LP concentration in animals with alimentary hypercholesteremia it is thus possible to use the same values of R as were established for normal rabbits.

The coefficient of proportionality R for each class of LP has similar values for different species of animals; however, for the same class of LP and the same species of animal it remains unchanged in experimental atherosclerosis.

The procedure of measuring is simple and can be reduced to addition of the ANS solution to the LP solution, after which the intensity of fluorescence is measured. Its value is stable for 2 h after addition of the probe. The concentration is determined with the aid of a coefficient R, values of which are given in Table 1. Only one reagent (the solution of ANS) is required for the work, and it can be kept in a refrigerator at 4°C for up to 3 months. The method has high sensitivity; to measure the quantity of HDLP only 0.5 ml of solution containing phospholipids in a concentration not exceeding 0.005 mg/ml is required. This is several times less than is needed for chemical methods of phospholipid assay [2, 14].

TABLE 1. Values of Coeffcient of Proportionality (R) for Lipoproteins of Man and Various Animals (M ± m)

Source of lipoproteins	Class of lipoproteins	R
Man	HDLP	485,5±10,4
	LDLP	90,0±7,5
Pig	HDLP	339,5±7,4
	LDLP	68,5±3,3
	VLDLP	16,0±0,2
Guinea pig	HDLP	329,0±13,6
	LDLP	61,6±1,8
Rabbit	HDLP	331,5±5,2
	LDLP	54,8±1,4
Rat	HDLP	426,6±17,4

TABLE 2. Coefficient of Proportionality (R) for Lipoproteins of Rabbits of Control Group and Rabbits with Alimentary Hypercholesteremia (M ± m)

Group of animals	HDLP	LDLP
Control (n = 4)	332,8±7,3	50,7±3,4
Hypercholesteremia	330,2±6,7	61,4±8,1
	(n=5)	(n=3)
P	>0,5	>0,5

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LITERATURE CITED

1. M. D. Barratt, A. Badley, R. B. Leslie, et al., Eur. J. Biochem., 48, 595 (1974).
2. J. R. Bartlett, J. Biol. Chem., 234, 466 (1959).
3. G. D. Calvert, in: Light Density Lipoproteins, New York (1976), p. 281.
4. S. Ghosh, M. Basu, and J. Schweppe, Biochim. Biophys. Acta, 337, 395 (1974).
5. C. J. Hart, R. Leslie, and A. Scanu, Chem. Phys. Lipids, 61, 367 (1970).
6. R. J. Havel, H. A. Eder, and J. H. Bregdon, J. Clin. Invest., 34, 1345 (1955).
7. J. B. Karlin, D. J. Juhn, G. Fless, et al., J. Lipid Res., 19, 197 (1978).
8. K. Kirkley, Scand. J. Clin. Lab. Invest., 18, 437 (1966).
9. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
10. G. L. Mills and C. E. Tanlaur, Comp. Biochem. Physiol., 40-B, 489 (1971).
11. G. K. Radda and J. Vanderkooi, Biochem. Biophys. Acta, 265, 509 (1972).
12. E. J. Schaefer, S. Eisenberg, and R. I. Levy, J. Lipid Res., 19, 667 (1978).
13. G. Schonfeld, A. Bailey, and R. Steelman, Lipids, 13, 951 (1978).
14. A. Svannborg and M. Svennerholm, Acta Med. Scand., 25, 72 (1964).
15. P. Weisweiler and R. Schwandt, Atherosclerosis, 31, 53 (1978).