Charge Heterogeneity of Human Low Density Lipoprotein (LDL) (37232)

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Hyperlipoproteinemia in man is characterized by the elevation of one or more of the serum lipoproteins. Fredrickson, Levy and Lees have proposed a classification (1) of human hyperlipoproteinemia into five types depending on the particular lipoprotein elevated. It is not known if any of these types of hyperlipoproteinemia is associated with a lipoprotein that is qualitatively different from the normal. However, Slack and Mills (2) have recently shown that the low density lipoprotein (LDL) of type II has an abnormal flotation coefficient S_f. Differences in the electrophoretic mobility of the pre-β-lipoprotein between the different phenotypes have also been sometimes noted (3–4). However, these differences in mobility have not been studied in detail, probably because of difficulty in the interpretation of gel electrophoretic data.

These difficulties have been resolved and we have recently reported (5) how agarose gel electrophoretic data for a protein may be used to calculate its true mobility, isoelectric point (IEP) and molecular size. As a further application of this technique, the present work reports a systematic investigation of the electrophoretic mobility of LDL isolated from the serum of different individuals. Agarose gel electrophoresis has been used to study the isoelectric point and the variation of surface charge with a change in the concentration of NaCl or $CaCl_2$, for LDL isolated from several normolipemic students, South African Bantus and from several hyperlipemic patients with history of atherosclerotic vascular disease.

Materials and Methods. EDTA plasma was obtained from fasting (16 hr) subjects and stored with thimerosal at refrigerator temperature $\sim 4°$. Plasma samples of the Bantus were supplied by Dr. A. R. P. Walker of Johannesburg, South Africa and were flown in refrigerated. Plasma samples of patients were obtained mainly from the Northwestern Memorial Hospital and St. Joseph Hospital in Chicago. The following chemical determinations were made on aliquots of the plasma: total and esterified cholesterol, triglyceride (TG) and phospholipid. Agarose gel electrophoresis of serum was performed to aid in the phenotyping of the patients.

Isolation of LDL. The low density lipoprotein (LDL) was prepared from the plasma samples by ultracentrifugation. The method employed was similar to one described by Hatch and Lees (6). A Spinco No. 65 rotor was used with 13 ml ultracentrifuge tubes. The details of the separation procedure using this rotor have been given elsewhere (5). The samples of LDL obtained by ultracentrifugation were dialyzed to remove excess bromides and chlorides. The dialyzed samples were stored with drops of EDTA and thimerosal as recommended by Hatch and Lees (6). While the electrophoretic mobilities would not be affected by traces of other proteins, all the samples were tested for purity by immunoelectrophoresis and/or by Coomassie blue stain after electrophoresis. All samples tested gave single bands in both the tests.

Electrophoresis. For the electrophoretic determination of IEP of purified LDL, 0.6% agarose was used as the supporting medium. The details of this procedure have been published previously (5). In the present work, in order to investigate the effects of different concentrations of NaCl and $CaCl_2$ on the electrophoretic mobility at pH 6.8, a stock Tris buffer (0.005 M) was used and its ionic strength was adjusted by adding NaCl or $CaCl_2$. The agarose gel was prepared in the

experimental buffer. From the experimental data, the surface potential (ζ_{mv}) was calculated using Smoluchowski equation after Henry correction; the ionic charge density (σ, esu/cm^2) was calculated using the relation valid for small particles (7).

The electrophoretic titration curve of LDL (5) shows the mobility to be fairly linear with pH between pH values 4.5 and 6.8; the IEP of the different samples was determined by interpolation of the mobilities at these two values of pH. The values of surface potential and IEP obtained from electrophoresis in 0.6% agarose, are only slightly different from the values obtained by the extrapolation method described previously (5); for the sake of comparison between a number of samples, the electrophoretic data obtained in 0.6% gel were used for the calculations in this paper.

Results. Figure 1 shows the range of values of the surface potential at pH 6.8, as well as the isoelectric point of LDL of the various groups obtained by electrophoresis in 0.6% agarose gel. The isoelectric point seemed to be fairly constant within the groups of students and Bantus. However, the values of LDL of hyperlipidemic patients taken as one group ranged from 4.7 to 5.6. It was initially observed (8) that the IEP was relatively high for LDL of individuals with very high cholesterol and β-lipoprotein concentrations in serum. This suggested that this group may be of type II in Fredrickson-Levy-Lees classification. The isoelectric point of LDL obtained from type II patients was then compared in Table I with that of LDL obtained from the two groups of controls, students and Bantus.

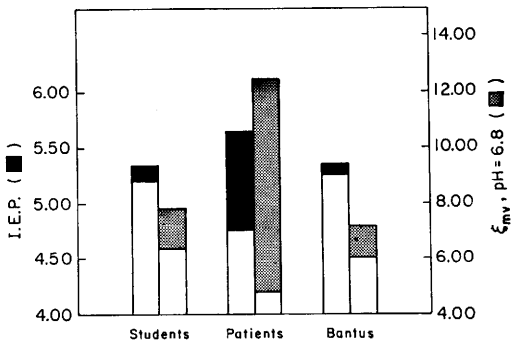

FIG. 1. The range (shaded area) of surface potential (ζ_{mv}) at pH 6.8 and IEP determined at ionic strength 0.05 for LDL obtained from normolipidemic U.S. whites (students) and hyperlipidemic U.S. whites (patients) and from normolipidemic South African Bantus.

TABLE I. Values (Means ± SD) for Serum Lipids and Isoelectric Point (IEP) of LDL Isolated from Normolipidemic U.S. White Students (Group 1), Hypercholesteremic-Type II U.S. Whites (Group 2) and Normolipidemic South African Bantus (Group 3).

Group	Total cholesterol (mg/100 ml)	TG (mg/100 ml)	IEP[a]
1	192 ± 34	71 ± 37	5.28 ± 0.05
2	410 ± 115	231 ± 141	5.45 ± 0.11
3	111 ± 25	58 ± 22	5.28 ± 0.025

[a] $p < .002$ for the difference between Groups 1 and 2: and $p < .002$ for the difference between Groups 2 and 3.

The effect of varying concentrations of NaCl or $CaCl_2$ at pH 6.8, was studied in several samples of LDL (type II as well as controls). The surface charge calculated from electrophoretic data obtained at different concentrations of NaCl has been plotted in Fig. 2 against the square root of the ionic strength. The values of surface potential of LDL obtained at different concentrations of $CaCl_2$ have been plotted in Fig. 3.

Discussion. It has been shown that the apoprotein of LDL of normolipidemic persons is not different from the apo-protein of LDL obtained from hyperlipidemic persons (9). It has been assumed in general that the physicochemical properties of LDL do not vary. However, the range of the values obtained in this work for the IEP (Fig. 1) of LDL of the patients, is much larger than can be explained on the basis of experimental error, and shows the existence of charge heterogeneity among LDL of different individuals in this group. The patient population represented in Fig. 1 contained different Fredrickson types. If one considers only the type II patients in our experimental group and compares the IEP of their LDL with that of the LDL of controls (Table I) a significant difference ($p < 0.002$) is observed between the two. This shows that indeed the LDL from type II has different surface charge characteristics than the control samples.

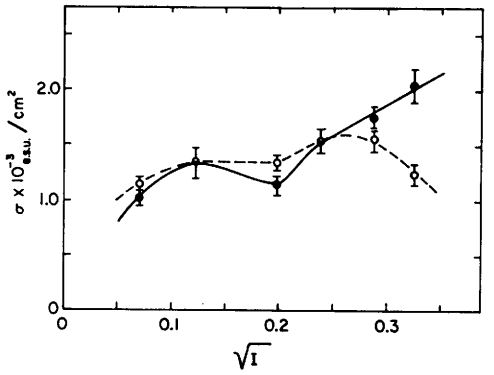

FIG. 2. Plot of surface charge densities of LDL against the square root of the ionic strength I, at pH 6.8. Normolipidemic students (○) and hypercholesteremic type II patients (●). The bar represents standard deviation.

The number of patients of types III, IV, and V in our experimental group was too small to calculate any mean value for these types; however, the LDL of these patients, along with two who are borderline between IIB and IV, had IEP lower than the control groups and mostly below 5.2. The average TG/cholesterol ratio for this group was higher than for the type II patients.

At the initial stage of this work, the mobility of β-lipoprotein was determined using whole serum and the values of mobility determined this way were in the same general order as those determined with isolated LDL. The mobility at pH 4.5 using serum agreed reasonably well with the mobility of the separated lipoprotein. These evidences, apart from the precautionary measures taken to avoid deterioration and oxidation, show that the differences are real and not produced later on during preparation.

Further studies would, however, be necessary to establish if a phenotype is characterized by a particular IEP for the LDL, or whether the differences in the IEP observed in this work are due to some other parameters like free fatty acid concentration, and its partitioning between LDL and albumin in serum.

Haydon (10) has pointed out that the variation of surface charge with ionic strength may give valuable information about the disposition of charged groups on the surface. It may be observed from Figure 2 that the σ is not significantly different at ionic strength ~0.01, for LDL of students and hypercholesteremic patients, whereas the difference is significant ($p < 0.002$) at ionic strength ~0.1. This suggests the possibility of a difference in the relative orientation of the charged groups rather than in their number. Burnett and Bull (11) have produced evidence of the existence of hidden charged groups in proteins. In the present case, however, in the absence of detailed analyses of the LDL lipid, small differences in the lipid composition, especially of the phospholipids or of free fatty acids, cannot be ruled out.

Figure 3 shows that Ca^{2+} has a drastic effect on the surface charge of LDL. Under the conditions of the experiment, bovine serum albumin did not show any change in the charge, but the lipoproteins become positively charged even at pH 6.8. Phospholipids have high affinity for Ca^{2+} and the existence of exposed phospholipid groups on cell membranes has sometimes been inferred from the effect of Ca^{2+} on the electrophoretic mobility of the cells (12). The charge reversal of LDL may be related to the binding of Ca^{2+} ions by the exposed phospholipids. The charge reversal concentrations of Ca^{2+} for the LDL of type II, students and Bantus are, respectively, 0.015 ± 0.001 M, 0.021 ± 0.003 M, 0.021 ± 0.003 M with a $p < 0.002$

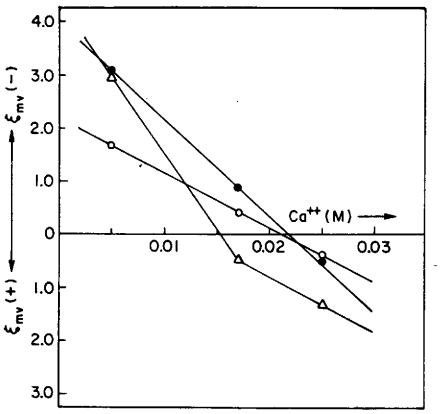

FIG. 3. The variation of surface potential with the concentration of $CaCl_2$, at pH 6.8 for LDL isolated from three different groups. Normolipidemic U.S. whites (○), hypercholesteremic U.S. type II patients (△) and normolipidemic South African Bantus (●).

for the difference between the type II and the controls (Fig. 3). They are in the order of the net charge of the LDL at the lower ionic strength (*e.g.*, 0.05). A detailed analysis of these data together with information on the extent and affinity of Ca^{2+} binding by lipoproteins would give important information about the role of phospholipids at the surface of the lipoprotein molecules.

The above evidences show the existence of significant differences in the surface charge characteristics of the LDL isolated from different groups of human subjects. Several workers (13, 14) have suggested that electrical charge of the lipoprotein may be important in its interaction with the constituents of the arterial wall, in particular with the acid-mucopolysaccharides. Such interactions have been suggested to be the primary event in atherogenesis. The present work suggests that because of the charge heterogeneity, the LDL of different individuals may react differently in the arterial wall. The surface charge heterogeneity should be considered in dealing with the problem of LDL interaction with polyanions or with the arterial wall.

Summary. Plasma samples were obtained from patients with known history of atherosclerosis as well as from a group of Bantus of South Africa and a group of medical students without any history of atherosclerotic vascular disease. The isoelectric point (IEP) of LDL of the different persons was determined by electrophoresis in agarose gel at different pH values. The IEP of the LDL of the students or of the Bantus, were fairly constant at pH 5.28; for the patients the IEP varied widely. The IEP of LDL from hypercholesteremic persons (Fredrickson type II) showed consistently a significantly higher IEP than for the controls. Electrophoretic mobility of the different samples of LDL was determined at pH 6.8 at different ionic strengths using NaCl or $CaCl_2$. The effect of ionic strength (using NaCl) on the mobility was different for LDL isolated from different persons. Low concentrations of $CaCl_2$ reverse the charge of lipoprotein from a negative to a positive one at pH 6.8. The drastic effect of Ca^{2+} on the net charge of LDL at pH 6.8, possibly due to the exposed phospholipid groups of LDL, shows that this ion may be important in the interaction between the lipoprotein and the mucopolysaccharides; such an interaction has been suggested by other workers as occurring in the arterial wall.

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