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A Comparative Dielectric Study of Human Serum Low Density Lipoprotein Before and After Partial Digestion by Trypsin

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The relative permittivity of aqueous solutions of human serum low density lipoprotein (LDL) and partially trypsin digested lipoprotein (T-LDL) has been determined for various concentrations at 20°C over the frequency range 0.15–100 MHz. Comparison of the dielectric dispersion curves for the digested lipoprotein with those for the native preparation revealed a larger low-frequency dielectric increment, which may be attributed to an increase in the number of counterions moving over the surface of the molecule. An explanation of this observation is an elevation of 70% in the net negative charge on the surface of the trypsin-treated particle as compared to its native counterpart.

Key words: lipoprotein, trypsin, dielectric measurements, counterions, α-dispersion

Although dielectric methods have been in use for studying biological molecules for over 40 years, it is only recently that these have been applied to natural lipoproteins [1, 2], phospholipid vesicles [3], and similar systems [4, 5]. The structure of human serum low-density lipoproteins (LDL) has been of considerable interest in recent years [6, 7] and still remains a matter of conjecture [8–10]. The detailed arrangement of the molecular constituents remains unresolved, and in particular there is controversy regarding the location of the protein moiety. One classical [11] technique for studying the structure of proteins concerns their specific breakdown into smaller peptides, the use of proteolytic enzymes of high substrate specificity being a good method of achieving this [12–13]. This approach may also be employed to investigate the structure of more complex macromolecules such as serum lipoproteins [14, 15].

Limited tryptic treatment of human serum LDL permits the removal of 20–25% of its total protein, which is liberated in the form of low molecular weight peptides [15]. Such trypsin-accessible protein is specifically removed from surface-exposed regions of the lipoprotein particle. Moreover, the internal structure of the particle is essentially un-

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altered, as determined by small-angle X-ray scattering studies [16]. Application of dielectric techniques to the study of native and trypsin-treated LDL may provide information on the nature of the surface charge resulting from trypsin treatment. Dieletric studies may also be used to reveal whether any changes in the internal organization occur [2]. The protein-deficient LDL – ie, T-LDL – may, after purification [15], serve as a model for evaluation of the contribution of the surface protein to the structure of this macromolecular complex.

The objectives of the present study were therefore to obtain dielectric dispersion curves for T-LDL and to compare them with those obtained from the native preparation.

MATERIALS AND METHODS

Isolation of LDL

The isolation of human serum LDL (density 1.024–1.045 g/ml) was carried out as described prevously [17]. The preparations were shown to be homogeneous and of high purity by the techniques of negative-stain electron microscopy, agarose gel electrophoresis, and immunological methods [18]. The final LDL preparation was exhaustively dialysed at 4°C against a solution containing 50 mM NaCl, 0.04% EDTA, 0.02% sodium azide, and 0.005% merthiolate (pH 7.6).

Preparation of T-LDL

The T-LDL preparations were prepared as follows [15]. LDL was first centrifuged (2,000 g for 10 min at 5–8°C) to remove any denatured material and then concentrated by ultrafiltration (Amicon, Lexington, MA) on a Diaflo XM50 ultrafilter under nitrogen to give a final protein content of 10–18 mg protein/ml. Tryptic treatment (TPCK-Trypsin, Worthington; protein:trypsin ratio 50:1 w/w) was then performed under carefully controlled conditions for 5 h at 37°C in 50 mM Tris buffer (pH 7.6). The degree of proteolysis was determined as the amount of acid-soluble protein after precipitation of a small portion (250 μg) of the digest in 5% trichloroacetic acid. Gel filtration chromatography was performed on a Sephadex G-75 column equilibrated with the eluting buffer containing 25 mM Tris, 0.02% sodium azide, 0.01% EDTA and 0.001% merthiolate (pH 7.6); this column permitted separation of the T-LDL fraction from trypsin and the liberated low molecular weight peptides. When LDL and T-LDL preparations were required with protein content in the range 20–100 mg/ml they were concentrated by ultrafiltration as outlined above.

Chemical, Physical, and Immunological Studies

Comparison of T-LDL with the native preparation revealed a loss of 20–25% of the total protein, an increase in the net negative charge on the particle surface, and a greater heterogeneity in particle size. The higher peak s_f rate observed for T-LDL (range 6.9–8.9 S for 6 preparations) was consistent with the lower hydrated density (range 1.022–1.027 g/ml for 5 preparations) of the lipoprotein after tryptic treatment. For native LDL, the peak s_f rate ranged from 5.6 to 8.5 S (6 preparations) and the hydrated density from 1.023 to 1.033 g/ml (4 preparations). A partial immunological identity was established between LDL and the corresponding trypsin-treated preparations. By SDS-polyacrylamide gel elecrophoresis, the protein moiety of T-LDL was distinct from that of its native counterpart in lacking high mol wt ($>$ 250,000) protein and displaying a series of polypeptide bands ranging from 165,000 to 12,000. No loss of lipids was detected upon tryptic treatment. The peptides liberated from LDL were enriched in basic amino acid residues ($\sim$ 20% as compared to $\sim$ 14% in the native particle).

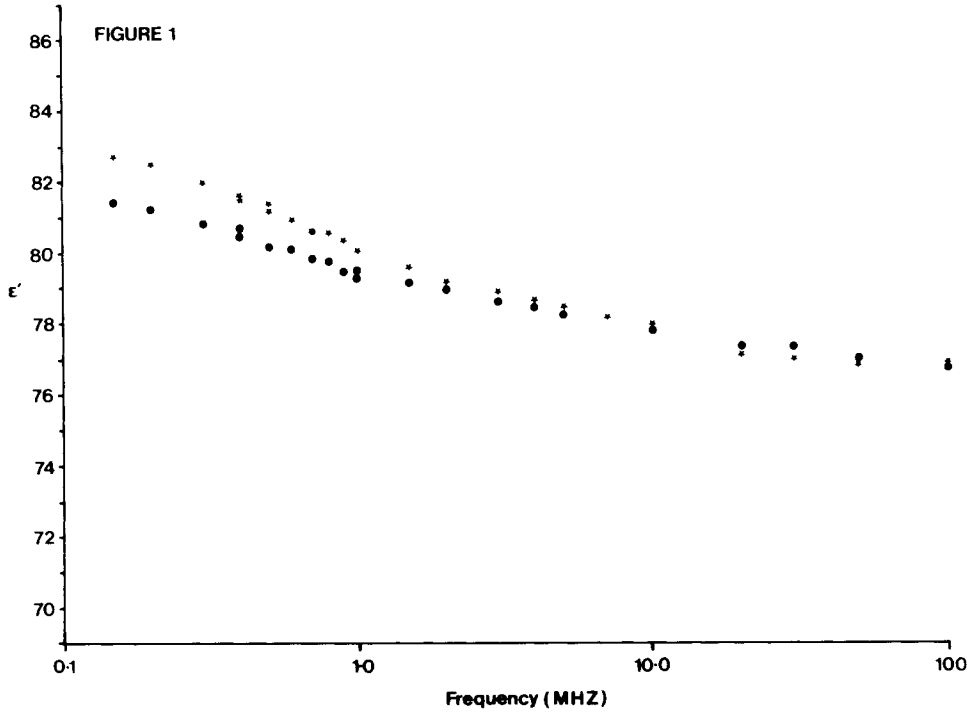

Fig. 1. Variation with frequency of the electrical permittivity of human serum LDL (●) and T-LDL (*) in aqueous solutions. (Concentrations 31.9 and 32 mg/ml, respectively; temperature, 20°C; pH, 7.6).

Dielectric Measurements

Prior to measurement native and T-LDL preparations were dialysed at 4°C against a solution of low ionic strength as previously outlined [2]. The relative permittivity ϵ' of the solutions was determined at various concentrations over the frequency range 0.15–100 MHz using AC bridge techniques described previously [19–21]. For each sample, the permittivity was measured at a minimum of 20 frequencies in the above range.

RESULTS

Previous dielectric measurements on LDL [2] had shown the existence of two dispersion regions in the frequency range of measurement: a low frequency, or α-dispersion, centered around 0.5 MHz and a higher frequency, or β-dispersion, centered around 5 MHz. The present data, typical examples of which are shown in Figure 1, were therefore represented mathematically by the sum of two Debye dispersion regions, ie,

$$\epsilon'(\omega) = \frac{\Delta\alpha}{1 + \omega^2\tau_\alpha^2} + \frac{\Delta\beta}{1 + \omega^2\tau_\beta^2} + \epsilon_\infty \qquad (1)$$

where Δ = the dielectric increment
T = the relaxation time
ω = the angular frequency
ϵ_∞ = the value of the high-frequency plateau

and the subscripts apply to the appropriate dispersion. The permittivity data were analysed with the aid of a computer using a least-squares minimization technique [22].

TABLE I. Values for the Relaxation Frequencies of LDL and T-LDL in Aqueous Solutions

Sample	f_α (MHz)	f_β (MHz)
LDL	0.42 ± 0.06	3.9 ± 0.5
T-LDL	0.41 ± 0.05	3.8 ± 0.4

The four parameters resulting from the analysis of the permittivity data are the two relaxation frequencies ($f_i = (2\pi\tau_i)^{-1}$) f_α and f_β and the two dielectric increments, $\Delta\alpha$ and $\Delta\beta$. The values for f_α and f_β of T-LDL were found to agree with the 95% confidence intervals with those obtained for LDL (Table I).

The variation of the increment of the α dispersion ($\Delta\alpha$) is shown as a function of concentration for both LDL and T-LDL in Figure 2. It can be seen that, although the two curves exhibit a similar shape, there are detailed differences.

Both curves show an initial linear dependence of $\Delta\alpha$ with concentration up to a certain critical solute concentration. At higher concentrates, there is an abrupt change, and $\Delta\alpha$ attains a constant value resulting in a plateau region. However, it appears from Figure 2 that T-LDL exhibits a lower critical solute concentration than LDL, with respective values of approximately 25 mg/ml compared with 30 mg/ml. The plateau height is also different, being about 4 for T-LDL and about 3 for LDL. This unusal form of variation of $\Delta\alpha$ with concentration had been observed previously [2] for LDL.

The variation of $\Delta\beta$ with concentration was similar for both T-LDL and LDL samples and showed a linear dependence with concentration as predicted by Pauly-Schwan equation [23].

DISCUSSION

The origin of the α-dispersion had been attributed [2] to counterion relaxation [19] on the particle surface. The higher value of $\Delta\alpha$ obtained for the T-LDL would therefore be consistent with an increase in the net negative charge on the particle surface. This elevation in negative charge was suggested previously on the basis of immunoelectrophoretic studies [15]. The present investigations show that the value of the charge on a particle surface calculated from the dielectric data is 2.5×10^{-18}C for T-LDL compared with 1.5×10^{-18}C for LDL. Assuming no differences between the particle diameters of LDL and T-LDL, the corresponding surface charge densities are 0.9×10^{-3} and 1.5×10^{-3} Cm^{-2} respectively.

The origin of the β-dispersion has been attributed [2] to a Maxwell-Wagner type of mechanism [23] and is therefore due in general to the fact that the interior and superficial regions of the LDL particle are of differing permittivity and conductivity. The values of $\Delta\beta$ and f_β are particularly sensitive to the dielectric properties of the central region of the particle. The fact that the dielectric behaviour of the β-dispersion for T-LDL did not differ from that for LDL is in agreement with conclusions derived from small-angle X-ray scattering studies [16]; namely, that the interior of the particle appears unaltered by tryptic treatment. The present dielectric work does not, however, permit any conclusions regarding the core structure over and above to those proposed previously [2]. In conclusion our data clearly indicate that the effect of trypsin on the LDL particle is essentially localized to its surface, where it is manifested as a markedly increased (70%) net negative charge resulting from the preferential removal of basic amino acid residues.

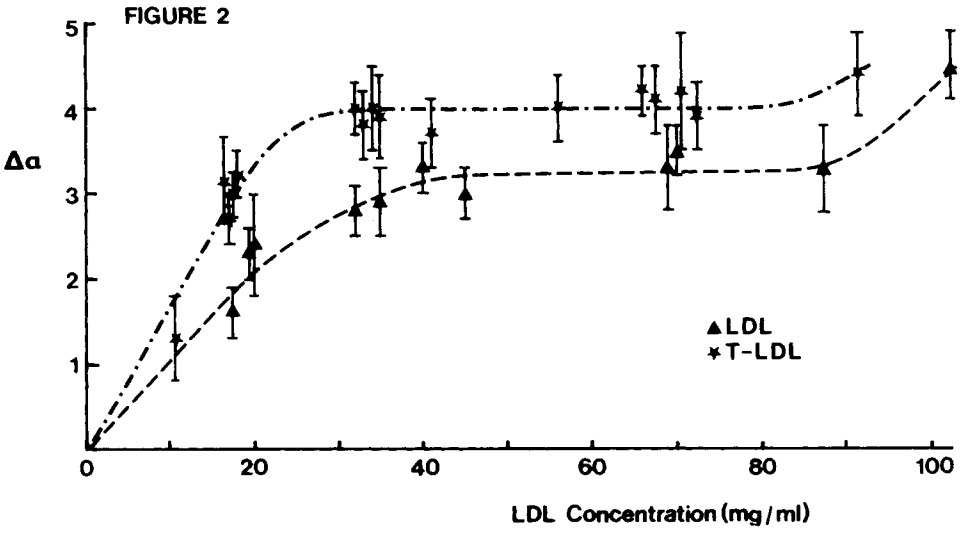

Fig. 2. Variaton of the dielectric increment of the α-dispersion with concentration (temperature, 20°C; pH, 7.6).

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