The Surface Charge of Apolipoproteins, Phospholipid Liposomes, and Human Very Low Density Lipoproteins*

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The surface charge of isolated apolipoprotein A-I, apo-A-II, apo-C-II, and apo-C-IIIz as well as of lipoproteins from synthetic highly purified phospholipids and isolated very low density lipoprotein (VLDL) particles from different donors (n = 35) was determined by polyelectrolyte titration. The particle size of apolipoproteins was evaluated from their molecular weight and specific volume, while that of VLDL particles was determined by photon correlation spectroscopy. The surface charge density of apolipoproteins and of VLDL at pH 7 was calculated from the number of surface charges of the particle and the specific area. The experimental net charge versus pH curves for apo-A-I and apo-A-II are very similar to the theoretical data. The differences between experimental and calculated results for apo-C-II and apo-C-IIIz are believed to result from "polyelectrolyte" effects of neighboring charged centers within the protein molecule causing a decrease of dissociation of carboxylic residues. Neutral phospholipid liposomes do not exhibit anionic or cationic properties between pH 3 and 9. Liposomes from anionic phospholipids behave similar to polymeric carboxylic acids, i.e., their degree of dissociation increases with pH. The number of surface charges of VLDL particles increases with the particle size, while their surface charge density is about 1.10 ± 0.36 charges/nm² of the surface area.

A variety of biochemical studies have shown that the chemical composition of VLDL changes during lipolytic catabolism in vivo and in vitro (1–5). The relative composition both of lipids and of apolipoproteins is altered with a simultaneous decrease of particle size (6, 7). The loss of apolipoproteins during lipolysis of VLDL results in the final formation of low density lipoproteins (8–10) and of HDL (10, 69). By contrast, apolipoproteins are transferred from HDL to nascent VLDL (11). After intravenous administration of a lipid emulsion, a transfer of apolipoproteins to the chylomicron-like particles is observed (12, 13).

From a biochemical point of view, until now no clear answer has been presented to explain the observed transfer phenomena. The compositional changes of VLDL during lipolysis have not yet been investigated with respect to changes of the colloid chemical properties. These properties, however, are fundamental for the stability of composite lipid-apolipoprotein particles since they determine the tendency for aggregation and adhesion.

From a physicochemical point of view, the stability of a colloidal system is governed by steric and electrostatic factors. It was the purpose of this investigation to study electrostatic properties of isolated apolipoproteins and individual lipoproteins. These observations should be related to changes of lipoprotein particles during lipolysis. The study concentrated on VLDL and excluded investigations on breakdown products of this lipoprotein class.

EXPERIMENTAL PROCEDURES

Chemicals and Biochemicals—MGC (M = 17,000) and KPVS (M = 320,000) were purchased from ICN K & K Laboratories Inc., Plainview, NY. o-Tb was ordered from Fluka, New Ulm, Federal Republic of Germany. Polyethyleneimine (Polymin G 500, Mr = 25,000) was provided by Badische Anilin und Soda Fabrik AG, Ludwigshafen/Rhein, Federal Republic of Germany. Phospholipids PC, PE, PS, PI, LPC, and sphingomyelin were ordered from Roth GmbH, Karlsruhe, Federal Republic of Germany. The phospholipids were extracted with hexane/ether (2:1) (v/v) to remove traces of fatty acids. The purity of each phospholipid fraction was assessed by thin layer chromatography on silica gel plates as described by Skipsey et al. (14). The absence of free fatty acids was controlled by gas liquid chromatography according to Wirth et al. (15). The other reagents were used without further purification.

Isolation of Human Serum Lipoproteins—Lipoproteins were isolated from serum obtained from subjects with a primary disorder in lipoprotein metabolism (types IIa, IIb, IV, and V) according to Friedrickson et al. (16) 14 h after the last food intake. The serum was fractionated by discontinuous gradient ultracentrifugation according to Havel et al. (17) (Beckman ultracentrifuge L-2; 40.3 rotor and 50 Ti rotor; 4°C; t = 20 h for VLDL at a density of 1.006 kg/liter and 48 h for HDL at a density of 1.063–1.21 kg/liter). The isolated lipoprotein fractions were recentrifuged at the same densities to remove traces of albumin. The purity of VLDL was investigated by agarose electrophoresis (18) and immunologically with the Ouchterlony technique.

Isolation of Apolipoproteins—The isolated VLDL and HDL were lyophilized and delipidated with ether/ethanol (1:2) (v/v).

Apo A-I and Apo-A-II were fractionated from delipidated HDL by gel chromatography on Sephadex G-200 in 0.1 M guanidine chloride and subsequently by DEAE-cellulose chromatography in 6 M urea as previously described (19). Apo C-IIIz and Apo C-II were isolated from VLDL by a modification of the method of Herbert et al. (20) Gel filtration chromatogra-
of delipidated VLDL apolipoproteins was conducted on Sephacryl S-200 (Pharmacia, Freiburg, Federal Republic of Germany) in 6 M urea, 0.1 M Tris-HCl at pH 8.4. Ion exchange chromatography on DEAE-cellulose was conducted in 6 M urea with a linear NaCl gradient from 0.01 to 0.2 M. The apo-C peptides were further purified by preparative isoelectric focusing in 4% Ultraloe gels (LKB, Munchen, Federal Republic of Germany), 6 M urea, and 1.8% ampholyte (Serva, Heidelberg, Federal Republic of Germany) at pH 4–5. The high purity of the isolated apolipoproteins was confirmed by sodium dodecyl sulfate-acrylamide gel electrophoresis (21), isoelectric focusing (22, 23), and immunoelectrophoresis for the absence of other apolipoproteins.

Chemical and Immunochemical Analysis—Triacylglycerol and choles- terol in VLDL were chemically determined according to the procedure of the lipid research clinics (24). Phospholipids were measured after extraction with methanol/chloroform (1:2) (v/v) according to the procedure of Bartlett (25). Protein measurements of isolated apo- lipoproteins were conducted gravimetrically. Protein in VLDL was measured according to Bradford with the Bio-Rad assay (26).

The particle size of isolated apolipoproteins was evaluated from their molecular weight and their previously published specific volumes at a hydration of 0.34 g of water/g of protein (37). Their surface charge density at pH 7 was obtained from the net charge divided by the surface area. The amount of isolated lipoprotein in the solution was evaluated from the sum of the lipid and protein moieties. Using a specific volume of 1.000 kg/liter and a specific surface as determined by photon correlation spectroscopy (square meter/cm³) (listed in Table II), the surface charge density was evaluated from the amount of the polyelectrolyte charge neutralized by the lipidprotein particles divided by the specific surface area. The average number of charged centers/particle was determined from the product of surface charge density and the surface area ( = d, d = equivalent diameter of monodisperse particles with the same specific surface area as obtained for the polydisperse system from autocorrelation spectroscopy) multiplied by Avogadro’s number. The Student’s t test was applied for statistical analysis.

Theoretical Calculations—The calculation of the net surface charge of given proteins, phospholipid, or lipoproteins was evaluated from the difference of the total number of charged residues of polyelectrolyte used for preincubation and the back-titratable number of charges in the presence of the analyte. The calculations were based on the assumption that KPVS is completely dissociated above pH 2 and that complex formation between oppositely charged polyelectrolytes occurs through a 1:1 ion pair complex formation (34–36).

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**RESULTS**

Surface Charge Distribution of Apolipoproteins—The amino acid composition of the investigated proteins has been previously determined (Table I). Based on this analysis the calculated surface charge is compared with the experimental data obtained from polyelectrolyte titration (Fig. 1). For apo-A-I and apo-A-II, the curves almost coincide at pH values below the isoelectric point. Even above the isoelectric point the difference between the experimental and calculated data is not more than 1.5 charge equivalents. This difference is not surprising since the calculations are based on the assumption that the dissociation of the ionic residues within the amino

**Table I**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Molecular weight</th>
<th>Partial specific volume</th>
<th>Isoelectric point</th>
<th>No. COOH-terminal groups</th>
<th>No. NH₂-terminal groups</th>
<th>No. Asp</th>
<th>No. Glu</th>
<th>No. His</th>
<th>No. Lys</th>
<th>No. Arg</th>
<th>No. sialic acid residues</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apo-A-I</td>
<td>28,400</td>
<td>0.74</td>
<td>5.36–6.62</td>
<td>1</td>
<td>1</td>
<td>16</td>
<td>25</td>
<td>5</td>
<td>21</td>
<td>16</td>
<td>0</td>
<td>23, 58, 59, 61</td>
</tr>
<tr>
<td>Apo-A-II</td>
<td>17,380</td>
<td>0.74</td>
<td>4.56</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>16</td>
<td>0</td>
<td>18</td>
<td>0</td>
<td>0</td>
<td>58, 60, 61</td>
</tr>
<tr>
<td>Apo-C-I</td>
<td>8,837</td>
<td>0.73</td>
<td>4.78</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>8</td>
<td>0</td>
<td>6</td>
<td>1</td>
<td>0</td>
<td>6, 22, 58, 60</td>
</tr>
<tr>
<td>Apo-C-III</td>
<td>9,960</td>
<td>0.72</td>
<td>4.54</td>
<td>1</td>
<td>1</td>
<td>7</td>
<td>4</td>
<td>1</td>
<td>6</td>
<td>2</td>
<td>2</td>
<td>6, 22, 62, 63</td>
</tr>
</tbody>
</table>

pKₐ value

3.0 8.3

3.9 4.3

6.0 10.8

12.5 3.0

39
Acid sequence of the protein occurs at the same pH as for the corresponding isolated amino acids. Furthermore, the isolated apolipoproteins were not separated into their isomorphs. These, however, may show slight differences with respect to their charge distribution as can be concluded from shifts of their isoelectric points (23).

The calculated number of negatively charged residues at physiological pH is higher for the apo-C than for the apo-A proteins. Furthermore, there is less agreement between the calculated and experimental data for apo-C proteins within the range of pH 4 and 8. Polyelectrolyte titration reveals an isoelectric point for apo-A-I at pH 5.2, for apo-A-II at pH 4.5, for apo-C-II at pH 4.8, and for apo-C-III_{2} at pH 4.6. Clearly, the experimental data are in better agreement with the previously published isoelectric points than the calculations from the amino acid composition.

Taking the data as listed in Table I, the calculated particle size of apo-A-I is 4.6 nm, for apo-A-II is 3.8 nm, for apo-C-II is 3.0 nm, and for apo-C-III_{2} is 3.4 nm. Assuming a spherical shape for isolated apo-A-I, the negative surface charge density evaluated from the net charge and the surface area is 0.06 eq/nm^{2}, for apo-A-II it is 0.08 eq/nm^{2}, for apo-C-II it is 0.12 eq/nm^{2}, and for apo-C-III_{2} it is 0.15 eq/nm^{2}. The data indicate that at physiological pH apo-C-III_{2} has the highest negative surface charge density while it is lowest for apo-A-I.

The calculated ζ-potentials in a solution of 0.15 M saline at pH 7 and 25 °C for the isolated hydrated apolipoproteins were 8.1 mV for apo-A-I, 11.3 mV for apo-A-II, 20.3 mV for apo-C-II, and 17.0 mV for apo-C-III_{2}. The data are consistent with the observation that apo-A proteins are more hydrophobic than the apo-C proteins.

**Surface Charge of Phospholipids**—The experiments with liposomes of synthetic phospholipids should give information about the potential contribution of phospholipids to the surface charge density of lipoprotein particles. Identical results were obtained from titrations with both polycations, MGC, and polyethyleneimine. Therefore, only the data from measurements with polyethyleneimine are presented in Fig. 2.

As expected, neutral phospholipids (PC, LPC, and sphingomyelin) exhibit minimal binding properties for counterionic polyelectrolytes over the entire pH range. Since measurements were made with low concentrations of phospholipids, the minimal degree of ionization was almost within the limits of detection and could be easily caused by the presence of minimal amounts of fatty acids which, however, were not detected by gas liquid chromatography. The absence of interaction between polycations and phospholipid liposomes indicates that the anionic phosphate groups are intra- and/or intermolecularly neutralized by neighboring quaternary ammonium groups that prevent an interaction with the counterionic polyelectrolyte. Our observations confirm the previous studies of Gosh et al. (40) showing that an electrophoretic mobility of the neutral phospholipids was not detectable over the entire pH range.

In contrast, the surface charge of PI and PS liposomes increases with the pH. Also, PE liposomes exhibit anionic properties at pH 9 due to the lower pK of the free amino group, which is apparently only partially protonated in the alkaline pH range.

**Particle Size of Lipoproteins**—The particle size of lipoproteins with a density d < 1.006 kg/liter isolated from subjects with different forms of hyperlipoproteinemia and determined by photon correlation spectroscopy (41, 42) is listed in Table II. Their estimated average diameter of 40–126 nm is in good agreement with observations by electron microscopy (43, 44). Interestingly, the particle size does not correlate with the type of hyperlipoproteinemia.

**Surface Charge of Lipoprotein with a Density d < 1.006 kg/liter at pH 7**—The curves of polyelectrolyte titration of VLDL with polyethyleneimine/KPVS are presented in Fig. 3. The end point of titration is marked by a steep decrease of relative absorbance of the metachromatic indicator dye at 665 nm.
TABLE II
Mean particle size of lipoproteins with a density d < 1.006 kg/liter from donors with different forms of hyperlipoproteinemia

The particle size was determined with photon correlation spectroscopy as described under "Experimental Procedures."

<table>
<thead>
<tr>
<th>No.</th>
<th>Donor</th>
<th>Diffusion coefficient $\times 10^{-7}$ cm$^2$/s</th>
<th>S.D. of $z$-weighted diffusion coefficient</th>
<th>Mean diameter of particle</th>
<th>Specific surface (nm$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normolipemic</td>
<td>0.624</td>
<td>71</td>
<td>69</td>
<td>126.7</td>
</tr>
<tr>
<td>2</td>
<td>Normolipemic</td>
<td>0.680</td>
<td>50</td>
<td>63</td>
<td>118.3</td>
</tr>
<tr>
<td>3</td>
<td>Normolipemic</td>
<td>0.902</td>
<td>41</td>
<td>48</td>
<td>147.3</td>
</tr>
<tr>
<td>4</td>
<td>Normolipemic</td>
<td>0.757</td>
<td>46</td>
<td>57</td>
<td>128.0</td>
</tr>
<tr>
<td>5</td>
<td>IIb</td>
<td>0.580</td>
<td>57</td>
<td>74</td>
<td>106.2</td>
</tr>
<tr>
<td>6</td>
<td>IIb</td>
<td>0.679</td>
<td>69</td>
<td>63</td>
<td>135.8</td>
</tr>
<tr>
<td>7</td>
<td>IIb</td>
<td>0.550</td>
<td>55</td>
<td>78</td>
<td>99.2</td>
</tr>
<tr>
<td>8</td>
<td>IIb</td>
<td>0.712</td>
<td>61</td>
<td>60</td>
<td>134.3</td>
</tr>
<tr>
<td>9</td>
<td>IIb</td>
<td>0.799</td>
<td>46</td>
<td>54</td>
<td>135.1</td>
</tr>
<tr>
<td>10</td>
<td>IV</td>
<td>0.516</td>
<td>51</td>
<td>83</td>
<td>90.4</td>
</tr>
<tr>
<td>11</td>
<td>IV</td>
<td>0.630</td>
<td>52</td>
<td>68</td>
<td>111.2</td>
</tr>
<tr>
<td>12</td>
<td>IV</td>
<td>0.669</td>
<td>41</td>
<td>64</td>
<td>109.2</td>
</tr>
<tr>
<td>13</td>
<td>IV</td>
<td>0.714</td>
<td>43</td>
<td>60</td>
<td>118.2</td>
</tr>
<tr>
<td>14</td>
<td>IV</td>
<td>0.669</td>
<td>37</td>
<td>64</td>
<td>106.4</td>
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<tr>
<td>15</td>
<td>IV</td>
<td>0.690</td>
<td>34</td>
<td>63</td>
<td>106.2</td>
</tr>
<tr>
<td>16</td>
<td>IV</td>
<td>0.874</td>
<td>46</td>
<td>49</td>
<td>147.7</td>
</tr>
<tr>
<td>17</td>
<td>IV</td>
<td>0.758</td>
<td>40</td>
<td>57</td>
<td>122.9</td>
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<tr>
<td>18</td>
<td>IV</td>
<td>0.814</td>
<td>47</td>
<td>53</td>
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</tr>
<tr>
<td>19</td>
<td>IV</td>
<td>0.709</td>
<td>41</td>
<td>60</td>
<td>115.8</td>
</tr>
<tr>
<td>20</td>
<td>IV</td>
<td>0.769</td>
<td>41</td>
<td>56</td>
<td>125.6</td>
</tr>
<tr>
<td>21</td>
<td>IV</td>
<td>1.101</td>
<td>30</td>
<td>42</td>
<td>154.1</td>
</tr>
<tr>
<td>22</td>
<td>IV</td>
<td>0.680</td>
<td>53</td>
<td>63</td>
<td>120.9</td>
</tr>
<tr>
<td>23</td>
<td>IV</td>
<td>0.874</td>
<td>35</td>
<td>49</td>
<td>137.3</td>
</tr>
<tr>
<td>24</td>
<td>IV</td>
<td>0.732</td>
<td>37</td>
<td>57</td>
<td>119.6</td>
</tr>
<tr>
<td>25</td>
<td>IV</td>
<td>0.840</td>
<td>41</td>
<td>51</td>
<td>137.2</td>
</tr>
<tr>
<td>26</td>
<td>IV</td>
<td>0.779</td>
<td>47</td>
<td>55</td>
<td>132.6</td>
</tr>
<tr>
<td>27</td>
<td>IV</td>
<td>0.746</td>
<td>60</td>
<td>57</td>
<td>139.7</td>
</tr>
<tr>
<td>28</td>
<td>IV</td>
<td>0.770</td>
<td>39</td>
<td>56</td>
<td>121.1</td>
</tr>
<tr>
<td>29</td>
<td>IV</td>
<td>0.874</td>
<td>30</td>
<td>49</td>
<td>133.4</td>
</tr>
<tr>
<td>30</td>
<td>IV</td>
<td>0.556</td>
<td>46</td>
<td>77</td>
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</tr>
<tr>
<td>31</td>
<td>IV</td>
<td>0.721</td>
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<td>59</td>
<td>118.5</td>
</tr>
<tr>
<td>32</td>
<td>IV</td>
<td>0.698</td>
<td>45</td>
<td>61</td>
<td>117.2</td>
</tr>
<tr>
<td>33</td>
<td>IV</td>
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<td>34</td>
<td>V</td>
<td>0.341</td>
<td>53</td>
<td>126</td>
<td>60.6</td>
</tr>
<tr>
<td>35</td>
<td>V</td>
<td>0.739</td>
<td>33</td>
<td>58</td>
<td>114.7</td>
</tr>
</tbody>
</table>

Fig. 4. Change of the amount of the counterionic polyelectrolyte polyethyleneimine (PEI) for neutralization of the surface charge of a lipoprotein with a density $d < 1.006$ kg/liter as a function of the amount of lipoprotein preincubated with the polycation at pH 7.

Fig. 5. Correlation between the number of charged residues ($n$) at the surface of lipoproteins with a density $d < 1.006$ kg/liter as measured with polyelectrolyte titration with polyethyleneimine/KPVS and their mean particle volume as determined by photon correlation spectroscopy.

Fig. 6. Correlation between the surface charge density ($n$) of lipoproteins with density $d < 1.006$ kg/liter and their surface area (nm$^2$).

Titrating a dilution series of the lipoprotein, the portion of the back-titratable charge equivalents of polyethyleneimine decreases linearly with the amount of VLDL. This observation indicates a stoichiometric interaction between the lipoprotein particles and the counter-polyelectrolyte. In some cases, at higher concentrations of the lipoprotein, the stoichiometry of the neutralization reaction of polyethyleneimine is disturbed (Fig. 4). Presumably, this is caused by flocculated lipoprotein-polyethyleneimine complexes. Therefore, it is advantageous to perform the titration at the lowest possible concentrations of VLDL. The analysis results in a linear correlation ($r = 0.869, n = 35$) between the surface of the
lipoprotein particles and the number of charged residues/particle (Fig. 5), indicating that the number of charged residues/particle decreases with the particle size.

The surface charge density is obtained by dividing the number of charged residues with the specific surface area (Fig. 6). The mean of the surface charge density of VLDL particles is determined as 1.10 ± 0.36 eq/nm². It is noteworthy that it does not correlate with the size of VLDL particles (r = 0.004).

**DISCUSSION**

The importance of the surface charge of proteins has been discussed for a variety of biochemical reactions (45-48). The electrostatic properties of lipoprotein particles have been emphasized in metabolic processes involving apolipoproteins and in the regulation of cholesterol synthesis (49). Recently, we could demonstrate that the surface charge of water-soluble enzymes is measurable by polyelectrolyte titration (28). The close agreement between experimental and calculated data demonstrates that the method can also be successfully applied for studies of lipophilic proteins.

The data show that at a given pH the back-titratable portion of charged amino acid residues reflects the difference of ionized cationic and anionic sites within the protein. Apparently, the tertiary structure of isolated apolipoproteins is governed by the intramolecular electrostatic neutralization of oppositely charged residues. Our findings essentially agree with structural investigations showing that the polar part of the apolipoproteins is formed by intramolecular salt bridges between oppositely charged residues (50, 51).

Although close agreement between calculated and experimental data was observed for the apo-A proteins, significant differences were found for apo-C-II and apo-C-III, between pH 5 and 8. Similarly, experiments with pepsin, which has an isoelectric point in the acidic range (pH 2-3), resulted in a lower degree of dissociation in this range of pH than one would expect from the amino acid composition (29). Therefore, we conclude that the lower dissociation is due to a "polyelectrolyte" effect, as it is observed with uniformly charged polycarboxylic acids at neutral pH (52, 53).

Phospholipid liposomes with an anionic charge (PI, PS, and PE) exhibit properties similar to uniformly charged polyelectrolytes, i.e., the degree of ionization increases with the pH. However, even at pH 9 the degree of dissociation is still smaller than 1, indicating that either the dissociation is incomplete or the anionic residues in the interior of the liposomes are dissociated but not accessible for interaction with the cationic polyelectrolyte.

Our data indicate that the contribution of phospholipids to the surface charge of lipoproteins seems to be restricted to the anionic phospholipids, that is about 5% of the total phospholipid moiety. However, their degree of dissociation at the lipoprotein surface may well be higher than one would expect from our observations with pure anionic liposomes. Anionic phospholipids form co-micelles with neutral phospholipids (54) and with free cholesterol (55). In such aggregates the polyelectrolyte effect of equally charged sites causing a suppression of the dissociation should be less pronounced, since the incorporation of the neutral spacer molecules into the phospholipid layer increases the distance between charged groups.

It is tempting to compare our results with previous potentiometric analyses by Rosseneu et al. (56) of apo-A-I complexed with dimyristoylphosphatidylcholine. In such peptide-phospholipid complexes, 43 charged residues/apo-A-I molecule at pH 3, 20 eq/apo-A-I at pH 4, and 2 eq/apo-A-I at pH 5 were found. These data correspond closely with our results for uncomplexed apo-A-I. The close agreement suggests that the surface charge of the apolipoprotein remains unaffected by complex formation with the neutral phospholipid. Again, the agreement of our data for isolated apo-A-II and apo-C-III, in the range below pH 5 is fairly close with results from another potentiometric investigation of the corresponding phospholipid complexes (57). However, the measurements with isolated proteins in that study differ significantly from ours. They could be explained by differences of the isoelectric point used for reference (near pH 6) for the potentiometry. In view of the more recent determinations of the isoelectric points (22, 23), this value is questionable.

Remarkably, the surface charge density of VLDL from different donors scatters only slightly. Furthermore, it is independent of particle size. Since the surface charge density determines the surface potential and thereby the electrophoretic mobility, this finding, in fact, agrees with the observation that VLDL migrate uniformly on electrophoresis even when the size of the lipoprotein particles differs from one sample to another.

The conditions of sampling after a period of 14 h of fasting were such that steady state conditions were assumed. In this case, the variance in particle size would indicate considerable differences in the kinetics of VLDL metabolism within one type of hyperlipoproteinemia from one subject to another. If on the other hand, sampling was made under nonsteady state conditions, then it is tempting to speculate about the significance of our results for the understanding of the metabolic processes in vivo. During lipolytic breakdown apolipoproteins and lipid apolipoprotein complexes are transferred from the surface of VLDL particles in a sequence of decreasing surface charge density of the proteins (4, 44, 64). The driving forces of these processes are not clearly identified. If the decrease of particle size reflects nonsteady state conditions during lipolysis, the maintenance of the surface charge density would be a consequence of desorption processes of charged fragments. They occur to such an extent that the balance between hydrophobic and electrophoretic forces of the resulting particle is maintained. The formation of anionic sites (i.e. fatty acids) during lipolysis must, therefore, result in a disaggregation since the number of equally charged centers within one particle increases. A transfer of fragments then will occur, when the balance between electrostatic forces promoting dissociation and hydrophobic forces of attraction is altered.

In summary, we conclude that polyelectrolyte titration can be successfully applied for measurement of the surface charge of lipophilic proteins, liposomes, and lipoproteins. The results of this study are consistent with previous biochemical observations and may give a further explanation for processes that occur in the metabolism of VLDL.

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