Intermolecular Ionic Interaction in Aggregates of a Lipoprotein of the *Escherichia coli* Outer Membrane†

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ABSTRACT: The lipoprotein isolated from the outer membrane of *Escherichia coli* was labeled with fluorescamine. The extent of labeling decreases as the concentration of the sodium dodecyl sulfate ($NaDodSO_4$) increases. When fluorescamine was added in the absence of NaCl, only one out of five lysine residues in the lipoprotein was labeled by the reagent. This reactive lysine was identified as the carboxyl-terminal lysine since very low labeling was observed when it was removed by carboxypeptidase B before the addition of fluorescamine. Upon the addition of 0.5 M NaCl, at least three additional lysine residues became susceptible to fluorescamine. On the basis of circular dichroism neither fluorescamine labeling nor treatment with NaCl caused conformational changes in the lipoprotein. The rate of modification of arginine residues of the lipoprotein was also enhanced by the presence of NaCl, although the rate of modification of free arginine by 2,3-butanedione was unaffected by NaCl. The Sephadex gel filtration chromatogram of the lipoprotein in 0.1% $NaDodSO_4$ showed a broad distribution of $NaDodSO_4$–protein micelles with different effective radii. When the lipoprotein was incubated in $NaDodSO_4$ in the presence of 0.5 M NaCl, prior to chromatograph, only aggregates with smaller effective radii were observed. These results are consistent with the idea that ionic interactions play a major role in forming the lipoprotein aggregates in $NaDodSO_4$ and that breaking the ionic interactions by NaCl makes more lysine and arginine residues accessible to chemical modifications.

The outer membrane of *Escherichia coli* contains a lipoprotein having a molecular weight of 7200 (Inouye et al., 1972; Bosch and Braun, 1973; Lee and Inouye, 1974). This lipoprotein consists of 58 amino acid residues and its complete amino acid sequence has been determined by Braun and Bosch (1972). The lipoprotein has been shown to have a very high helical content (Braun, 1973; Inouye et al., 1976; Lee et al., 1977). In its helical structure, all the hydrophobic amino acid residues are aligned on one side of the helical rod. Thus, it is conceivable that the protein molecules can interact with each other to form dimers or ordered aggregates through hydrophobic and/or hydrophilic interactions. A three-dimensional molecular model of the lipoprotein assembly has been proposed, in which six helices of the lipoprotein are assembled to form a tubular hydrophilic channel with a hydrophobic outer surface (Inouye, 1974). In this model, the superhelical structure is stabilized by forming seven salt linkages between adjacent α helices. A procedure to purify the lipoprotein in high yield has been recently developed (Inouye et al., 1976). It has also been shown that the purified lipoprotein forms paracrystals with a highly ordered ultrastructure (DeMartini et al., 1976).

In the present paper, we have examined the possible role of ionic interaction in the lipoprotein aggregates formed in different concentrations of sodium dodecyl sulfate ($NaDodSO_4$[1]). By using fluorescamine to titrate the lipoprotein aggregates, we observed that, among ϵ-amino groups of 5 lysine residues in the lipoprotein, only the one at the carboxyl-terminal end reacts with fluorescamine in high $NaDodSO_4$ concentrations. However, in the presence of NaCl, at least three more amino groups react with the reagent. Similarly, in 0.1% $NaDodSO_4$, the rate of arginyl residue modification by 2,3-butanedione is enhanced by the presence of NaCl. The gel filtration data also indicate that in the presence of NaCl observed aggregates have smaller effective Stokes' radii. These results are consistent with the idea that ionic interaction is playing an important role in the formation of the lipoprotein aggregates and that destruction of the ionic interaction leads to the formation of smaller protein–$NaDodSO_4$ micelles exposing lysine and arginine residues to modification.

Materials and Methods

Purification of the Lipoprotein. The lipoprotein was purified from *E. coli* B (late exponential phase, Grain Processing Corp., Muscatine, Iowa) according to the method of Inouye et al. (1976).

Labeling of the Lipoprotein with Fluorescamine. Five microliters of 35 mM fluorescamine in acetone was added to each of 3 mL of $2.5–3.0 \times 10^{-6}$ M lipoprotein solutions in 5 mM sodium phosphate buffer, pH 7.0, containing different amounts of $NaDodSO_4$ and NaCl. The mixtures were shaken vigorously at room temperature. The amounts of fluorescamine that bonded to the protein were estimated from fluorescence intensities of the labeled samples which were excited at 390 nm. The fluorescence intensities were measured by integrating the emission spectra which were recorded with a Farrand Mark I spectrofluorimeter. Similarly, fluorescence intensities were obtained for known concentrations of the difluorescamine derivative of lysine in an identical medium. Using these intensities, the amounts of fluorescamine labeled lysine derivatives were determined as described by Weigele et al. (1972). Lysine with both amino groups labeled with fluorescamine was prepared by adding 50 μL of 0.1 M lysine–HCl in water containing 15 nmol of triethylamine to 2.5 mL of 4 mM fluorescamine in acetone. The reaction was complete within a few minutes at room temperature. The fluorescent product was

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[1] Abbreviation used is: $NaDodSO_4$ (or SDS), sodium dodecyl sulfate.

isolated by thin-layer chromatography. The product had absorption maxima at 266 and 387 nm with extinction coefficients of 3.6×10^4 and 1.2×10^4 M^{-1} cm^{-1}, respectively, in 10 mM sodium phosphate buffer at pH 8.0. Lysine labeled with one fluorescamine was prepared in a similar manner, except that a one to one ratio of fluorescamine to lysine was used in the synthesis. The product so obtained had absorption maxima at 266 and 386 nm with extinction coefficients of 1.8×10^4 and 6.0×10^3 M^{-1} cm^{-1}, respectively, in phosphate buffer at pH 8.0. Both mono- and difluorescamine labeled lysines exhibit emission maxima at 475 nm upon excitation at 390 nm in aqueous medium at pH 8.0. The fluorescence intensities are linear in the concentration range of 0 to 5×10^{-5} M of the labeled lysine. The relative quantum yields of those standard compounds are only slightly affected (±5%) by the compositions of all the aqueous media used in the present report.

Carboxypeptidase Digestion of the Lipoprotein. The lipoprotein was digested with carboxypeptidase B as follows: 10 μL of 0.1 mg/mL carboxypeptidase B was added to 1 mL of 0.46 mg/mL lipoprotein solution (0.02 M $NaHCO_3$, pH 8.0, 0.1 M NaCl, and 0.01% sodium dodecyl sulfate). The mixture was incubated for 30 min at 37 °C and then the reaction was stopped by precipitating the lipoprotein with 2 mL of acetone. The lipoprotein precipitate was collected by centrifugation, washed twice with 2 mL of acetone, dissolved in 1 mL of 0.1% $NaDodSO_4$, and immediately used for the fluorescence experiment with the same method used for untreated lipoprotein. We found that carboxypeptidase B in the digestion mixture produces negligible fluorescence under these conditions. An aliquot (0.05 mL) of the reaction mixture was applied to an amino acid analyzer at the end of the digestion to determine the amino acids released by the enzyme. It was found that 0.5 to 0.7 mol of lysine per mol of lipoprotein was released but no other amino acids were detected. This indicates that only the carboxy-terminal lysine residue was removed from the lipoprotein by the digestion.

Modification of Arginyl Residues by 2,3-Butanedione. The modification of arginyl residues in the lipoprotein by 2,3-butanedione was performed essentially as described by Riordan (1973). Lipoprotein (0.05 mg) in 2 mL of 10 mM phosphate, pH 7.2, containing 0.1% $NaDodSO_4$ with or without 0.5 M NaCl was incubated at 27 °C for 24 h. At the end of incubation, 0.2 mL of 2,3-butanedione was added to the solution. The pH was quickly readjusted to 7.2 with 6 N sodium hydroxide. The reaction mixture was withdrawn at specified time intervals and was added to 10 volumes of chilled acetone to stop the reaction. The modified lipoprotein precipitate was collected by centrifugation at 20 000*g* for 10 min at 2 °C. The precipitate was washed twice with distilled water. The washed precipitate was then dissolved in 0.1% $NaDodSO_4$ and hydrolyzed in 6 N HCl at 110 °C under vacuum for 16 h. The content of unmodified arginine was then determined by amino acid analysis.

Gel Filtration. The apparent molecular weights of the lipoprotein aggregates formed in 0.1% $NaDodSO_4$ were determined by gel filtration with a Sephadex G-150 column. A 0.2-mL solution of the lipoprotein (0.1 mg/mL) in 5 mM sodium phosphate buffer, pH 7.0, containing $NaDodSO_4$ was applied to a Sephadex G-150 column (10 × 160 mm) equilibrated with the same buffer solution. The column was then eluted (with the buffer solution) at a constant flow rate. The elution patterns of the lipoprotein aggregates were continuously monitored at 280 nm and recorded with an ISCO UA-5 monitor. For the estimation of the effective radius, the column was calibrated with $NaDodSO_4$ denatured bovine serum albumin, carbonic anhydrase, chymotrypsin, lysozyme, and cytochrome *c* applied in the same manner as for the lipoprotein aggregates. Blue dextran 2000 and bromophenol blue were used as exclusion and inclusion volume markers, respectively. The apparent Stokes radii of the lipoprotein aggregates were estimated as follows: The apparent Stokes radius of a macromolecule is proportional to the distribution coefficient K_{av} (Nazaki et al., 1976) which is defined as

$$K_{av} = \frac{V_e - V_0}{V_t - V_0} \quad (1)$$

in which V_e is the elution volume for the macromolecule, V_0 the void volume, and V_t the total volume of the column. When the column is eluted at a constant flow rate, F, the equation (eq 1) can be expressed as

$$K_{av} = \frac{F(t_e - t_0)}{F(t_t - t_0)} = \frac{t_e - t_0}{t_t - t_0} \quad (2)$$

in which t_t, t_e, and t_0 are the elution times for bromophenol blue, the macromolecule, and the blue dextran 2000, respectively. Thus, the apparent effective radius of the macromolecule is proportional to $(t_e - t_0)$ values when both the flow rate F and total volume of the column are kept constant.

In order to detect small amounts of the lipoprotein aggregates during the gel filtration, 1 mol of fluorescamine was linked to 1 mol of the lipoprotein so that the absorbance at 280 nm increased about threefold. This modification of the lipoprotein with fluorescamine was carried out as follows: 1 μL of 35 mM fluorescamine in acetone was added to a 0.5-mL solution of the lipoprotein (0.4 mg/mL) suspended in 5 mM sodium phosphate buffer, pH 7.2. The mixture was vigorously shaken. The reaction was usually complete within a few seconds. The labeled lipoprotein was precipitated by adding 1 mL of acetone, collected by centrifugation at 18 000*g* for 10 min, and washed twice with 5 mM sodium phosphate buffer, pH 7.2. Under these conditions, about 1 mol of fluorescamine was bonded to 1 mol of the lipoprotein. This treatment did not cause any significant change in the apparent molecular weights of the lipoprotein aggregates.

Circular Dichroism Measurements. The possible conformational changes induced by fluorescamine labeling and NaCl treatment were examined by circular dichroism measurements with a JASCO J-20 automatic recording spectropolarimeter.

Protein Concentration Determinations. The concentrations of the lipoprotein were determined by amino acid analysis with a JEOL 6-AH analyzer. The lipoprotein was hydrolyzed in 6 N HCl at 110 °C (for 16 h) in a vacuum sealed tube before being applied to the amino acid analyzer.

Materials. Electrophoresis grade $NaDodSO_4$ and fluorescamine were purchased from Bio-Rad Laboratories and Roche Diagnostics, respectively. Egg white lysozyme and carboxypeptidase B treated with diisopropyl fluorophosphate were obtained from Worthington Biochemical Corp. Bovine serum albumin, carbonic anhydrase, chymotrypsin, cytochrome *c*, and 2,3-butanedione were purchased from Sigma Chemical Co. Sephadex G-150 and blue dextran 2000 were the products of Pharmacia Co.

Results

Effect of $NaDodSO_4$ Concentration of Fluorescamine Labeling. It has been reported that the labeling of soluble proteins by fluorescamine can be inhibited by the presence of $NaDodSO_4$ (Tu and Grosso, 1976). The inhibitory effects of $NaDodSO_4$ are interpreted as the low permeability of fluorescamine toward the highly charged envelopes of $NaDodSO_4$-protein micelles. The inhibitory effect was also ob-

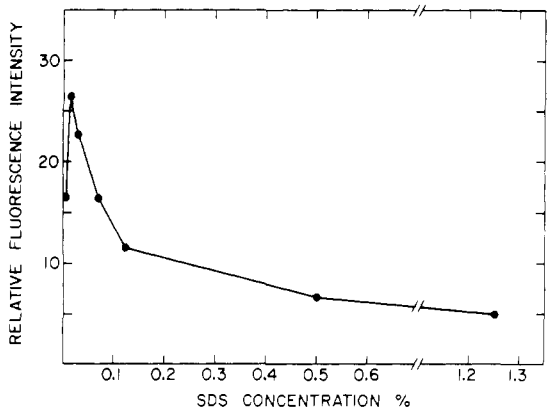

FIGURE 1: Effect of $NaDodSO_4$ (SDS) concentration on fluorescamine labeling of the lipoprotein. The lipoprotein was labeled with suboptimal amount of fluorescamine in 10 mM phosphate, pH 7.2, with different $NaDodSO_4$ concentrations as described in Materials and Methods. The molar ratio of fluorescamine to the lipoprotein was 2 at labeling stage.

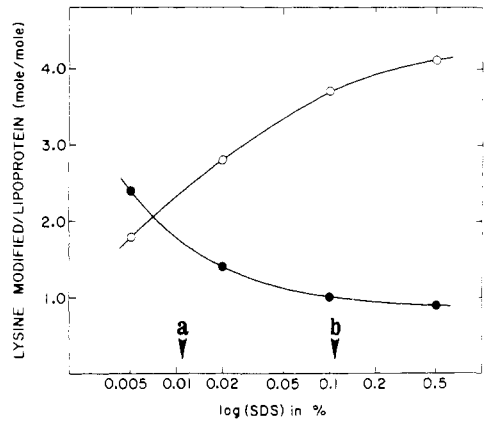

FIGURE 2: Effect of NaCl on fluorescamine labeling of the lipoprotein in various $NaDodSO_4$ concentrations. The lipoprotein was labeled with the saturated amount of fluorescamine in 10 mM phosphate, pH 7.2, with different $NaDodSO_4$ concentrations in the absence and the presence of 0.5 M NaCl as described in Materials and Methods. The molar ratio of fluorescamine to the lipoprotein was 15 at labeling stage. (●—●) In the absence; (○—○) in the presence of 0.5 M NaCl. Arrows a and b indicate the critical micelle concentrations of $NaDodSO_4$ in the presence and the absence of 0.5 M NaCl, respectively.

served in the present study. Figure 1 shows the extent of labeling of the lipoprotein in different $NaDodSO_4$ concentrations. It can be seen that fluorescamine labeling first increased and then started to decrease as $NaDodSO_4$ concentrations increased. Since the lipoprotein is insoluble in aqueous medium in the absence of detergents, the initial increase shown in Figure 1 may be due to the dissolution process of the insoluble lipoprotein. The decrease of fluorescamine labeling upon further addition of $NaDodSO_4$ is consistent with the inhibitory effects of $NaDodSO_4$ upon fluorescamine labeling of proteins as described by Tu and Grosso (1976).

Effect of NaCl on Fluorescamine Labeling. Fluorescamine is known to react with only primary amino groups exposed on the surface of $NaDodSO_4$ micelles (Tu and Grosso, 1976) or biological membranes (Nakaya et al., 1975; Hawkes et al., 1976). Since the amino terminus of the lipoprotein is acylated, it has only 5 free amino groups from ϵ-amino group of lysine residues (Braun and Bosch, 1972). Furthermore it has been reported that the lipoprotein has a very high α-helical content even in the presence of $NaDodSO_4$ (Braun et al., 1976; Lee et al., 1977). When an α helix is constructed from the amino acid sequence, it was found that all basic amino acid residues except

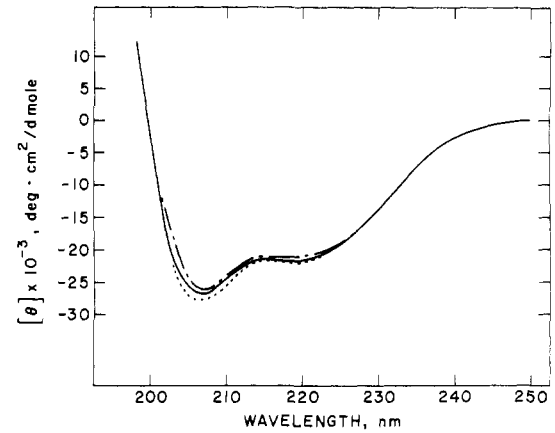

FIGURE 3: Effects of fluorescamine and NaCl on circular dichroism spectra of the lipoprotein. (—) Spectrum of the untreated lipoprotein in 10 mM sodium phosphate buffer, pH 7.1, containing 0.1% $NaDodSO_4$; (···) spectrum of the fluorescamine-labeled lipoprotein in 10 mM sodium phosphate buffer, pH 7.1, containing 0.1% $NaDodSO_4$. Four moles of fluorescamine was bonded to 1 mol of the lipoprotein; (– – –) spectrum of the untreated lipoprotein in 10 mM sodium phsophate buffer, pH 7.1, containing 0.1% $NaDodSO_4$ and 0.5 M NaCl.

for -Arg-Lys at the carboxyl-terminal end can form salt linkages with acidic amino acid residues of the adjacent lipoprotein α helices (Inouye, 1974). It has been proposed that the lipoprotein may be assembled in a superhelical structure stabilized by such ionic interactions (Inouye, 1974).

In order to examine the existence of ionic interactions between the lipoprotein molecules in $NaDodSO_4$ solution, we have investigated the accessibility of lysine residues to fluorescamine in the absence and presence of 0.5 M NaCl. Figure 2 shows the number of lysine residues reactive to fluorescamine in different $NaDodSO_4$ concentrations with and without 0.5 M NaCl. In the absence of NaCl the number of lysine residues reactive to fluorescamine decreased as $NaDodSO_4$ concentrations increased. On the other hand, in the presence of 0.5 M NaCl the number of fluorescamine-reactive lysine residues increased substantially as $NaDodSO_4$ concentrations increased. In 0.5% $NaDodSO_4$, only 1 mol of lysine was accessible to fluorescamine, whereas upon the addition of 0.5 M NaCl, a little more than 4 mol of lysine became reactive to fluorescamine. These data are consistent with the interpretation that the presence of NaCl facilitates the breakage of the ionic interaction between lysine and acidic amino acid residues in the lipoprotein aggregates resulting in more fluorescamine-reactive lysine residues. The following two alternative explanations, although possible, seem unlikely in view of the present data. Since the critical micelle concentration of $NaDodSO_4$ decreases in higher salt concentrations (see Figure 2), the lipoprotein aggregates may dissociate into smaller units by being incorporated into $NaDodSO_4$ micelles as suggested by Robinson and Tanford (1975) for the interaction of detergents and membrane proteins. This may allow more lysine residues to be accessible to fluorescamine labeling. However, this interpretation is unlikely because the extent of the fluorescamine labeling decreased in higher $NaDodSO_4$ concentrations in the same salt concentration as shown in Figure 1. Secondly, NaCl may induce conformational changes in the lipoprotein molecule which cause more lysine residues to be exposed to fluorescamine labeling. This is also unlikely because circular dichroism measurements showed no conformational changes in 0.1% $NaDodSO_4$ in the absence and presence of 0.5 M NaCl (Figure 3).

Effect of Carboxypeptidase Digestion on Fluorescamine

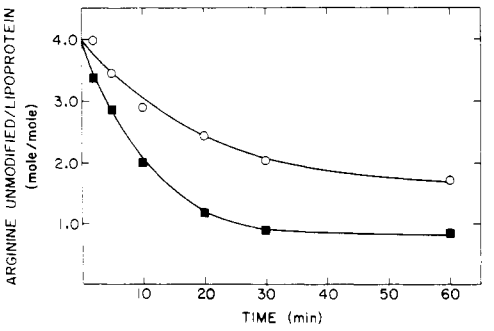

FIGURE 4: Effect of NaCl on the modification of arginyl residues of the lipoprotein with 2,3-butanedione in the absence and the presence of NaCl. The reaction was carried out as described in Materials and Methods. (○—○) In the absence; (■—■) in the presence of 0.5 M NaCl.

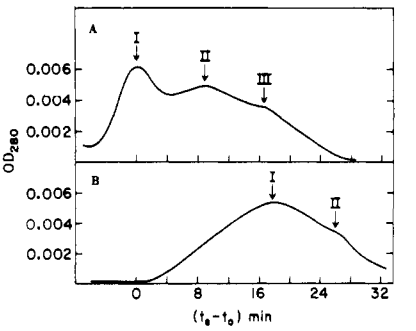

FIGURE 5: Gel filtration of the lipoprotein aggregates formed in 0.1% $NaDodSO_4$ in the absence and the presence of 0.5 M sodium chloride. Gel filtration was carried out with Sephadex G-150 as described in Materials and Methods. (A) The lipoprotein was dissolved in 0.1% $NaDodSO_4$ and the solution was incubated for 24 h at room temperature to reach equilibrium before gel filtration. Gel filtration was carried out in the absence of NaCl. (B) Gel filtration was carried out in the same way as described in A except that the lipoprotein solution was incubated in 0.1% $NaDodSO_4$ containing 0.5 M NaCl and the gel filtration was also carried out with the same medium. Arrows indicate the positions of peaks of the lipoprotein aggregates.

Labeling. As shown in Figure 2, there is only one lysine residue reactive to fluorescamine in the absence of NaCl in 0.5% $NaDodSO_4$. We examined whether this fluorescamine-reactive lysine residue is derived from the carboxyl-terminal one. Since it has been reported that carboxy-terminal residues of the lipoprotein can be easily removed by carboxypeptidase treatment (Braun et al., 1975), we prepared the lipoprotein missing the carboxyl-terminal lysine residue as described in Materials and Methods. When about 60% of carboxyl-terminal lysine residue was removed, the number of fluorescamine-reactive amino groups (in 0.1% $NaDodSO_4$ without NaCl) dropped from 1.1 to 0.4 mol, indicating that the carboxyl-terminal lysine residue is not involved in salt-linkage formation.

Modification of Arginine Residues by 2,3-Butanedione. In addition to lysine residues, it is possible that arginine residues of the lipoprotein are also involved in salt-linkage formation in the lipoprotein aggregate. We tested this possibility with use of 2,3-butanedione, which is reactive to arginine residues (Harden and Norris, 1971; Yankulov, 1970). However, since 2,3-butanedione is much more stable than fluorescamine in aqueous solution, we measured the rate of modification of arginine residues in the absence and presence of NaCl, instead of measuring final number of arginine residues modified by the chemical. The results are shown in Figure 4. It can be seen that the rate of modification increased about twofold in the presence of 0.5 M NaCl. Since the addition of NaCl did not have any effect on the rate of modification of free arginine by 2,3-butanedione (data not shown), the results in Figure 4 are assumed to be due to better accessibility of arginine residues of the lipoprotein to the chemical in the presence of NaCl. This again supports the existence of salt linkages in the lipoprotein aggregates.

Gel Filtration of the Lipoprotein Aggregates. If the lipoprotein aggregates are formed by salt linkages as discussed above, one can also expect that the size of the lipoprotein aggregates should become smaller in the presence of NaCl than in the absence of NaCl. Thus, we examined the effect of NaCl on gel filtration of the lipoprotein aggregates in 0.1% $NaDodSO_4$. As shown in Figure 5A in the absence of NaCl the equilibrium mixture of the lipoprotein was eluted in a wide range of molecular weights. However, one can observe three distincts peaks I, II, III, of which molecular weights approximately correspond to larger than 75 000, 40 000, and 17 000, respectively. When NaCl was added, peaks I and II of Figure 5A disappeared. Correspondingly a major peak and a minor peak appeared at I and II, respectively, as shown in Figure 5B. Their molecular weights correspond to about 16 000 for peak I and 7500 for peak II. It is clear that upon the addition of NaCl the effective Stokes radii of the lipoprotein aggregates became smaller. These results cannot be explained by the fact that the critical micelle concentration of $NaDodSO_4$ was decreased from 0.1 to 0.0125% by 0.5 M NaCl (Reynolds and Tanford, 1970), since even in 1% $NaDodSO_4$, a considerable amount of the lipoprotein aggregates (more than 50%) was eluted at the void volume (data not shown). Thus it is most likely that NaCl breaks the ionic interactions between the lipoprotein molecules to form smaller aggregates.

Discussion

In the present paper, we showed that the addition of NaCl to the lipoprotein solution in $NaDodSO_4$ caused the following three events: (i) increase in the number of lysine residues reactive to fluorescamine; (ii) enhancement of arginine modification by 2,3-butanedione; and (iii) reduction of the effective Stokes radii. These results are consistent with the idea that the ionic interactions between the lipoprotein molecules are playing an important role in forming the lipoprotein aggregates in $NaDodSO_4$, and that the ionic interactions are broken by NaCl making more lysine and aringine residues accessible to chemical modifications. At the same time, the lipoprotein aggregates apparently larger than hexamer or pentamer (peak II in Figure 5A) disappear to form smaller aggregates, which have the effective Stokes radii of the size of a dimer of the lipoprotein (peak I in Figure 5B).

Although from the present study one cannot deduce lipoprotein assembly in the intact membrane, the present results are consistent with the proposed three-dimensional molecular assembly model (Inouye, 1974) in which lipoprotein helices are assembled together with ionic interactions. Since it has been shown that the lipoprotein has a very high α-helical content even in $NaDodSO_4$ (Braun et al., 1975; Lee et al., 1977), it is conceivable that under the present condition several molecules of the lipoprotein are in fact assembled in superhelical structures in which ionic interactions are playing a major role in the assembly.

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Interaction of Plasma "Arginine-Rich" Apolipoprotein with Dimyristoylphosphatidylcholine†

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ABSTRACT: Very low density lipoproteins isolated from the plasma of cholesterol-fed rabbits contain abnormally high amounts of cholesterol, phospholipid, and an apoprotein referred to as the "arginine-rich" protein (ARP). It is generally assumed that the major interaction between apolipoproteins and lipids is between the protein and the phospholipids. Therefore, we have studied in the present report the lipid-binding properties of ARP to dimyristoylphosphatidylcholine (DMPC) vesicles in order to determine the importance of this interaction for ARP. The interaction was studied by ultracentrifugal flotation, circular dichroism, and microcalorimetry. The binding studies were performed using low protein-to-lipid ratios so as to minimize protein–protein interaction and vesicle disintegration. The ARP–DMPC complexes were isolated by salt density ultracentrifugation in KBr and had an average DMPC to protein molar ratio of 625 to 1. The complexes were stable for several days. The addition of DMPC to ARP induced an increase in the α helicity of the protein; the maximal change (from 45% to 65%) in α-helical content required 90 min with a $t_{1/2}$ of approximately 15 min. The enthalpy of association of ARP with DMPC was highly exothermic with a value $\Delta H = -614$ kcal/mol of protein. The rate of heat release in this measurement was time dependent, requiring in excess of 20 min; however, the enthalpic changes were totally finished when the helical increase was only about one-half complete. Based on the kinetics of interaction, we suggest that the high enthalpy of binding may be associated with the increase in helicity of the protein; these two processes, though, are not sufficiently concomitant to account unequivocally for the heat release in terms of either protein–lipid interaction or protein structural changes.

Very low density lipoproteins (VLDL)[1] of human plasma contain several apolipoproteins in variable amounts. (For a review, see Scanu 1972a,b; Scanu et al., 1975; Jackson et al., 1976.) These apoproteins have been designated apoB, apoC-I, apoC-II, apoC-III (Alaupovic, 1971) and a protein rich in arginine termed the "arginine-rich" protein (ARP) (Shore and Shore, 1972, 1973) or apoE (Utermann, 1975). Normal subjects, as well as those with hyperlipidemia, show individual quantitative differences in these VLDL proteins which may reflect variances in genetic, hormonal, and dietary factors. The "arginine-rich" protein of normal human VLDL, which was originally described by Shore and Shore (1970) and characterized by Shelburne and Quarfordt (1974), comprises about 5–15% of the total VLDL proteins. Shore et al. (1974) found that the proportion of ARP is preferentially increased in VLDL

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[1] Abbreviations used are: VLDL, very low density lipoproteins; ARP, "arginine-rich" protein; apoB, apoC-I, apoC-II, apoC-III, and ARP, apoprotein constituents of VLDL; DMPC, dimyristoylphosphatidylcholine; CD, circular dichroism; DSC, differential scanning calorimetry; T_c, gel → liquid crystalline transition temperature; R_s, Stokes radius; LP-X_1 and LP-X_2, two lipoproteins isolated from plasma of obstructive jaundice patients; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; DEAE, diethylaminoethyl.