Nature of the Interaction of Dextran Sulfate with High and Low Density Lipoproteins in the Presence of Ca^{2+}

Young C. Kim† and Toshiro Nishida§

From the Burnsides Research Laboratory, Department of Food Science, University of Illinois, Urbana, Illinois 61801

The interactions of dextran sulfate with high density lipoproteins (HDL) and with low density lipoproteins (LDL) were studied to differentiate their mechanisms and to provide some information of the charge characteristics of these lipoproteins. The insoluble complex formation with high density lipoprotein 3 (HDL₃) progressively increased with increases of the Ca²⁺ concentration; complete conversion occurred with 60 mM CaCl₂. Between the two major subclasses, HDL₂ was more effectively converted to insoluble complex at lower CaCl₂ concentrations than HDL₃. It appeared that primarily the phospholipid components of HDL were involved in the formation of the insoluble complex and that the protein components reduced the complex forming ability of HDL. However, both apolipoproteins A-I (apo-A-I) and A-II (apo-A-II) possessed a definite reactivity with dextran sulfate in the presence of divalent metal ions, and a reduction in the ionic strength progressively enhanced their complex formation. In addition, modification of the apo-A-I carboxyl groups with glycine methyl ester, after activation with carbodimide, was found to greatly increase its complex forming ability. Although LDL strongly interacted with dextran sulfate, primarily involving the positive charges of the protein moiety, increases in the Ca²⁺ concentration seemed to cause a progressive increase in the participation of the zwitterionic polar heads of the LDL phospholipids in complex formation. The study of the interaction of the preincubated apo-A-I and lyssolecithin mixtures with dextran sulfate in the presence of Ca²⁺ revealed that these two components produced a molecular complex which interacted with dextran sulfate as an organized unit.

Sulfated polysaccharides and other polyanions interact with human plasma lipoproteins under a variety of conditions. LDL and very low density lipoproteins form insoluble complexes with sulfated polysaccharides even in the absence of divalent metal ions (1, 2). However, HDL requires high concentrations of both divalent metal ions and sulfated polysaccharides (2). It has recently been postulated on the basis of epidemicological studies that, while low density lipoproteins are atherogenic, high density lipoproteins exert antiatherogenic effects (3-5). Unlike LDL, HDL does not possess an affinity with aortic glycosaminoglycans (6, 7), and uptake by human arterial smooth muscle cells is rather limited (6, 9). These different behaviors may reflect in part differences in the charge characteristics and organization of the lipoprotein surfaces. Some basic information on the surface properties may be derived from a systematic study of the lipoprotein-polyanion interaction since the interaction is primarily governed by polar forces (1, 2).

We previously studied the interaction of HDL with dextran sulfate (10-13). In the absence of divalent metal ions, the interaction involved primarily the positively charged groups of the protein moiety (7, 19). However, in the presence of divalent metal ions, the charged groups of the phospholipids seemed also to contribute to the interaction (13, 14). To provide basic information on the nature of the involvement of lipoprotein phospholipids, we examined the interaction utilizing lecithin dispersions and lyssolecithin micelles (15). Although the interaction between HDL and sulfated polysaccharides has not been well understood, the abundance of zwitterionic phospholipids at the surface as well as the possible presence of a large proportion of the protein charged groups as ion pairs (16, 17) suggest a mechanism of interaction similar to that for lecithin dispersions or lyssolecithin micelles (15). The present communication describes the differences in the mode of interaction of dextran sulfate with HDL and LDL, and the charge characteristics of their constituents influencing the interaction.

MATERIALS AND METHODS

Buffer—Unless otherwise indicated, experiments were performed with Tris buffer, pH 7.4, ionic strength of 0.1.

Preparation of Dextran Sulfate—The sodium salt of dextran sulfate, with a sulfur content of 16.1% corresponding to 1.7 sulfate groups/hexose unit, was prepared from dextran with an average molecular weight of 150,000 according to the method of Ricketts (18) as previously described (10).

Preparation of LDL and HDL—Human plasma LDL of the Sr₅ to class 10 class was isolated from fresh nonturbid human plasma and purified by ultracentrifugation at 4°C (10, 13). Total HDL (d = 1.063 to 1.210 g/cm³) was isolated and purified as described previously (19). To obtain HDL₃ and HDL₄, the total HDL fraction was dialyzed against a NaCl/NaBr solution of d = 1.125 g/cm³ and centrifuged at 105,000 x g for 36 h. The HDL₃ fraction was removed from the top of the tube. The bottom fraction containing HDL₄ was adjusted to a density of 1.210 g/cm³ with NaBr and then centrifuged at 105,000 x g for 36 h to float the HDL₄ to the top of the tube. Both HDL₃ and HDL₄ fractions were purified by recentrifugation after a 1:4 dilution with NaCl/NaBr solution of d = 1.125 and 1.210 g/cm³, respectively. All media used for centrifugation contained 0.025% EDTA (pH 7.4) and 2 mM NaN₃. After purification, all lipoprotein fractions were dialyzed against a buffer containing 0.025% EDTA (pH 7.4) and 2 mM NaN₃ under nitrogen at 4°C. Typical lipoprotein preparations used in this study contained 21% protein and 7% lipids for LDL, 48% protein and 52% lipids for total HDL, 40% protein and 90% lipids for HDL₃, and...
54% protein and 46% lipids for HDL. The methods used for the
determination were previously described (19).

**Succinylatation and Acetylation of HDL**—HDL was succinylated in a manner similar to that described for LDL (13). The molar ratio of succinic anhydride/lysine residues was 14. Acetylation of HDL was performed as previously described (10) using a molar ratio of acetic anhydride/lysine residues of 6. The extent of acylation of the free amino groups was assayed by the trinitrobenzene sulfonic acid procedure (13). The succinylation and acetylation modified 96 and 97%, respectively, of the amino groups of HDL.

**Separation of Apo-A-I and Apo-A-II**—The purified total HDL in an NAB medium of d = 1.21 g/cm³ was dialyzed against 0.025% EDTA, pH 7.4, at 4°C, and extracted with ethanol/ether mixtures by the method of Shore and Shore (20). Apo-A-I and apo-A-II were fractionated by gel permeation chromatography on Sephadex G-100 and subsequent DEAE-cellulose column chromatography (21) at 4°C. The purified apo-A-I and apo-A-II exhibited well defined single bands on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The apolipoproteins were stored in 6 M urea at 4°C under nitrogen and were dialyzed against buffer prior to each use.

**Modification of Apo-A-I Carboxyl Groups**—The carboxyl groups of apo-A-I were modified by reaction with [1-14C]glycine methyl ester and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide HCl essentially according to the method of Hoare and Koshland (22). [1-14C]Glycine methyl ester was prepared according to the procedure of Wells et al. (23). Thin layer chromatography of the labeled preparation using 1-butanol/acetic acid/H₂O (60:20:20, v/v) as the developing solvent showed a single yellow spot with ninhydrin with a Rf value higher than that of glycine. The spot completely superimposed with the radioactivity on the chromatogram. The modification of apo-A-I (4 mg/ml) was carried out at 25°C in 6 M urea at pH 4.75 using glycine methyl ester and the carboxylate at the final concentrations of 1.0 and 0.4 M, respectively. The reaction was stopped by the addition of an equal volume of 4 M acetic buffer (pH 4.7). The mixtures were first dialyzed against 0.1 M Tris buffer (pH 8.0) containing 6 M urea and then against regular Tris buffer. The extent of carboxyl modification was determined by the specific activity of the modified apo-A-I. Preparations so modified to the extents of 16 and 36% were used in the experiments.

**Preparation of Lecithin Dispersions and Lysolecithin Micelles**—Egg lecithin was purified by neutral alumina column chromatography according to the method of Wells and Hanahan (24) from crude egg lecithin prepared by the method of Faure (25). Lysolecithin (1-acyl-sn-glycero-3-phosphorylcholine) prepared from egg lecithin was purchased from Pierce Chemical Co. (Rockford, Ill.) and was freed of a trace of contaminating free fatty acids as previously described (15). Thin layer chromatography of the purified lecithin and lysolecithin on Silica Gel G plates with chloroform/methanol/water (66:25:4, v/v) as the developing solvent gave well defined single spots. Lecithin dispersions were prepared by the sonication method and lysolecithin micelles by dissolving a weighed amount of lysolecithin in Tris buffer as previously described (15).

**Formation and Analysis of Insoluble Complexes**—The formation of insoluble complexes with dextran sulfate was studied as described previously (11, 13, 15). Mixtures containing a known amount of lipoprotein, apolipoprotein, or lipid dispersion, and various amounts of dextran sulfate and CaCl₂ in 1.5 ml of Tris buffer.

**RESULTS**

The interaction of HDL₃ with dextran sulfate was studied at various concentrations of CaCl₂ in Tris buffer (Fig. 1). Although no insoluble complex was formed from HDL₃ in the absence of Ca²⁺, increasing amounts of complex were formed as the concentration of CaCl₂ was increased. An equivalence dextran sulfate/HDL₃ weight ratio was present for the formation of a maximal amount of insoluble complex and was shifted to higher values by increases in the CaCl₂ concentration. Complete conversion of HDL₃ to the insoluble complex was achieved when the CaCl₂ concentration was raised to 60 mM (Fig. 1, Curve 5).

In contrast to HDL₃, LDL has a high reactivity with dextran sulfate and did not require the presence of Ca²⁺ for the formation of insoluble complex as described previously (10, 13). Complete conversion of LDL to the insoluble complex was attained at the dextran sulfate/LDL weight ratio of 0.04 in the absence of Ca²⁺ (Fig. 2, Curve 1). When CaCl₂ was added to a concentration of 5 mM, the equivalence weight ratio for maximal complex formation was shifted to a value of approximately 0.07 (Fig. 2, Curve 2). Above CaCl₂ concentrations of 10 mM, the complete conversion of LDL to insoluble complex was not observed at any concentration of dextran sulfate.

**Lipid and Protein Determinations**—Lipid phosphorus was determined essentially according to the method of Bartlett (26) as modified by Parker and Peterson (27). The amount of phospholipid in the samples is given as the amount of phosphorus x 25. Protein was determined according to the method of Lowry et al. (28) with crystalline bovine serum albumin (Sigma Chemical Co.) dried over phosphorus pentoxide as a standard. Interference with the protein assay by Tris buffer was eliminated by incorporating the same amount of Tris buffer in the standards as described by Rej and Richards (29).

**Fig. 1.** The conversion of HDL₃ to insoluble dextran sulfate-HDL₃ complex in the presence of 5 (Curve 1), 10 (Curve 2), 20 (Curve 3), 30 (curve 4), and 60 (Curve 5) mM CaCl₂. Insoluble complex formation was assayed with mixtures containing 320 μg of HDL₃ and various amounts of dextran sulfate and CaCl₂ in 1.5 ml of Tris buffer.

**Fig. 2.** Effect of calcium ions on the formation of insoluble dextran sulfate-LDL complex. Curve 1, conversion of LDL to insoluble complex as a function of the dextran sulfate/LDL weight ratio in the absence of Ca²⁺; Curves 2, 3, 4, and 5, conversion in the presence of 5, 10, 20, and 30 mM CaCl₂, respectively. Insoluble complex was formed with 0.8 mg of LDL, various amounts of dextran sulfate, and CaCl₂ in 1.5 ml of Tris buffer.
complex occurred over a wide range of dextran sulfate/ LDL weight ratios (Fig. 2, Curves 3 to 5). The lower reactivity of HDL3 with dextran sulfate in comparison to LDL might have been caused in part by the presence of a greater proportion of free negative charges on HDL3. Thus, the complex forming ability of HDL3 was expected to be further reduced by chemical modifications that increase the number of negative charges or the net negative charge of HDL3. When approximately 96% of the HDL3 amino groups were modified by acetylation and succinylation, the formation of insoluble complex was drastically reduced (Fig. 3, Curves 2 and 3) as compared to untreated HDL3 (Fig. 3, Curve 1) in the presence of 60 mM CaCl2. Succinylation effected a slightly larger reduction than did acetylation. When the concentration of CaCl2 was reduced below 30 mM, neither succinylated nor acetylated HDL3 produced a significant amount of insoluble complex.

To obtain some information concerning the contribution of the protein components of HDL3 to the interaction, the most abundant HDL apoproteins, apo-A-I and apo-A-II, were used in the interaction study. It was observed that apo-A-I and apo-A-II produce insoluble complexes with dextran sulfate in the presence of Ca2+ (Fig. 4), although the insoluble complex formation was less pronounced than with HDL3.

Zwitterionic phospholipids, such as lecithin and lysolecithin, can also be converted into insoluble complexes by dextran sulfate in the presence of relatively low concentrations of Ca2+ (15). The effectiveness of the conversion of LDL, lecithin, lysolecithin, HDL, HDL2, HDL3, apo-A-I, and apo-A-II to insoluble complex at the equivalence ratios as a function of CaCl2 concentration. Experimental conditions for the conversion of LDL, HDL3, and HDL2 were the same as those described for Fig. 1; the conditions for the conversion of lecithin and of apo-A-I or apo-A-II were described for Figs. 2 and 4, respectively. For the conversion of lecithin or lysolecithin to insoluble complex, the mixtures containing 200 μg of lecithin or 133 μg of lysolecithin and various amounts of dextran sulfate and CaCl2 in 0.75 ml of Tris buffer were used.

The conversion of apo-A-I and apo-A-II to insoluble complex was compared by plotting the amount of insoluble complex formed for each substance at the equivalence ratios as a function of NaCl concentration. Accordingly, the conversion of LDL to the insoluble complex was most efficient with near complete conversion occurring even in the absence of Ca2+. This was followed by lecithin dispersions which required 1 to 2 mM CaCl2 for near complete conversion. The enhanced polar character of lysolecithin micelles caused a reduction in the efficiency of conversion to insoluble complex, thus requiring higher concentrations of CaCl2. The conversion of high density lipoproteins was less efficient than either lecithin or lysolecithin. Among high density lipoproteins, HDL3 was most effectively converted to insoluble complex, followed by total HDL and HDL2. The least effective conversion was observed with apo-A-I and apo-A-II: only 83% of apo-A-I and 60% of apo-A-II were converted to insoluble complex at CaCl2 concentrations as high as 30 mM.

The conversion of apo-A-I and apo-A-II to insoluble complex was enhanced by a decrease in the ionic strength of medium (Fig. 6). At NaCl concentrations below 0.01 M, near complete conversion of apo-A-I to the insoluble complex was
observed, while maximal conversion of apo-A-II was approximately 89%. When NaCl concentration was increased from 0.05 to 0.2 M, a sharp decrease in complex formation occurred with both apo-A-I and apo-A-II. In order to study the effect of the negative charges of apo-A-I on the formation of insoluble complex, the carboxyl groups of the dicarboxylic amino acid residues were modified with glycine methyl ester and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide HCl to the extent of 16 and 35%. These preparations were found to produce insoluble complex even in the absence of Ca++. Approximately 46% conversion to insoluble complex was observed at the equivalence weight ratio of 0.05 when 16% of the carboxyl groups were modified (Fig. 7, Curve 1). With modification of 35% of the carboxyl groups, the equivalence ratio was shifted to approximately 0.2 and at this ratio complete conversion to insoluble complex was effected (Fig. 7, Curve 1). Upon addition of CaCl₂ to apo-A-I with 16% modification, the formation of insoluble complex was enhanced and the equivalence weight ratio shifted to higher values, these effects being less pronounced at 10 mM CaCl₂ (Fig. 7, Curve 2) than at 20 mM CaCl₂ (Fig. 7, Curve 3). Since the apo-A-I modified to 35% was completely converted even in the absence of CaCl₂, the main effect of the addition of CaCl₂ (Fig. 7, Curves 4 and 5) was the formation of an extended plateau region as observed with LDL.

Fig. 7. Effect of the modification of apo-A-I carboxyl groups on the conversion of apo-A-I to insoluble complex. Curves 1, 2, and 3, precipitation curves for the apo-A-I with 16% of the carboxyl groups modified in the presence of 0, 10, and 20 mM CaCl₂ respectively. Curves 4, 5, and 6, precipitation curves obtained with the apo-A-I with 35% modification at the CaCl₂ concentrations of 0, 10, and 20 mM respectively. Experimental conditions were the same as those described for Fig. 4.

Fig. 8. Interaction of dextran sulfate with the lyssolecithin/apo-A-I mixture in the presence of 20 mM CaCl₂. The conversion of lyssolecithin and apo-A-I alone to insoluble complex is shown by Curves 1 and 2, respectively. Curve 3, conversion of the lyssolecithin/apo-A-I mixture to the insoluble complex as determined by both phosphorus (●-●-●) and protein (△-△-△) assays. The complex was formed from 80 μg of lyssolecithin or 80 μg of apo-A-I, or a mixture of 80 μg of lyssolecithin and 80 μg of apo-A-I in the presence of various amounts of dextran sulfate at a CaCl₂ concentration of 20 mM in 0.75 ml of Tris buffer.

Fig. 9. Effect of the concentration of NaCl on the stability of the insoluble complexes of dextran sulfate with apo-A-I alone (Curve 1), apo-A-I/lyssolecithin mixtures of the apo-A-I to lyssolecithin weight ratios of 1:1 (Curve 2), 1:2 (Curve 3), and 1:3 (Curve 4), and lyssolecithin alone (Curve 5). Insoluble complexes were formed at the equivalence ratios in the presence of 20 mM CaCl₂ in 10 mM Tris buffer of pH 7.4 in a similar manner as given for Fig. 8.

DISCUSSION

Although the major HDL apoproteins, apo-A-I and apo-A-II, possessed a definite reactivity with dextran sulfate in the presence of divalent metal ions, their presence seemed to reduce the complex forming ability of the phospholipids in the intact lipoproteins. The interfering effect of HDL proteins on the complex formation appears to be caused by the presence of a large number of free negative charges. The succinylation or acetylation of HDL apoprotein would exacerbate the interfering effect by...
increasing the number of negative charges or the net negative charge of the HDL proteins.

The differences in the complex forming abilities of LDL and HDL can be best explained by comparing the plots of insoluble complex formed from these lipoproteins and some of their major constituents as a function of Ca²⁺ concentration (Fig. 5). The effectiveness of LDL in producing insoluble complex even in the absence of Ca²⁺ appears to reflect the participation of the protein positively charged groups in the interaction despite the net negative charge present on the surface (13). The conversion of lecithin vesicles or lysolecithin micelles to insoluble complex required the presence of Ca²⁺ and appeared to represent mutually enhancing interactions involving both positive and negative charges of the zwitterionic phospholipids (15). These are the direct electrostatic interaction between the phospholipid choline nitrogen and the sulfate groups of dextran sulfate, and the calcium cross-linking of the phosphate groups to the sulfate groups (Model A) or to the phosphate groups of neighboring phospholipids (Model B). It is possible that a large portion of the positive and negative charges on apo-A-I and apo-A-II may exist as ion pairs (16, 17) which could interact with dextran sulfate in a manner similar to that of the zwitterionic polar heads of lecithin or lysolecithin. The inefficient conversion of the apolipoproteins as compared to the zwitterionic phospholipids suggests that the interaction of the ion pairs may be reduced by the abundance of free negative charges present on apo-A-I and to a greater extent on apo-A-II. This explanation was substantiated by the formation of insoluble complex by apo-A-I with the carboxyl groups modified, even in the absence of Ca²⁺. The decrease in the negative charges of apo-A-I by the modification and the consequent exposure of some positive charges which were involved in ion pairs apparently facilitated the interaction. Conversely, untreated apo-A-I and apo-A-II required high concentrations of Ca²⁺ for the interaction of the ion pairs with the sulfate groups of dextran sulfate and for the charge destruction or neutralization of ionized carboxyl groups. We previously showed that an increase in the net negative charge of LDL by association with fatty acid carboxylate ions (11, 12) or by succinylation or acetylation of the LDL amino groups (13) increases the requirement for divalent metal ions.

Although HDL appears to be considerably heterogeneous (30, 31), the differences in the Ca²⁺ requirement between the two major subfractions, HDL₂ and HDL₃, may be related largely to the different amounts of lipid and protein components exposed at their surfaces. The complex forming ability of HDL₃ is greater than that of HDL₂, possibly due to a higher proportion of zwitterionic phospholipids on the surface of HDL₂ (32). Since apo-A-II was less effective in the complex formation than apo-A-I, the somewhat smaller proportion of apo-A-II present on HDL₂ (30) could have provided a favorable effect on the conversion of HDL₂ to the insoluble complex. However, HDL₂ and HDL₃ may possess a fixed ratio of apo-A-I and apo-A-II (33), and HDL₂ could be produced by the combination of 2 HDL₃ molecules plus extra lipids as recently speculated (34). The phospholipid components of the extra lipids may effectively participate in the complex formation. It is expected that the Ca²⁺ requirements of HDL lie between those of the major constituents, lecithin, and the HDL apoproteins. However, the conformation of apo-A-I and apo-A-II in the intact lipoproteins are different from those in bulk solution (17, 35-38). Thus, the complex forming abilities of these apoproteins may be altered upon association with the lipids. The effect of apo-C and apo-D which constitute approximately 5% of the HDL proteins could be relatively minor as compared to that of apo-A-I and apo-A-II.

To explore the possibility that sulfated polysaccharides can be used to obtain some information on the nature of lipid-protein associations, the interaction of the lysolecithin-apo-A-I mixtures with dextran sulfate was studied. This system was chosen in view of the efficient association of lysolecithin with apo-A-I, availability of some basic information on the interaction (39-42), and the micellar organization of lysolecithin molecules, which ensures that all polar heads present in the micelles are accessible to dextran sulfate, unlike those in lecithin vesicles which have a bilayer organization (15). The results showed that apo-A-I and lysolecithin in the mixtures produced a molecular complex and interacted with dextran sulfate as organized units. The equivalence weight ratio obtained with a lysolecithin/apo-A-I mixture of a weight ratio of 1 (molar ratio, 57) was approximately 2.0, which was about 80% higher than the means of 0.6 and 1.5 obtained with lysolecithin and apo-A-I, respectively. At the equivalence weight ratios which give maximal complex formation, the sulfate negative charges of dextran sulfate are most effectively neutralized (13, 15). Thus, the higher equivalence weight ratio obtained for the mixture indicates a large increase in the participation of the sulfate groups in the interaction. This increased participation may be explained by the differences in the mode of the interaction of dextran sulfate with lysolecithin molecules in micelles and with those in lysolecithin-apo-A-I complex. When lysolecithin is present in the form of micelles, calcium cross-linking may occur between the phosphate groups of neighboring lysolecithin molecules due to their proximity (Model B) (15). However, the separation of individual lysolecithin molecules in the lysolecithin-apo-A-I complex may favor calcium cross-linking between the phosphate groups of lysolecithin and the sulfate groups of dextran sulfate (Model A), which requires twice the number of sulfate groups that are involved in Model B. These considerations support the contention that the lysolecithin-apo-A-I interaction occurs through penetration of monomeric lysolecithin molecules into apo-A-I (40, 42) rather than insertion of the apo-A-I into the lysolecithin micelles (41). Sufficient spacing out of the lysolecithin molecules between preformed or newly formed amphiphatic helices (16, 17), or both, may be important in enhancing the flexibility of the peptide chain (42).

The higher equivalence ratio obtained for the apo-A-I-lysolecithin complex also indicates the primary involvement of hydrophobic interactions in the association of apo-A-I with lysolecithin. If the electrostatic interaction between the phospholipid polar heads and the protein ion pairs was substantially involved in the association, the number of lysolecithin polar heads and protein ion pairs available for interaction with dextran sulfate would be greatly reduced. Nevertheless, weak ionic interactions or hydrogen bonding could occur between the zwitterionic polar heads and the charged amino acid residues of apo-A-I since the comparison of the ionization behavior of apo-A-I and of its complex with dymiristoyl lecithin revealed some masking of the acidic and basic residues in the complex (43). Such weak interactions, however, can be effectively eliminated by the strong interaction of dextran sulfate with the lysolecithin-apo-A-I complex. The importance of the hydrophobic association was previously indicated by observations that the lysolecithin-apo-A-I interaction was not influenced appreciably by changes of pH, the presence of salts at high concentrations, or the succinylation of apo-A-I (41). Valuable information on the surface organization of the lipoprotein molecules can be obtained by extending the present study with a variety of polyanion molecules differing in their charge characteristics.

Although the dextran sulfate-LDL interaction occurs in the absence of divalent metal ions through electrostatic interactions between the positive charges of the LDL basic amino
acid residues and the negative charges of dextran sulfate (13), the addition of Ca**+ seems to cause the participation of LDL phospholipids in the interaction. The extent of this participation appeared to become greater with increases in the Ca**+ concentration as evidenced by the upward shift and broadening of the equivalence weight ratios. These changes were indicative of the involvement of more sulfate groups of dextran sulfate in the interaction with the zwitterionic polar heads of the LDL phospholipids by the mechanism we previously proposed from the study of the interaction of dextran sulfate with lecithin vesicles and lysolecithin micelles (15). We noted that, although the insoluble lecithin-calcium-dextran sulfate complex is easily dissociated upon the addition of EDTA, the LDL-calcium-dextran sulfate complex was not dissociated by EDTA alone; it was necessary to raise the pH of the medium above 10 or to increase the ionic strength by the addition of NaCl. Therefore, the dissociation apparently required not only the abolishment of the interaction between the LDL phospholipids and dextran sulfate, but also a reduction in the electrostatic interactions involving the positive charges of LDL either by deprotonation of a large portion of the e-amino groups of the lysine residues or by an increase in the ionic strength of the medium.

It may be generalized that, in the presence of divalent metal ions, the interaction of all sulfated polysaccharides and glycosaminoglycans with LDL, VLDL, and the lipoproteins containing apo-E (44, 45) probably occurs by the joint participation of the lipoprotein phospholipids in the interaction. The joint participation of the protein and phospholipid moieties may also be involved in the interaction of the lipoproteins with the cell surface receptors on human fibroblasts. The interaction which requires the presence of divalent metal ions (46) was speculated to occur by a mechanism similar to that for the interaction with glycosaminoglycans (45, 47). Although zwitterionic phospholipids, such as lecithin, when present alone, do not bind Ca**+ to any appreciable extent (48-50), lecithin dispersions interact effectively with dextran sulfate at CaCl2 concentrations as low as 0.5 mM (15). Thus, the phospholipid-receptor interaction could occur at physiological Ca**+ concentrations. While the chemical modification of the lipoprotein lysine or arginine residues (51, 52) may interfere primarily with the direct electrostatic interaction involving the positive charges, it may also nullify the phospholipid-receptor interaction, which may be cooperatively involved in the binding of the lipoproteins. Verification of the possible role of the phospholipids in the lipoprotein-receptor binding would be important in the clarification of the binding mechanism.

REFERENCES
Nature of the interaction of dextran sulfate with high and low density lipoproteins in the presence of Ca2+.
Y C Kim and T Nishida