Physical–Chemical Interaction of Heparin and Human Plasma Low-Density Lipoproteins†

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ABSTRACT: This study characterizes the physical–chemical interactions of heparin with human plasma low-density lipoproteins (LDL). A high reactive heparin (HRH) specific for the surface determinants of LDL was isolated by chromatography of commercial bovine lung heparin on LDL immobilized to AffiGel-10. HRH was derivatized with fluoresceinamine and repurified by affinity chromatography, and its interaction with LDL in solution was monitored by steady-state fluorescence polarization. Binding of LDL to fluoresceinamine-labeled HRH (FL·HRH) was saturable, reversible, and specific; HRH stoichiometrically displaced FL·HRH from the soluble complex, and acetylation of lysine residues on LDL blocked heparin binding. Titration of FL·HRH with excess LDL yielded soluble complexes with two LDL molecules per heparin chain (M_r 13 000) characterized by an apparent K_d of 1 μM. Titration of LDL with excess HRH resulted in two classes of heparin binding with two and five heparin molecules bound per LDL and apparent K_d values of 1 and 10 μM, respectively. At physiological pH and ionic strength, the soluble HRH–LDL complexes were maximally precipitated with 20–50 mM Ca^{2+}. Insoluble complexes contained 2–10 HRH molecules per LDL with the final product stoichiometry dependent on the ratio of the reactants. The affinity of HRH for LDL in the insoluble complexes was estimated between 1 and 10 μM. Insoluble LDL–heparin complexes were readily dissociated with 1.0 M NaCl, and their formation was prevented by acetylation of the lysine residues on LDL.

There is considerable interest in elucidating the structural components of the human plasma low-density lipoproteins (LDL)[1] that mediate their binding to specific cell surface receptors (Brown et al., 1981; Pittman et al., 1979) and to sulfated glycosaminoglycans (GAG) of plasma (Nakashima et al., 1975; Staprans & Felts, 1985) and arterial tissue (Bihari-Varga & Végh, 1967; Radhakrishnamurthy et al., 1982). The major mechanism determining plasma cholesterol levels in normal man is the hepatic clearance of LDL. This process involves the binding of LDL to specific cell surface receptors. Binding is followed by the internalization and degradation of the lipoprotein [for a review, see Brown and Goldstein (1986)]. It has been suggested that receptors play a protective role as reduced levels of functional receptors are associated with increased risk of atherosclerosis (Brown & Goldstein, 1986). However, recent studies in pigs (Rapacz et al., 1986) and humans (Vega & Grundy, 1986) have demonstrated in vivo evidence for reduced binding of LDL to normal receptors as a cause for primary moderate hypercholesterolemia and associated atherosclerosis, suggesting that a structural defect in the lipoprotein is responsible. These findings are consistent with previous hypotheses that LDL may contain subpopulations of particles with increased avidity for the arterial wall [see Rudel et al. (1986) for a review]. Such particles, if defective in receptor binding, may escape the circulation and by direct insudation penetrate arterial tissue where they bind GAG and other components of the extracellular matrix (Hollander, 1976). Since the interactions of LDL and GAG may contribute to the accumulation of lipid in the arterial wall, we have undertaken the present study to identify the heparin-binding domains of LDL.

The major protein constituent of LDL, apolipoprotein B-100 (Kane et al., 1980), is a high molecular weight monomeric protein consisting of 4536 amino acid residues (Chen et al., 1986). Although the primary structure of apoB-100 has been elucidated, the structural domains which determine LDL function and metabolism are not known. Recently, we reported that trypsin treatment of LDL diminished the lipoprotein's ability to bind the LDL receptor of cultured fibroblasts without significantly affecting its binding to the acidic mucopolysaccharide heparin (Cardin et al., 1986b). In the present study, we show multiple heparin-binding sites on LDL.

Materials and Methods

Materials. Bovine lung heparin was a generous gift from Hepar Industries, Inc. (Franklin, OH). Fluoresceinamine (isomer 1) and dialysis tubing (2000 molecular weight exclusion) were obtained from Sigma; PD-10 disposable columns were from Pharmacia; Iodo-Gen (1,3,4,6-tetrachloro-3α,6α-diphenylglycoluril) was from Pierce; $Na^{125}I$ was from New England Nuclear; 0.45-μm Metricel filters were from Gelman Filtration Products; and 0.025-μm type VS filters were from Millipore. The purification of LDL and high reactive heparin (HRH) and the preparation of LDL AffiGel-10 were as described in the preceding paper (Hirose et al., 1987).

Modification of HRH with Fluoresceinamine. HRH was labeled with fluoresceinamine by the method of Glabe et al. (1983) with modifications according to Smith and Knauer (1987). Briefly, CNBr (94 μmol) in 0.2 mL of H_2O was added to a solution of 20 mg of HRH (dry weight) in 1 mL of H_2O. The mixture was adjusted to pH 11 and maintained at that

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[1] Abbreviations: LDL, low-density lipoprotein(s); apoB-100, apolipoprotein B-100; GAG, glycosaminoglycans; EDTA, ethylenediaminetetraacetic acid; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HRH, high reactive heparin; FL (F in figures), fluoresceinamine.

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pH for 5 min with 0.2 M NaOH. The solution was then chromatographed on a PD-10 column equilibrated in 0.2 M sodium borate, pH 8.0. The first 2.5 mL of column eluate was discarded, and the next 3.5 mL was collected into a 13 × 100 mm test tube containing 4 mg of fluoresceinamine. The mixture was allowed to react overnight at 4 °C. The sample was then dialyzed extensively against distilled water to remove unreacted reagent and then against 10 mM HEPES, 10 mM $CaCl_2$, and 0.01% NaN_3, pH 8.0 (LDL AffiGel-10 column equilibration buffer). The sample was then chromatographed on a 1.5 × 25 cm column of LDL AffiGel-10 and eluted with 0.5 M NaCl in column equilibration buffer; prior to the experiments, FL·HRH was dialyzed against 50 mM HEPES and 0.1 M NaCl, pH 7.4. The labeling stoichiometry was 0.2 mol of fluoresceinamine per HRH of M_r = 13 000.

Radioiodination of FL·HRH. The radioiodination of FL·HRH was performed by the method of Fraker and Speck (1978) with 1,3,4,6-tetrachloro-3α,6α-diphenylglycoluril (Iodo-Gen). A 1-mg sample of Iodo-Gen in a 13 × 100 mm glass tube was dissolved in 1 mL of $CHCl_3$ and the solvent removed by lyophilization. FL·HRH (1 mg of uronic acid) in 0.4 mL of borate buffer, pH 8.0, was added to the tube followed by the rapid addition of 1 mCi of $Na^{125}I$. After 20 min at 0 °C, the reaction was terminated by removing the mixture from the insoluble Iodo-Gen, and then NaI was added to a final concentration of 250 mM. The sample was desalted on a PD-10 column in 10 mM HEPES, 10 mM $CaCl_2$, and 0.01% NaN_3, pH 8.0, and fractionated on an LDL AffiGel-10 column in equilibration buffer as described above. ^{125}I-HRH was dialyzed against 50 mM HEPES, 0.1 M NaCl, and 0.01% NaN_3, pH 7.4. The specific radioactivity was 100 dpm/ng of uronic acid.

Heparin-Binding Assays. Steady-state fluorescence polarization measurements of the binding of LDL to fluoresceinamine-labeled HRH were performed on an SLM–Aminco 4800 spectrofluorometer (SLM Instruments Inc., Urbana, IL). Polarization measurements were performed with the instrument configured in the T format with Glan–Thompson polarizers; all readings were signal-averaged × 10. Experiments were performed in quadruplicate and analyzed as the mean ± SEM. The emission (channel B) was observed through an Ealing (South Natick, MA) filter (35-3557) with an E_{max} of 510 nm and 7.7-nm bandwidth at half-height; the A channel was observed through an emission monochromator set at 510 nm and 8-nm slitwidth. Polarization values of LDL alone were insignificant and therefore disregarded. Excitation–emission wavelengths were 465 and 510 nm, respectively. FL·HRH (1.3 μM in heparin) was titrated with increasing amounts of a 7.5 mg of protein/mL solution of LDL (from 0.1 to 4.7 μM lipoprotein). Titrations were performed in 2.0 mL of 50 mM HEPES, 0.1 M NaCl, and 0.01% NaN_3, pH 7.4, and reactant concentrations were corrected for dilution. In some titrations, 3 mM $CaCl_2$ was included. In other titrations, the ability of unmodified HRH to displace FL·HRH from its soluble complex with LDL was determined. In these experiments, soluble complexes of FL·HRH (0.98 μM) and LDL (4.7 μM) were titrated with increasing amounts of HRH (from 1 to 20 μM), and the decrease in steady-state fluorescence polarization was recorded. All experiments were corrected for viscosity effects due to added lipoprotein by performing control titrations with acetylated LDL which does not bind heparin. Curves were generated by fitting the experimental data to the relation $\Delta P/\Delta P_{max} = [LDL]/(K_d + [LDL])$, where ΔP is the incremental change in polarization, ΔP_{max} the maximal change in polarization, and K_d the apparent dissociation constant for the LDL–heparin interaction. The polarization measurements were utilized as plots of $\Delta P/\Delta P_{max}$ vs. [LDL]/[FL·HRH] or vs. [HRH]/[FL·HRH].

Soluble complexes of LDL and ^{125}I-HRH were quantitated by a filtration assay described previously (Cardin et al., 1986b). Briefly, LDL (1.2 μM) were titrated with increasing amounts of ^{125}I-HRH (from 2.8 to 52 μM). In these experiments, a fixed amount of ^{125}I-HRH was diluted with unmodified HRH to achieve the desired molar concentrations of heparin. Titrations were performed in a total volume of 1 mL of 50 mM HEPES, 0.1 M NaCl, and 0.01% NaN_3, pH 7.4, and incubated for 30 min before being filtered. Complexes were collected on 0.025-μm filters and subjected to a single wash with 1.0 mL of incubation buffer, and the amount of ^{125}I-HRH bound to LDL was determined by γ counting. Nonspecific binding was determined to be less than 2% when titrations were conducted in the presence of 1 M NaCl to dissociate the complexes or with acetylated LDL.

Insoluble complexes of LDL and heparin were quantitated as previously described (Cardin et al., 1986b). Briefly, LDL (0.42 μM) were titrated with increasing amounts of HRH (from 2 to 55 μM) in 1-mL total volume of 50 mM HEPES, pH 7.4, 0.1 M NaCl, 0.01% NaN_3, and 20 mM $CaCl_2$. The samples were incubated for 30 min at 25 °C, and insoluble complexes were pelleted by low-speed centrifugation. Phospholipid in the pellets was determined by the method of Bartlett (1959). Uronic acid was measured according to Bitter and Muir (1962); the weight ratio of HRH to total uronic acid was 6.0. In other experiments, insoluble complexes of LDL and ^{125}I-HRH were collected by vacuum filtration on 0.45-μm Metrical filters. The amount of ^{125}I-HRH in the complexes was determined by γ counting. Nonspecific binding was less than 5% after washing with 1.0 M NaCl or when titrations were conducted with acetylated LDL, as described above for the soluble complexes. In other experiments, identical concentrations of HRH, FL·HRH, and ^{125}I-HRH (0.8 μM) were titrated with increasing amounts of Ca^{2+} (5–60 mM) in the presence of LDL (0.2 μM) and then quantitated by light scattering at 620 nm. These experiments established that radioiodination and fluoresceinamine labeling of HRH did not alter the reactivity of heparin for LDL.

Other Methods. Protein was determined by the method of Lowry et al. (1951) with bovine serum albumin (Sigma) as standard. LDL were acetylated according to the method of Fraenkel-Conrat (1957). Briefly, 6 mL of LDL (20 mg of protein/mL) in 0.15 M NaCl, pH 7.4, was mixed with an equal volume of saturated sodium acetate at 0 °C. A total of 16 4-μL samples of acetic anhydride (Aldrich) were added over 90 min. After the last addition, the reaction was allowed to incubate for an additional 30 min. The sample was then dialyzed against 5 × 1 L changes of 50 mM HEPES, 0.1 M NaCl, and 0.01% NaN_3, pH 7.4.

Results

Commercial heparin was chromatographed on a column of LDL AffiGel-10 to purify the fraction specific for LDL (Cardin et al., 1984a). This fraction, designated high reactive heparin (HRH), was modified with fluoresceinamine and refractionated on LDL AffiGel-10. Greater than 90% of the applied material eluted with 0.5 M NaCl (data not shown), indicating that chemical modification did not reduce the binding of HRH to LDL.

The interaction of LDL and fluoresceinamine-labeled HRH (FL·HRH) in solution was monitored by steady-state fluorescence polarization. The data of Figure 1 (open circles) represent a nearly 2-fold increase in the observed steady-state

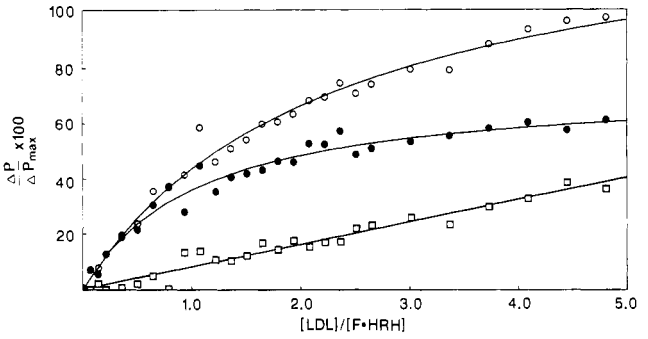

FIGURE 1: Effect of LDL on the fluorescence polarization of fluoresceinamine-labeled HRH in solution. Increasing amounts of LDL were added to FL·HRH in 50 mM HEPES and 0.1 M NaCl, pH 7.4, at 22 °C (see Materials and Methods). The binding of LDL to FL·HRH was monitored by the increase in steady-state fluorescence polarization. Results are expressed as the incremental change in fluorescence polarization (ΔP) divided by the maximal change (ΔP_{max}) normalized to 100%. The values represent four different experiments and are expressed as the mean ± SEM. The data were corrected for viscosity effects as described under Results. (○) Total change in polarization with added LDL; (□) change in polarization with added acetylated LDL; (●) viscosity-corrected binding calculated as the difference between the curve for LDL (○) and acetylated LDL (□).

fluorescence polarization of FL·HRH with added LDL. The polarization values increased from 0.117 in the absence of LDL to 0.213 in the presence of excess lipoprotein (4.7 μM). As can be seen, the titration curve does not show a well-defined plateau characteristic of saturable binding. One possible explanation for the ascending nature of the curve is an increase in the viscosity of the medium due to added lipoprotein. If so, the observed polarization at each point in the titration would represent a sum of polarization contributions due to binding and viscosity effects. The fluorescence polarization of FL·HRH in 100% glycerol is 0.265. The magnitude of this value indicates that the viscosity contribution to the observed polarization change is likely to increase throughout the titration and not obtain a limiting value. Therefore, LDL were acetylated to destroy heparin binding (Iverius, 1972), and the effect of lipoprotein viscosity on the fluorescence polarization of FL·HRH was then examined. The addition of acetylated LDL to a solution of FL·HRH (Figure 1, open squares) resulted in a gradual monotonic increase in fluorescence polarization, e.g., from 0.117 in the absence of acetylated LDL to a maximal value of 0.145. Subtracting the titration curve for acetylated LDL (open squares) from that of LDL (open circles) yields a curve corrected for viscosity (closed circles). This curve shows a well-defined plateau characteristic of saturable binding. Identical curves were obtained in the presence of 3 mM $CaCl_2$, indicating that this concentration of divalent cation does not affect the affinity or stoichiometry of the LDL–heparin interaction (not shown). Analysis of the data indicates a stoichiometry of two LDL particles per heparin chain (M_r 13 000) characterized by an apparent K_d of approximately 1 μM.

Figure 2 shows that greater than 90% of FL·HRH is displaced from the complex by unlabeled HRH. A 45% reduction in fluorescence polarization occurs at a 1:1 molar ratio of HRH/FL·HRH. The stoichiometric displacement of FL·HRH from LDL by HRH shows reversibility and provides further evidence that chemical modification of HRH does not alter its binding properties to LDL.

In the next experiment, the lipoprotein was titrated with ^{125}I-HRH in order to determine the number of heparin-binding sites on LDL. The HRH–LDL complexes were collected on filters, and the amount of LDL-associated heparin was determined. As shown in Figure 3, the binding of ^{125}I-HRH to LDL was saturable. A plot of the data by the method of Scatchard (1949) showed curvilinear behavior (Figure 3, inset). The curvilinearity was analyzed in terms of two major classes of sites by linear regression. Apparent K_d values of 1 and 10 μM with corresponding B_{max} values of 2 and 5 heparins bound per LDL particle were obtained. Acetylation of lysine residues on LDL completely inhibited the interaction as determined by this assay.

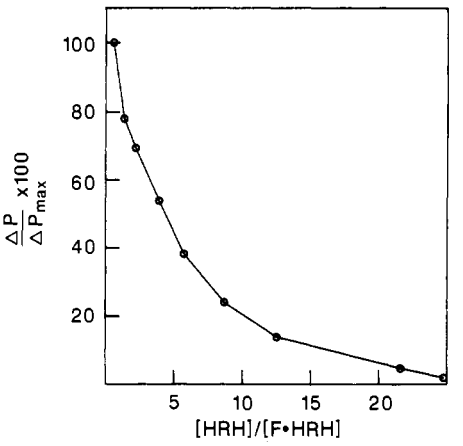

FIGURE 2: Displacement of fluoresceinamine-labeled HRH from LDL by HRH. Preformed soluble complexes of LDL and FL·HRH were titrated with HRH as described under Materials and Methods. The viscosity-corrected decrease in steady-state fluorescence polarization was taken as a quantitative measure of the amount of FL·HRH displaced from the complex. Results were analyzed as described in Figure 1.

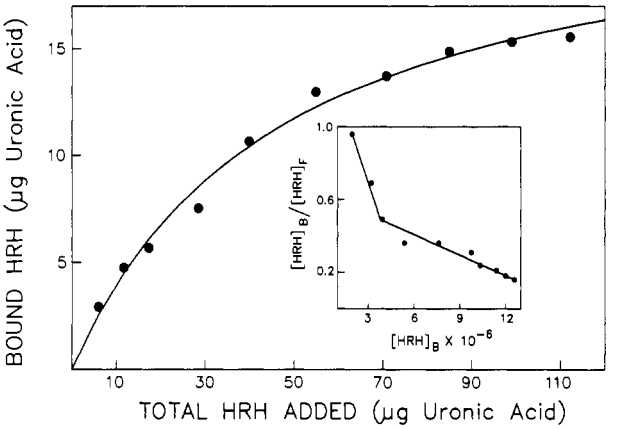

FIGURE 3: Binding of ^{125}I-HRH to human plasma LDL. LDL were titrated with increasing amounts of ^{125}I-HRH, and the resulting complexes were collected on 0.025-μm filters as described under Materials and Methods. The mass of ^{125}I-HRH bound was determined from the specific radioactivity. Results are expressed as micrograms of HRH bound vs. total micrograms of HRH added. Inset: Analysis of the binding data according to the method of Scatchard (1949).

In the presence of divalent metals, heparin precipitates LDL from solution (Burnstein & Scholnick, 1973). Figure 4 shows that preformed soluble complexes of LDL and HRH are maximally precipitated between 20 and 50 mM Ca^{2+}; the pH optimum for precipitation was approximately 7.4 (not shown). Acetylated LDL was not precipitated in the presence of HRH over the same range of Ca^{2+} concentrations, pH, and ionic strength of the medium (not shown).

Figure 5 shows that the Ca^{2+}-induced precipitation of the soluble HRH–LDL complexes is quantitative. In this experiment, increasing amounts of LDL were added to a constant amount of HRH (7.2 μg). After a 10-min incubation, $CaCl_2$ was added to a final concentration of 20 mM, and the amount of HRH in the precipitates was determined. The amount of

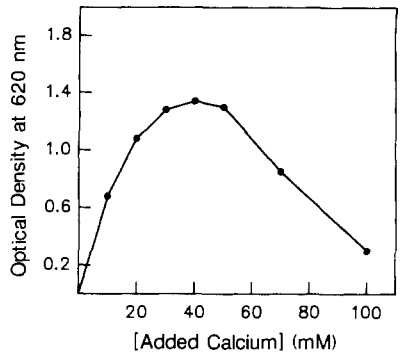

FIGURE 4: Calcium-dependent precipitation of LDL–HRH complexes. See Materials and Methods for details.

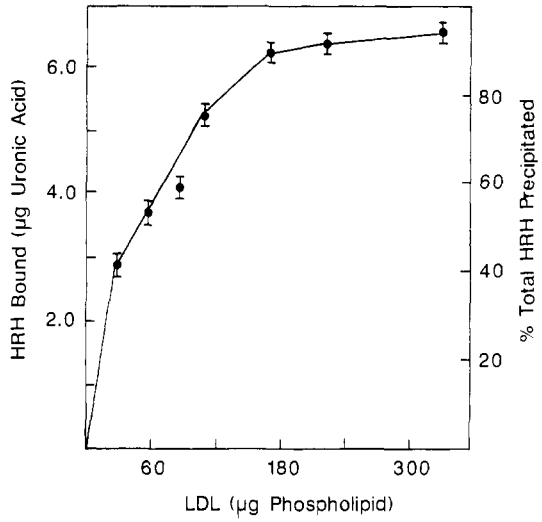

FIGURE 5: Quantitative precipitation of HRH by LDL and Ca^{2+}. HRH was precipitated by increasing the concentration of LDL in the presence of 20 mM Ca^{2+}. Complexes were collected, and the amount of heparin (as uronic acid) was determined in the pellet.

HRH precipitated at saturating levels of LDL accounted for 94% of the added HRH.

In the next experiment (Figure 6), the stoichiometry and affinity of HRH for LDL in the insoluble complexes were determined. LDL were titrated with increasing amounts of ^{125}I-HRH in the presence of 20 mM Ca^{2+}. The insoluble complexes were collected by filtration, and the amount of HRH in the complex was determined by γ counting. ^{125}I-HRH binding to LDL was saturable at 30–40 μg of uronic acid. Analysis of the binding by the method of Scatchard (1949) yielded a curvilinear plot having a major component characterized by an apparent K_d of 2 μM and a B_{max} of 10 HRH molecules per LDL particle.

Figure 7 shows that the stoichiometry of the insoluble products varies with the ratio of the reactants. At low ratios, insoluble complexes are formed with stoichiometries similar to those of soluble complexes, e.g., 2–5 heparin molecules per LDL, whereas complexes contain 10 HRH molecules per LDL at saturation. These values, obtained by direct determination of the mass of HRH and LDL in pellets recovered by centrifugation, agree with the range of stoichiometries obtained by Scatchard (1949) analyses of the binding data (Figures 3 and 6).

Discussion

The purpose of this study was to determine the physical-chemical parameters of LDL–heparin interactions. Previously, we showed that a specific fraction of heparin, designated high reactive heparin, accounts for the LDL-binding and precipitating activity of commercial heparin preparations (Cardin et al., 1984b). At present, however, there are no data concerning the affinity or stoichiometry of heparin-binding sites on the LDL surface, and hence no indication of the number of peptide domains involved in heparin binding.

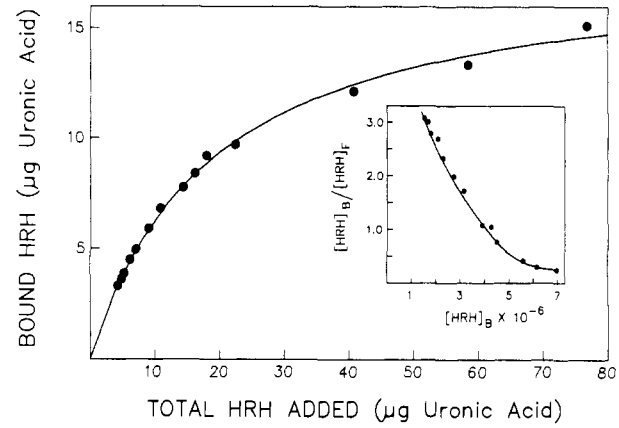

FIGURE 6: Quantitation of ^{125}I-HRH in insoluble heparin–LDL complexes. LDL were titrated with increasing amounts of ^{125}I-HRH in the presence of 20 mM Ca^{2+} as described under Materials and Methods. Complexes were isolated, and the amount of bound heparin was determined. Inset: Analysis of the binding data according to the method of Scatchard (1949).

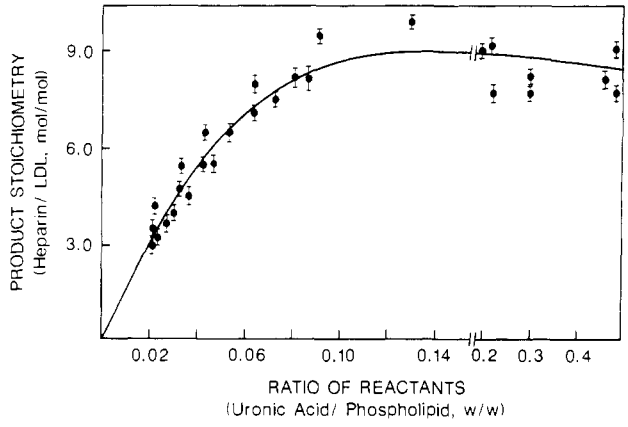

FIGURE 7: Stoichiometry of insoluble HRH–LDL complexes. HRH (2–36 μM) was added to LDL (5.3 μM) in the presence of 20 mM Ca^{2+}. Complexes were collected, and the amount of heparin and LDL in the complexes was determined. The data show the stoichiometry of the isolated complexes as a function of added HRH and LDL.

We devised a steady-state fluorescence polarization assay to monitor the binding of LDL to fluoresceinamine-labeled HRH; a similar assay was used to quantitate the binding of various hemostatic enzymes to a fluorescently tagged heparin (Jordan et al., 1980). The binding of LDL to FL-HRH was saturable, reversed by unlabeled HRH, and inhibited by chemical modification of lysine residues on LDL. The solution complex contained two LDL molecules per HRH (M_r 13000), indicating a bifunctional cross-linking property of the heparin. The interaction was characterized by an apparent K_d of approximately 1 μM, and neither the stoichiometry, affinity, nor solubility of the complex was affected by 3 mM Ca^{2+}. Mitterer et al. (1982) and Eigner et al. (1982) studied heparin–LDL interactions by viscometry and quasi-elastic light scattering and reported that one heparin molecule links to several LDL particles. In contrast to the affinity-fractionated HRH, these investigators used heparins that were size fractionated on Sephacyl-300, and, thus, relative affinities were not reported.

In addition to the fluorescence polarization assay, a filtration assay was used to study the interaction of heparin with LDL in solution. Since 1.0 M NaCl or acetylation of lysine residues on LDL reduced binding to below 5% of that obtained with

LDL, these results demonstrated that the filter-retained ^{125}I-HRH was specifically bound to the lipoprotein as opposed to being nonspecifically trapped on the filter. The binding data of Figure 3 indicate five to seven sites for the heparin interaction on the LDL surface with two sites of higher affinity. An alternative explanation for the curvilinearity of the data (Figures 3 and 6) is negative cooperativity in the binding. However, the multiple site interpretation is consistent with the stoichiometries of HRH and LDL in isolated complexes determined by mass measurements (see Figure 7) and with the recent purification and sequence analysis of multiple heparin-binding cyanogen bromide peptides of apoB-100 (Hirose et al., 1986a, 1987; Weisgraber et al., 1986b). Three of these peptides contain regions of high sequence homology with the heparin-binding domains of apoE (Cardin et al., 1986a; Weisgraber et al., 1986a) and vitronectin (Suzuki et al., 1985). Yamada et al. (1980) reported a similar method to quantitate the binding of heparin to fibronectin, a cell adhesive protein with multiple heparin-binding domains (Yamada et al., 1985).

Soluble complexes of LDL and HRH are maximally precipitated by Ca^{2+} in 50 mM HEPES and 0.1 M NaCl, pH 7.4. Srinivasan et al. (1970, 1975) proposed that Ca^{2+} bridges the anionic phospholipids on LDL with the acidic groups on heparin forming insoluble cross-linked aggregates. However, the experiments of Nishida and Cogan (1970) indicate a negligible role for phospholipids and a major role for protein in the formation of soluble and metal ion induced insoluble complexes of dextran sulfate and LDL. We show that acetylation of LDL inhibits both binding of HRH to LDL in solution and precipitation induced by Ca^{2+}. Others have reported that acetylation inhibits soluble complex formation (Iverius, 1972; Pan et al., 1978). Cyclohexanedione modification (Noel et al., 1981; Mahley et al., 1979), aceto-acetylation, and carbamylation (Mahley et al., 1979) of apoB inhibit the divalent metal cation induced precipitation of LDL–heparin complexes. These findings suggest that a major interaction between LDL and heparin to form soluble and insoluble complexes involves the electrostatic binding of basic amino acid residues on LDL with acidic groups on heparin. These studies, however, do not rule out a minor role for acidic groups on LDL participating with acidic groups on heparin bridged through Ca^{2+}. This mechanism would be consistent with reports that calcium and other ions bind to the LDL surface (Herak et al., 1984) and to heparin (Lerner & Torchia, 1986). In addition to heparin neutralizing protein positive charges, Ca^{2+} may neutralize negative charges on LDL and bound heparin, resulting in a complex with reduced surface charge. The precipitation of the complex may be analogous to the salting out and/or isoelectric precipitation of proteins (Scopes, 1982) in which hydrophobic patches on the surface promote aggregation and decreased solubility.

The present study shows multiple sites for heparin interaction and is consistent with the identification of five unique heparin-binding domains from apoB-100 (Hirose et al., 1986, 1987). In addition, the affinity of HRH for LDL is similar in magnitude to that determined recently for the binding of LDL to isolated human arterial proteoglycans (Camejo et al., 1985). Srinivasan et al. (1986) showed that heparin inhibits aortic uptake of LDL. In view of these findings, it is possible that the heparin-binding sites characterized in this study relate physiologically to the binding of LDL to arterial tissue proteoglycans and may have relevance to the pathogenesis of atherosclerosis.

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Registry No. Lys, 56-87-1; Ca, 7440-70-2; heparin, 9005-49-6.

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Dihydropyridine-Sensitive Calcium Channels in Cardiac and Skeletal Muscle Membranes: Studies with Antibodies against the α Subunits†

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ABSTRACT: Polyclonal antibodies (PAC-2) against the purified skeletal muscle calcium channel were prepared and shown to be directed against α subunits of this protein by immunoblotting and immunoprecipitation. These polypeptides have an apparent molecular weight of 162 000 without reduction of disulfide bonds. Under conditions where the functional properties of the purified skeletal muscle calcium channel are retained, β subunits (M_r 50 000) and γ subunits (M_r 33 000) are coprecipitated, demonstrating specific noncovalent association of these three polypeptides in the purified skeletal muscle channel. PAC-2 immunoprecipitated cardiac calcium channels labeled with [^{3}H]isopropyl 4-(2,1,3-benzoxadiazol-4-yl)-1,4-dihydro-2,6-dimethyl-5-(methoxycarbonyl)pyridine-3-carboxylate ([^{3}H]PN200-110) at a 3-fold higher concentration than skeletal muscle channels. Preincubation with cardiac calcium channels blocked only 49% of the immunoreactivity of PAC-2 toward skeletal muscle channels, indicating that these two proteins have both homologous and distinct epitopes. The immunoreactive component of the cardiac calcium channel was identified by immunoprecipitation and polyacrylamide gel electrophoresis as a polypeptide with an apparent molecular weight of 170 000 before reduction of disulfide bonds and 141 000 after reduction, in close analogy with the properties of the α_2 subunits of the skeletal muscle channel. It is concluded that these two calcium channels have a homologous, but distinct, α subunit as a major polypeptide component.

Voltage-sensitive calcium channels play important roles in the regulation of the calcium-linked cellular functions including muscle contraction, neurotransmitter and hormone release, and calcium-dependent phosphorylation of intracellular proteins by controlling calcium influx from the extracellular environment (Hagiwara & Byerly, 1981; Tsien, 1983). Dihydropyridine calcium channel antagonists block calcium channel function in smooth, cardiac, and skeletal muscle cells at low concentrations (Triggle, 1981; Janis & Scriabine, 1983). High-affinity binding sites for [^{3}H]dihydropyridines have been identified in skeletal, smooth, and cardiac muscles and brain (Janis & Scriabine, 1983; Janis & Triggle, 1984) and have been successfully solubilized by digitonin and 3-[(cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS)[1] (Curtis & Catterall, 1983, 1984; Glossmann &

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