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The effect of heparin on structural and functional properties of low density lipoproteins

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Abstract

Heparin binding to human low density lipoproteins (LDL) and the effect of heparin on the ability of LDL to bind to the LDL receptor has been investigated. Emphasis has been made on the physiological conditions of temperature, pH and the ionic strength. Intrinsic fluorescence spectroscopy of LDL has been applied to follow heparin binding. Fluorescence anisotropy has been measured to describe the changes in apoB and dansyl–heparin dynamics upon binding. Eu^{3+}-labeled LDL binding to the intact LDL receptor has been monitored by time-resolved fluorescence spectroscopy technique. We have found that heparin binds to LDL under the physiological conditions, probably by Van der Waals interactions and hydrogen bonding. Temperature seems to be the most important factor influencing the interaction. Furthermore, the presence of heparin inhibits LDL binding to the intact LDL receptor that might have consequences on the cholesterol metabolism in vivo.

Keywords: LDL; Heparin; Fluorescence; LDL receptor; Binding

1. Introduction

Proteoglycans (PGs) are important components of the environment of extracellular matrices, including cartilage, basement membrane and connective tissue. However, they are also found in abundance on the cell surface. They are defined as proteins with glycosaminoglycans (GAGs) attached to specific serine residues. GAGs are long, unbranched, polycarbohydrates consisting of disaccharide repeating units that are different for different classes of GAGs. For instance, heparin is a linear polymer consisting of $1\rightarrow4$ linked pyranosyluronic acid and 2-amino-2-deoxyglucopyranose (glucosamine) residues. It has the highest negative charge density of all known biological macromolecules as a result of the high content of sulfo and carboxyl groups [1]. Apart from being abundant at the surface of artery walls, it is widely used as an anticoagulant drug. With the increasing numbers of the discovered heparin-binding proteins, there was a need to characterize the properties of the sites on proteins responsible for specific recognition of heparin. Based on peptide analysis, it has been concluded that positively charged amino acid residues such as lysine or arginine, were responsible for heparin binding [2]. Although there were several attempts to determine a consensus sequence for heparin binding to proteolitically digested proteins in general, extensive research has shown that the motif hypothesis is not always valid for proteins in solution. Namely, according to the data obtained it was suggested that steric fit between heparin and proteins is important in mediating the interaction [1].

In this study the research of heparin binding to low density lipoproteins (LDL) was addressed with a special emphasis to the native integrity of the particle with respect to its constituents: apolipoprotein B100 (apoB), lipids and carbohydrates. The constituents are organized in a hydrophobic core of apolar lipids and the surface phospholipid monolayer stabilized

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by the amphipatic protein, apoB [3]. During its lifetime in plasma, LDL may interact with cells in several different ways: LDL-receptor-mediated endocytosis [4], LDL-receptor-independent pathway [5] and macrophage uptake of LDL by scavenger receptor [6]. The most important one is LDL binding to LDL receptor situated in plasma membranes, inducing the endocytosis of the entire particle [4]. In addition, Williams et al. [5] have found that cell surface heparan sulphate, together with the enzyme lipoprotein lipase, is crucial for LDL-receptor-independent LDL binding and uptake by cells.

As for other proteins, many aspects of LDL–heparin interaction are not yet fully understood and therefore call for further investigation. Namely, previous research has identified seven distinct heparin-binding apoB peptides. It should be stressed that these peptides were obtained by using combination of proteolytical digestion of apoB and heparin–sepharose affinity chromatography [7]. Therefore, the aim of this study was to describe the interaction between heparin and entire native LDL particles in solution using intrinsic fluorescence spectroscopy. Special emphasis has been made not only on physiological conditions in human plasma, but also on keeping the structural integrity of LDL particles. Fluorescence anisotropy has been measured to describe the changes in both apoB and dansyl–heparin dynamics upon binding. The conditions of temperature and ionic strength of the solution were modified in order to obtain information on the LDL–heparin interaction under non-physiological vs. physiological conditions. Furthermore, the effect of heparin on LDL binding to the intact LDL receptor in the cell culture was investigated using the europium labeled LDL samples. This analysis has been discussed in the framework of the functional properties of LDL in the bloodstream.

2. Experimental

2.1. Chemicals

Both pure heparin and dansyl-labeled heparin (purity 99.7%) were obtained from Calbiochem as a lyophilized powder isolated from porcine intestinal mucosa. Their molecular weight distribution is in the range from 13 500 to 15 000. All other chemicals were obtained from Kemika, Zagreb.

2.2. LDL isolation

LDL was isolated from 3 normolipidemic donors by density gradient ultracentrifugation as described previously [8,9] using the fixed angle rotor Ti70 on a Beckman preparative ultracentrifuge. To avoid the LDL oxidation during its isolation, EDTA (1 g/dm^3) was present in all steps of the process and all the buffers were flushed with argon. The purity of LDL fraction was checked by electrophoresis using Radiophor electrophoresis system with lipidophor agar medium. Upon isolation LDL samples were stored in 10 mM phosphate buffer (PB), pH 7.4 with 1 g/L EDTA and flushed with argon. Protein concentration was measured by the method of Lowry et al. [10]. LDL concentration is referred to its protein content.

2.3. Cell culture

The human endothelial cell line HEK-293 (CRL-1573; LGC Promochem, Molsheim Cedex, France) was used for this study. HEK-293 cells were grown in DMEM (Dulbecco modified Eagle medium) containing 10% heat-inactivated fetal calf serum and were maintained in a humidified incubator at 37 °C in the presence of 5% CO_2. Endothelial cells were grown on glass cover slips to app. 80% confluency.

2.4. Heparin binding assays

The interaction between LDL and heparin was studied using Varian Cary Eclipse spectrofluorimeter using quartz cuvettes (1 cm). All experiments were performed at 25 and 37 °C. The wavelength of excitation of LDL was 280 nm and the fluorescence emission was monitored at 332 nm, which is the wavelength of tryptophan emission maximum [11]. It should be noted that at these wavelengths heparin exhibits negligible absorbance ($A<0.04$). A data sampling interval of 0.5 nm was used.

Fluorimetric titrations of LDL with heparin were performed at constant ionic strengths of 0.01 and 0.2 by adding portions of heparin stock solution (1.2×10^{-5} M) into the LDL solution (0.02 μM). The obtained data were corrected for dilution. An attempt to determine the binding constants, K_s, and the binding ratio, $n=[\text{heparin}]_{\text{bound}}/[\text{LDL}]$, according to the Scatchard equation [12], failed due to small total change in fluorescence emission intensity, which did not allow the collection of enough data for accurate calculation. However, fitting of titration data by classical Langmuir isotherm (Scatchard equation with fixed value of ratio $n=1$) gave excellent correlation coefficients ($r>0.99$), strongly suggesting formation of one dominant type of heparin–LDL complex and therefore indicating that most heparin binding sites on LDL are of equivalent affinity. Binding constants obtained in this way, K_s^{cum}, can be considered only as cumulative since the real value of binding constant depends on the unknown number of binding sites as follows: $K_s=K_s^{\text{est}}\times n_{[\text{bound heparin}]/[\text{LDL}]}$, K_s^{est} being the estimated binding constant. Nevertheless, these values of K_s^{cum} are reproducible and therefore properly characterize heparin/LDL interactions. The results presented here refer to one of the three LDL preparations which all gave consistent results.

Steady state anisotropy measurements of LDL were performed at 300 nm excitation and 340 nm emission detection. LDL samples exposed to different amounts of heparin were excited with vertically polarized light. Steady state anisotropy measurements of dansyl–heparin were performed at 340 nm excitation and 500 nm emission detection. Vertically (I_{vv}) and horizontally (I_{vh}) polarized fluorescence intensities were measured using L-geometry optical path. The anisotropy (r) was calculated using the relationship:

$$r=(I_{vv}-GI_{vh})/(I_{vv}+2GI_{vh}) \quad (1)$$

The correction factor (G) is given by $G=I_{hv}/I_{hh}$ and is determined by measuring the vertically (I_{hv}) and horizontally

(I_{hh}) polarized fluorescence intensities using the horizontally polarized excitation light.

2.5. Labeling of LDL with europium

Europium labelling of LDL has been performed following the protocol described by Wang et al. [13]. In brief, 2 mg of LDL sample was dialysed for 48 h against 50 mM solution of $NaHCO_3$ (pH between 8.3 and 8.5). After dialysis, 0.2 mg of reagent containing N^1-(p-isothiocyanobenzyl)diethylenetriamine- N^1, N^2, N^3, N^3 -tetraacetic acid chelated with Eu^{3+} (Delfia Eu labeling kit, Wallac Oy) was added into LDL solution and stored for 16–18 h at 25 °C in the dark. The excess of reagent was removed by gel filtration on PD-10 column in 10 mM PBS, pH 7.4 in the presence of 1 g/L EDTA. This procedure was followed by 48 h dialysis against the same buffer.

2.6. Preparation of LDL–heparin complexes and their binding to LDL receptor

Stock solutions of 6 different complexes of Eu^{3+}-labeled LDL with heparin were prepared by adding portions of heparin stock solution into Eu^{3+}–LDL solution (5 μg/mL) so that the final heparin concentrations were: 0.025, 0.05, 0.075, 0.1, 0.15 and 0.2 μM. It should be noted that the concentrations of heparin are chosen according to the binding curves obtained in the previous stages of this study.

HEK-293 cells were plated on 96-well plates and incubated with different heparin–Eu^{3+}–LDL complexes in storage buffer (SB, in mM: 2 $CaCl_2$, 135 NaCl, 1 $MgCl_2$, 5 KCl, 10 HEPES, 2.6 $NaHCO_3$, 0.44 KH_2PO_4 and 10 D-glucose, pH 7.4) for 2 h at 4 °C. Unbound Eu^{3+}–LDL and Eu^{3+}–LDL–heparin complexes were washed out on time with 10 mM PBS, pH 7.4. Time-resolved fluorescence of bound LDL samples on HEK-293 was measured after addition of 200 μL enhancement solution with a VICTOR™ Multilabel Fluorescence Counter (EG&G® Wallac, Vienna, Austria) as described previously [13]. The decay of fluorescence is measured and integrated expressed in fluorescence counts [14]. The cell protein content was estimated with the bicinchoninic acid (BCA) Protein Assay Kit (Pierce, VWR, Vienna, Austria).

3. Results

The LDL emission maximum assigned to apoB tryptophan residues was used to describe heparin binding to LDL. The results of the LDL titrations with heparin at the temperature of 25 °C are presented in Fig. 1. At both ionic strengths of 0.01 and 0.2 (Fig. 1a and b, respectively), upon titration with heparin, apoB fluorescence quenching (10%) is detected suggesting that heparin indeed interacts with LDL under these conditions. Processing of the titration data by Scatchard equation, under the assumption of 1:1 stoichiometry, gave binding constants of $K_s^{cum}=(7.3\pm0.5)\times10^5$ mol^{-1} dm^3 at $I=0.01$ and $K_s^{cum}=(1.3\pm0.4)\times10^6$ mol^{-1} dm^3 at $I=0.2$. Furthermore, titration with heparin performed at 37 °C (Fig. 2), resulted in 7% quenching of LDL fluorescence at both $I=0.01$ and $I=0.2$.

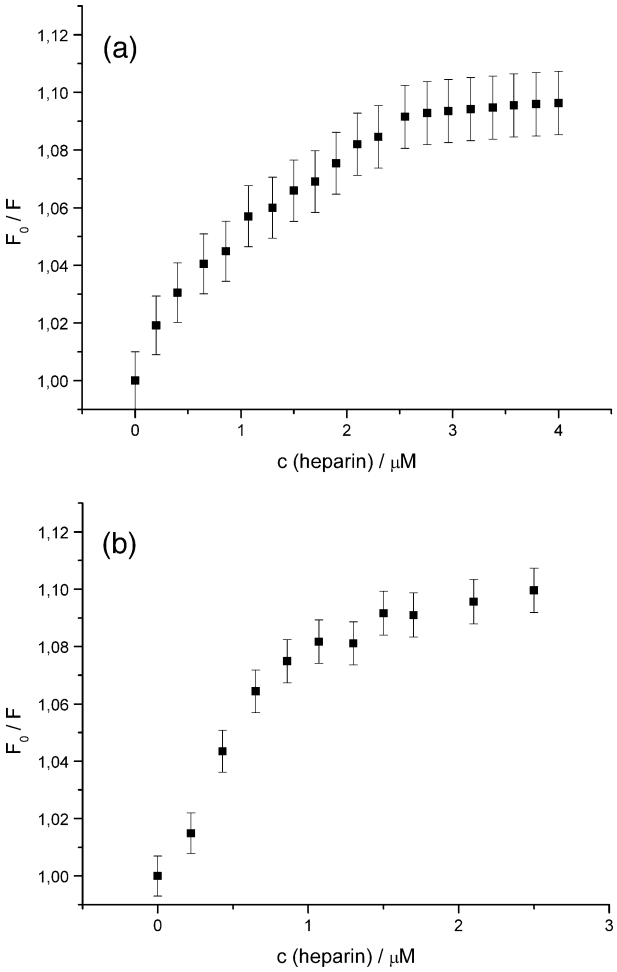

Fig. 1. Steady state fluorescence quenching of LDL detected in the presence of increasing amounts of heparin at 25 °C. LDL sample (0.02 μM) is titrated with 1.2×10^{-5} M heparin at the ionic strength of a) $I=0.01$ and b) $I=0.2$. The fluorescence intensity at 332 nm (F) is normalized to the intensity (F_0) for LDL in the absence of heparin. Standard error of the measurement is indicated by error bars.

Sigmoidal shape of the titration curves (Fig. 2) implies positive cooperativity of the interaction. Again, small total change of LDL fluorescence hampered collection of enough titration data for accurate processing by means of McGhee and von Hippel equation [15] given for cooperative binding of small molecules to large polymers. Therefore, it is only possible to state that the binding of heparin to LDL at 37 °C occurred in the similar concentration range of heparin as observed at 25 °C.

The changes in steady state fluorescence anisotropy of LDL upon titration with heparin are presented in Table 1. The obtained results indicate a decrease in anisotropy with the increase in heparin concentration, at all conditions of temperature and ionic strength applied.

The changes in steady state anisotropy of dansyl labelled heparin were measured upon titration with LDL. The results of these experiments are summarized in Table 2. Very low initial values of dansyl–heparin anisotropy are followed by the increase in its anisotropy upon successive additions of LDL solution, which is also consistent at all conditions of temperature and ionic strength applied.

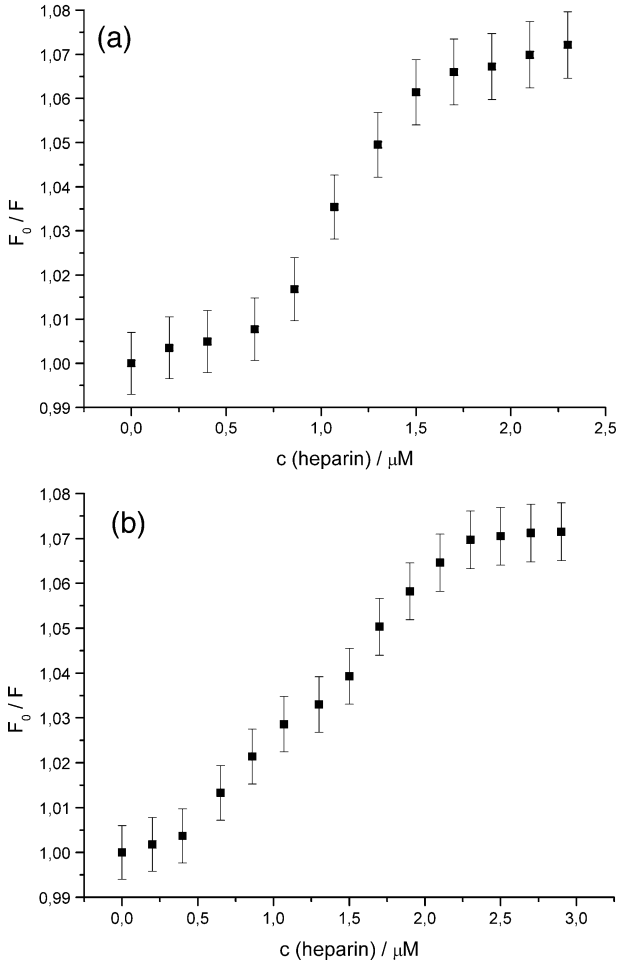

Fig. 2. Steady state fluorescence quenching of LDL detected in the presence of increasing amounts of heparin at 37 °C. LDL sample (0.02 μM) is titrated with 1.2×10^{-5} M heparin at the ionic strength of a) $I=0.01$ and b) $I=0.2$. The fluorescence intensity at 332 nm (F) is normalized to the intensity (F_0) for LDL in the absence of heparin. Standard error of the measurement is indicated by error bars.

In order to investigate the effect of heparin on functional properties of LDL particles, the influence of heparin on LDL capability to bind to LDL receptor was measured. The results of these experiments are presented in Fig. 3. The data reveal that LDL particles bind to LDL receptor with lower efficiency in the presence of heparin. Namely, already in the presence of the lowest concentration of heparin that showed the initial binding to LDL (0.02 μM), the binding of LDL to LDL receptor is significantly inhibited, as it remains for all heparin concentrations applied in the experiment.

Table 1
Fluorescence steady state anisotropy values of LDL during the titration of LDL (0.02 μM) with heparin

c (heparin)/μM	25 °C		37 °C	
	$I=0.01$	$I=0.2$	$I=0.01$	$I=0.2$
0	0.254±0.004	0.259±0.001	0.250±0.006	0.257±0.005
0.2	0.249±0.005	0.254±0.004	0.249±0.006	0.257±0.005
0.4	0.244±0.008	0.249±0.005	0.248±0.005	0.255±0.006
0.8	0.236±0.004	0.239±0.003	0.244±0.008	0.244±0.004
1.3	0.233±0.006	0.236±0.004	0.239±0.008	0.242±0.008
1.5	0.233±0.007	0.235±0.008	0.239±0.005	0.241±0.008

Table 2
Fluorescence steady state anisotropy values of dansyl–heparin (1.2×10^{-5} M) during its titration with LDL

c (LDL)/μM	25 °C		37 °C	
	$I=0.01$	$I=0.2$	$I=0.01$	$I=0.2$
0	0.003±0.001	0.008±0.003	0.005±0.002	0.003±0.003
0.01	0.005±0.002	0.008±0.003	0.006±0.002	0.006±0.004
0.02	0.005±0.001	0.009±0.004	0.006±0.003	0.007±0.004
0.05	0.009±0.003	0.018±0.002	0.009±0.003	0.019±0.005
0.06	0.018±0.003	0.019±0.005	0.013±0.002	0.029±0.005
0.08	0.019±0.004	0.020±0.003	0.014±0.005	0.030±0.003

4. Discussion

The aim of this study is to investigate the interaction between LDL and heparin molecules with the special emphasis on physiological conditions (phosphate buffer, $T=37$ °C, $I=0.2$). The conditions of temperature and ionic strength of the solution are varied in order to obtain information on the dominant type of interaction between heparin and LDL. The effect of heparin on the LDL binding to LDL receptor is investigated, reflecting the functional properties of LDL in the presence of heparin.

The results obtained from fluorescence titrations confirm the binding of heparin to LDL. Upon exposure of LDL to heparin, the intrinsic fluorescence of the particle is quenched under all conditions of temperature and ionic strength applied. This observation may indicate that: i) tryptophan residues are a part of the heparin binding site and are quenched by the direct interactions with heparin, or ii) a conformational change of LDL occurs upon heparin binding, which exposes tryptophan residues to the polar solvent resulting in fluorescence quenching. Furthermore, the analysis of the titration data obtained at 25 °C reveals a non-cooperative type of interaction and the values of the binding constants are in accord with the ones

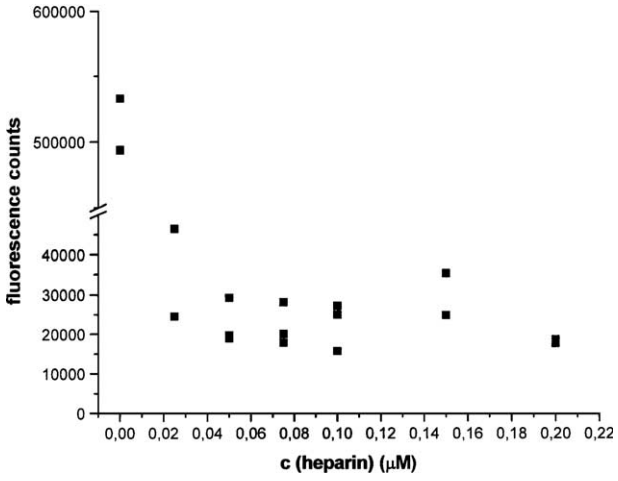

Fig. 3. Cell binding of LDL samples to HEK-293 in the absence and presence of increasing amounts of heparin. Cells were incubated with 10 μg/mL of Eu^{3+}-labeled LDL for 4 h at 4 °C. Squares indicate three repeated binding experiments.

obtained by Gigli et al. [16]. Namely, they have determined, using fluorescein-labeled heparin, that heparin binding to LDL is non-cooperative and characterized by the binding constant of 4×10^6 mol dm^{-3} under non-physiological conditions (HEPES buffer, I=0.1, 22 °C). In our study, no significant difference is observed between the binding constants at I=0.01 and I=0.2. However, due to the chemical and structural complexity of LDL particle, one cannot exclude the contribution of electrostatic interactions in the formation of LDL–heparin complexes based on this observation. In addition, hydrogen bonding possibly combined with Van der Waals interactions (steric fit) between heparin and LDL surface topology could contribute to heparin–LDL interaction. Interestingly, our results point to the temperature as a significant factor influencing the LDL–heparin interaction. This phenomenon could be correlated to the fact that LDL core experiences a temperature-dependent phase transition around 28 °C [17], which induces structural changes at the LDL surface [18]. Namely, the analysis of the titration data at 37 °C indicates that heparin binding to LDL is positively cooperative. This result is in accord with previously reported observation that, upon initial binding of GAGs to LDL, there is an apparent increase in the exposure of arginine and lysine containing apoB segments, suggested by increased susceptibility to trypsin hydrolysis and increased binding of GAGs [19]. Based on the obtained results, the following mode of heparin association with LDL at 37 °C might be proposed: after the initial binding of heparin to LDL surface structures, the exposure of arginine and lysine containing apoB segments increases, and heparin binding proceeds successively more efficient until it reaches saturation. The results of apoB anisotropy measurements support such reasoning. High initial values of apoB tryptophan anisotropy indicate a restricted environment of tryptophan residues. The decrease in anisotropy in the presence of heparin indicates an increase of motional freedom imposed on tryptophan residues in their environment. The collected data are consistent for all the conditions of temperature and ionic strength applied. Therefore, it is likely that upon heparin binding, a conformational change of LDL particle takes place leading to exposure of tryptophan residues towards the polar solvent. As a result, their fluorescence is quenched and their mobility increased. On the other hand, the increase in anisotropy of dansyl–heparin upon exposure to LDL reveals a decrease in its mobility. It is thus possible that heparin molecules are anchored at the LDL surface primarily by hydrogen bonding between arginine/lysine side chains and heparin molecule and possibly by additional Van der Waals interactions.

The importance of heparin interaction with LDL is demonstrated by the experiments of LDL binding to the LDL receptor. It should be stressed that according to Wang et al. [13] the method applied for LDL–LDL receptor interaction monitoring is indeed specific for this interaction. Therefore, the results indicate that the presence of heparin inhibits LDL binding to LDL receptor. This is consistent with the finding of Brown et al. [20] that certain basic proteins or peptides inhibit LDL binding to its receptor. Namely, several segments of the apoB form the LDL receptor binding domain. Positively charged segments of the polypeptide from 3145 through 3157 and from 3359 through 3367 are brought together by the disulfide bonding between cysteines 3167 and 3297 to form currently most plausible candidate for LDL receptor binding site exposed to the surface of the particle [21]. In addition, Borén et al. [22,23] have identified the segment of amino acid residues from 3359 to 3369 as an LDL receptor binding site which also shows proteoglycan-binding activity. Other positively charged polar segments that show great affinity for heparin seem to be clustered around the putative receptor binding regions [24]. Therefore, it is likely that the conformational change upon heparin binding causes the formation of such structures on LDL particle, which shield the LDL receptor binding site. The findings of Borén et al. [25] support such reasoning. Namely, according to their study the disruption of the interaction between arginine 3500 and tryptophan 4369 of the apoB results in a conformational change resulting in the impaired LDL receptor binding. Following the same reasoning, we have demonstrated that the tryptophan fluorescence emission is changed upon heparin binding, implying apoB conformational change which might be related to the diminished LDL receptor binding in the presence of this ligand. It cannot be excluded that heparin itself disrupts the interaction between arginine 3500 and tryptophan 4369 while competing for the arginine residue thus disrupting the interaction between LDL and the receptor. It can also be proposed that the abundance of negative charges on both heparin–LDL complexes and LDL receptor induce electrostatic repulsion. Due to such event, LDL particle interacting with heparin cannot approach the receptor. This finding is particularly important considering the fact that heparin is a widely used anticoagulant drug which can bind to LDL when present free in plasma. LDL is thus disabled to bind to its receptor and therefore its circulation time is prolonged. As a consequence, dietary cholesterol will fail to be delivered to target cells. Intracellular cholesterol concentrations would appear to be low, which would stimulate LDL receptor synthesis and their number in the cell membrane would increase. Structurally altered LDL may instead be taken up by a scavenger receptor into the macrophages. Therefore, these probably competitive associations may modulate the extracellular deposition and cellular uptake of LDL in both normal intima and during atherogenesis.

5. Conclusions

In conclusion, we have found that heparin binds to LDL under physiological conditions. The binding is described as positively cooperative at 37 °C but non-cooperative at 25 °C. Based on the fluorescence anisotropy measurements, the mobility of apoB tryptophan residues is increased upon heparin binding. On the other hand, heparin mobility is decreased, probably due to anchoring onto the LDL surface through hydrogen bonding and Van der Waals interactions. Furthermore, exposure to heparin inhibits LDL binding to LDL receptor. These findings raise interesting questions concerning the distribution of LDL particles interacting with GAGs at the cell surface, as well as with the LDL receptors in the cell membrane.

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References

[1] I. Capila, R.J. Linhardt, Heparin–protein interactions, Angew. Chem., Int. Ed. Engl. 41 (2002) 390–412.
[2] A.D. Cardin, H.J.R. Weintraub, Molecular modeling of protein–glycosaminoglycan interactions, Arteriosclerosis 9 (1989) 21–32.
[3] A.M. Gotto Jr., in: A. Neiberger, L.L.M. van Deenen (Eds.), New Comprehensive Biochemistry, Plasma Lipoproteins, vol. 14, Elsevier, New York, 1987.
[4] M.S. Brown, J.L. Goldstein, Familial hypercholesterolemia: defective binding of lipoproteins to cultured fibroblasts associated with impaired regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity, Proc. Natl. Acad. Sci. U. S. A. 71 (1974) 788–792.
[5] K.J. Williams, G.M. Fless, K.A. Petrie, M.L. Snyder, R.W. Brocia, T.L. Swenson, Mechanism by which lipoprotein lipase alters cellular metabolism of lipoprotein(a), low density lipoprotein, and nascent lipoproteins, J. Biol. Chem. 267 (1992) 13284–13292.
[6] M.S. Brown, J.L. Goldstein, Lipoprotein metabolism in the macrophage: implications for cholesterol deposition in atherosclerosis, Annu. Rev. Biochem. 52 (1983) 223–261.
[7] K.H. Weisgraber, S.C. Rall Jr., Human apolipoprotein B-100 heparin-binding site, J. Biol. Chem. 262 (1987) 11097–11103.
[8] G. Jürgens, H.F. Hoff, G.M. Chisolm, H. Esterbauer, Modification of human serum low density lipoprotein by oxidation–characterization and pathophysiological implications, Chem. Phys. Lipids 45 (1987) 315–336.
[9] H. Puhl, G. Waeg, H. Esterbauer, in: L. Parcker (Ed.), Methods to Determine Oxidation of Low-Density Lipoproteins, Methods in Enzymology, vol. 233, Academic Press, London, 1994, pp. 4235–4441.
[10] O.H. Lowry, N.J. Rosenbrough, A.L. Farr, R.J. Randall, Protein measurement with the Folin phenol reagent, J. Biol. Chem. 193 (1951) 265–275.
[11] A. Giessauf, E. Steiner, H. Esterbauer, Early destruction of tryptophan residues of apolipoprotein B is a vitamin E-independent process during copper-mediated oxidation of LDL, Biochim. Biophys. Acta 1256 (1995) 221–232.
[12] G. Scatchard, The attractions of proteins for small molecules and ions, Ann. N. Y. Acad. Sci. 51 (1949) 660–664.
[13] X. Wang, J. Greilberger, G. Jurgens, Time-resolved fluorometric assay for measuring cell binding and association of native and oxidized low-density lipoproteins to macrophages, Anal. Biochem. 267 (1999) 271–278.
[14] E. Soini, H. Kojola, Time-resolved fluorometer for lanthanide chelates—a new generation of non-isotopic immunoassays, Clin. Chem. 29 (1983) 65–68.
[15] J.D. McGhee, P.H. Von Hippel, Theoretical aspects of DNA–protein interactions: cooperative and non-cooperative binding of large ligands to a one-dimensional homogeneous lattice, J. Mol. Biol. 86 (1974) 469–489.
[16] M. Gigli, A. Consoni, G. Ghiselli, V. Rizzo, A. Naggi, G. Torri, Heparin binding to human plasma low-density lipoproteins: dependence on heparin sulfation degree and chain length, Biochemistry 31 (1992) 5996–6003.
[17] R.J. Deckelbaum, G.G. Shipley, D.M. Small, Structure and interaction of lipids in human plasma low density lipoproteins, J. Biol. Chem. 252 (1977) 744–754.
[18] S. Banuelos, J.L. Arrondo, F.M. Goni, G. Pifat, Surface–core relationship in human low density lipoproteins as studied by infrared spectroscopy, J. Biol. Chem. 270 (1995) 9192–9196.
[19] G. Camejo, E. Hurt, O. Wiklund, B. Rosengren, F. Lopez, G. Bondjers, Modifications of low-density lipoprotein induced by arterial proteoglycans and chondroitin-6-sulfate, Biochim. Biophys. Acta. 1096 (1991) 253–261.
[20] M.S. Brown, T.F. Deuel, S.K. Basu, J.L. Goldstein, Inhibition of the binding of low-density lipoprotein to its cell surface receptor in human fibroblasts by positively charged proteins, J. Supramol. Struct. 8 (1978) 223–234.
[21] U. Olsson, G. Camejo, E. Hurt-Camejo, K. Elfsber, O. Wiklund, G. Bondjers, Possible functional interactions of apolipoprotein B-100 segments that associate with cell proteoglycans and the ApoB/E receptor, Arterioscler. Thromb. Vasc. Biol. 17 (1997) 149–155.
[22] J. Borén, K. Olin, I. Lee, A. Chait, T.N. Wight, T.L. Innerarity, Identification of the principal proteoglycan-binding site in LDL, J. Clin. Invest. 101 (1998) 2658–2664.
[23] J. Borén, I. Lee, W. Zhu, K. Arnold, S. Taylor, T.L. Innerarity, Identification of the low density lipoprotein receptor-binding site in apolipoprotein B100 and the modulation of its binding activity by the carboxyl terminus in the familial defective apo-B100, J. Clin. Invest. 101 (1998) 1084–1093.
[24] L. Chan, Apolipoprotein B, the major protein component of triglyceride-rich and low density lipoproteins, J. Biol. Chem. 267 (1992) 25621–25624.
[25] J. Borén, U. Ekström, B. Ågren, P. Nilsson-Ehle, T.L. Innerarity, The molecular mechanism for the genetic disorder familial defective apolipoprotein B100, J. Biol. Chem. 276 (2001) 9214–9218.