Binding of lipoprotein lipase to α_2 -macroglobulin

Elisabet VILELLA,*†, Gunilla BENGTSOON-OLIVECRONA,†, Torgny STIGBRAND† and Poul Erik JENSEN†

*Center of Biomedical Research, Hospital de Sant Joan, C/ Sant Joan s/n, 43201 Reus, Spain

and †Department of Medical Biochemistry and Biophysics, University of Umeå, S-90187 Umeå, Sweden

The interaction between bovine lipoprotein lipase (bLPL) and human α_2 -macroglobulin (α_2 M) was studied by use of non-denaturing PAGE and gel-permeation, Zn^{2+} -Sephacrose and heparin-Sephacrose chromatography. It was demonstrated that bLPL *in vitro* binds non-covalently to native α_2 M, but not to the receptor-recognized form produced by treatment of α_2 M with chymotrypsin or methylamine. A small amount of bLPL was bound covalently to α_2 M by disulphide interchange, when incubated together with chymotrypsin or methylamine. Whereas

α_2 M in complex with bLPL still bound to Zn^{2+} -Sephacrose, bLPL lost the ability to bind to heparin-Sephacrose. Preincubation of bLPL with heparin prevented complex-formation with α_2 M, suggesting that α_2 M interacts with the heparin-binding domain of bLPL. Experiments in which ^{125}I -bLPL was incubated with human plasma at 20 °C demonstrated an 11–17 % binding of the labelled lipase to α_2 M, indicating that this interaction may be of physiological significance.

INTRODUCTION

Human α_2 -macroglobulin (α_2 M) is a large plasma glycoprotein (720 kDa). The protein consists of four identical subunits covalently held together as dimers and stabilized as tetramers by non-covalent forces [1,2]. α_2 M has three functional sites, i.e. a proteinase-sensitive region often denoted as the 'bait-region', an internal thiol ester, which is involved in covalent binding of proteinases on their inactivation, and a receptor-recognition site, which is exposed after bait-region cleavage [3]. α_2 M is a unique proteinase inhibitor able to inhibit proteinases from all four major classes of proteinases [4]. After proteolytic cleavage of the bait region, conformational changes cause cleavage of the thiol ester, and the proteinase can be covalently bound to α_2 M via an activated γ -glutamyl residue [5]. It has been demonstrated *in vitro* that non-proteolytic proteins, e.g. insulin, present during the proteolytic activation of α_2 M, can become covalently bound to α_2 M [6]. Recent investigations have demonstrated that α_2 M can bind a number of growth factors and cytokines such as nerve growth factor [7], platelet-derived growth factor [8], interleukin 1 β [9], interleukin 6 [10] and transforming growth factor α [11,12]. The binding of these proteins to α_2 M has been suggested to be non-covalent or covalent by disulphide interchange.

The α_2 M-proteinase complex is specifically cleared from the circulation by receptor-mediated endocytosis [13,14]. Much interest has been focused on the α_2 M receptor since it was demonstrated that this receptor is identical with the low-density-lipoprotein (LDL) receptor-related protein [15,16]. Besides α_2 M-proteinase complexes, the receptor can bind apolipoprotein E-containing lipoproteins [17,18], and this binding is enhanced by lipoprotein lipase (LPL) [19], which is shown to bind to the receptor as well [20].

LPL (EC 3.1.1.34) is a 110 kDa homodimeric glycoprotein which interacts with lipids, heparin and apoprotein CII [21]. It is a key enzyme in the catabolism of triacylglycerol-rich lipoproteins, i.e. chylomicrons and very-low-density lipoproteins (VLDL) [22]. The enzyme is only active in its homodimeric form

[23] and the presence of apoprotein CII is required for maximal activity [24]. After synthesis by parenchymal cells, LPL is transported to the luminal surface of the capillary endothelium, where it is anchored to membrane-associated heparan sulphate proteoglycans [21,22]. A low amount of LPL is found in circulating blood [25–28] and it was recently shown that this LPL is bound to lipoproteins [29]. The plasma concentration of LPL is kept low as the result of avid uptake and degradation by the liver [30].

During studies of the binding of LPL to lipoproteins by incubation of bovine ^{125}I -labelled LPL (^{125}I -bLPL) with plasma followed by non-denaturing electrophoresis for separation of lipoprotein particles [31], we observed a radiolabelled band with a Stokes' diameter similar to that of α_2 M. The purpose of the present study was to analyse the putative binding of ^{125}I -bLPL to α_2 M.

MATERIALS AND METHODS

Proteins and their derivatives

α_2 M was purified from human plasma as described [14] and stored in 0.1 M sodium phosphate, pH 8.0, at –20 °C. Radiolabelling with ^{125}I (Amersham) was performed using Iodobeads (Pierce). Free iodine was removed by passing the mixtures through a PD-10 column (Pharmacia) equilibrated with PBS, pH 7.4. The specific radioactivity of α_2 M was 100 c.p.m./ng. α_2 M was treated for 1 h at room temperature with 0.13 M methylamine with or without 1.5 mM iodoacetamide to produce the 'fast' form derivative of α_2 M. A twofold molar excess of α -chymotrypsin (EC 3.4.21.1) to α_2 M was used to produce an α_2 M-proteinase complex, with or without the presence of 1.5 mM iodoacetamide, for 3 min, and the proteinase activity was terminated by adding 1.2 mM phenylmethanesulphonyl fluoride (PMSF) for 5 min. Iodoacetamide and PMSF were removed by dialysis in PBS, pH 7.4, at room temperature for 1 h. bLPL was purified and iodinated as previously described [32]. The lipase was stored in buffer [10 mM Tris/HCl, 1 M

Abbreviations used: α_2 M, α_2 -macroglobulin; HDL, high-density lipoprotein; bLPL, bovine lipoprotein lipase; LDL, low-density lipoprotein; mAb, monoclonal antibody; PMSF, phenylmethanesulphonyl fluoride; VLDL, very-low-density lipoprotein.

† To whom correspondence should be addressed.

Table 1 Biometric, lipid and α_2 M data of subjects studied

Reference values in our population are: plasma cholesterol, 3.67–6.50 mmol/l; plasma triacylglycerols, 0.51–2.30 mmol/l; HDL cholesterol, 0.90–1.65 mmol/l, α_2 M in males, 1.5–3.5 g/l, and females, 1.8–4.2 g/l.

Subject	Sex	Age (years)	Plasma cholesterol (mmol/l)	Plasma triacylglycerol (mmol/l)	HDL cholesterol (mmol/l)	α_2 M (g/l)
I	F	29	5.0	2.11	2.4	1.1
II	M	36	6.5	3.35	1.1	1.4
III	M	34	6.3	1.29	2.4	0.9
IV	M	27	4.6	1.20	1.5	1.6
V	F	48	7.3	8.38	0.8	3.0

Figure 1 Non-denaturing PAGE of radiolabelled bovine LPL previously incubated with native α_2 M and its different derivatives

In the different incubations 30 μ l of dialysed 125 I-bLPL was incubated at a concentration of 7 nM with 1.44 μ M (10 μ l) native α_2 M and its derivatives in 0.1 M sodium phosphate, pH 8.0, for 1 h at 4 °C: lane 1, native α_2 M; lane 2, α_2 M treated with 0.13 M methylamine and 1.5 mM iodoacetamide; lane 3, α_2 M treated with 0.13 M methylamine in the presence of LPL; lane 4, α_2 M treated with a twofold molar excess of chymotrypsin and 1.5 mM iodoacetamide for 3 min and then terminated by adding 1.2 mM PMSF for 5 min; lane 5, α_2 M treated with a twofold molar excess of chymotrypsin in the presence of LPL; lane 6, α_2 M treated with a twofold molar excess of chymotrypsin in the presence of LPL; lane 7, α_2 M incubated with 0.13 M methylamine and 1.5 mM iodoacetamide in the presence of LPL; lane 8, α_2 M incubated with chymotrypsin in a twofold excess and 1.5 mM iodoacetamide in the presence of LPL; lane 9, 125 I-bLPL (not visible in a). The α_2 M derivatives (in lanes 2 and 4) were dialysed for 1 h at room temperature before incubation with LPL. (a) Gel stained with Coomassie Brilliant Blue; (b) autoradiography of the gel showing the binding of the 125 I-bLPL to native and derivatives of α_2 M.

NaCl, 20% (v/v) glycerol, 2 mg/ml BSA, pH 7.4]; the specific radioactivity was 7500 c.p.m./ng.

α_2 M- and LPL-binding assays

125 I-bLPL (45 ng) was incubated at a final concentration of 10 nM with 1.44 μ M (10 μ l) native α_2 M or derivatives of α_2 M in 0.1 M sodium phosphate, pH 8.0, for 1 h at 4 °C for non-denaturing PAGE assays. For gel-permeation chromatography 2 mg of native α_2 M and 750 ng of bLPL were incubated under the same conditions as above before application to the Superose 6 HR column. To assess the effect of heparin on the binding, parallel experiments were carried out with bLPL preincubated with heparin: iodinated or unlabelled bLPL was diluted and incubated with an equal volume of heparin (Fraxiparin, Sanofi Pharma SA; calcium heparinate, average molecular mass

4500 Da, range 1800–8000 Da, 10000 units/ml) for 30 min at 4 °C before incubation with native α_2 M.

Incubation of 125 I-bLPL with human plasma

Blood was obtained from five non-fasted volunteers and collected in tubes containing EDTA at a final concentration of 2.7 mM. A portion of plasma was used to measure cholesterol, acylglycerol, high-density lipoprotein (HDL) cholesterol and α_2 M concentrations. Plasma cholesterol, triacylglycerol and HDL cholesterol were measured by standard enzymic methods [33]. α_2 M was measured using a turbidimetric kit following the manufacturer's instructions (The Binding Site). The values are shown in Table 1. Plasma (400 μ l) was incubated with 100 μ l (150 ng) of 125 I-bLPL for 1 h at 20 °C; then 5 μ l of the mixture was diluted with sample buffer for the non-denaturing gradient gel electrophoresis, and, to visualize lipoproteins, the gel was stained with Oil Red O [34]. The rest of the mixture was diluted 1:1 with PBS, pH 7.4, and loaded on the Zn^{2+} -Sephacrose column in order to isolate the α_2 M- 125 I-bLPL complex. Incubations were performed at either 4 or 20 °C for 1 h. These temperatures, rather than 37 °C, were chosen in order to reduce hydrolysis of plasma lipoproteins by the lipase.

PAGE and autoradiography

Non-denaturing (5% gels) and SDS/PAGE (7.5% gels) was performed as described by Laemmli [35] in a Mini Protean System II (Bio-Rad). Non-denaturing electrophoresis in gradient gels (4–30%, Pharmacia) was performed in a buffer of Tris/borate/EDTA by the method of Krauss and Burke [34]. Gels to be autoradiographed were exposed at –70 °C to X-ray film using intensifying screens.

Gel-permeation chromatography

A Superose 6 HR 10/30 column installed in an h.p.l.c. system (LKB-Pharmacia) at 10 °C was equilibrated with PBS, pH 7.4, and run at a flow rate of 0.3 ml/min. Fractions of 0.3 ml were collected. The column was calibrated with a high-molecular-mass calibration kit (Sigma), and the void volume, determined by Blue Dextran elution, was at fraction 20. 125 I-bLPL run together with 130 μ g of unlabelled bLPL eluted with a peak of both activity and radioactivity in fraction 71.

Affinity chromatography

A chelating Zn^{2+} -Sephacrose 4B (Pierce) column was used to isolate α_2 M. Zn^{2+} -Sephacrose (1 ml of gel) was equilibrated with PBS, pH 7.4. After the samples (co-eluted α_2 M- 125 I-bLPL from

Figure 2 α_2 M and bLPL profiles from a gel-permeation chromatography

Purified human α_2 M (2 mg; ○) and bLPL (800 ng; ●) were incubated and applied to the Superose 6 HR column as described in the Materials and methods section. The collected fractions were tested by e.i.s.a. for detection of α_2 M and LPL (see the Materials and methods section). (a) α_2 M; (b) unlabelled bLPL plus 125 I-bLPL; (c) α_2 M incubated with unlabelled bLPL and 125 I-bLPL; (d) α_2 M incubated with 125 I-bLPL-heparin.

Table 2 Zn^{2+} -Sephacryl chromatography of the putative 125 I-bLPL- α_2 M complex isolated by gel-permeation chromatography

Native α_2 M (2 mg) and bLPL (750 ng) were incubated in a total volume of 0.6 ml for 1 h at 4 °C. Then 0.5 ml was loaded on to the Superose 6 HR column and chromatographed (for details see the Materials and methods section). The six peak fractions were pooled (1.8 ml total volume) and loaded on to the Zn^{2+} -Sephacryl column. A control of 125 I-bLPL was also run. Values represent the percentage of the total radioactivity loaded on the column and are means $\pm$ S.D. of triplicate experiments.

Sample	Wash I	Wash II	Elution of α_2 M		Wash III
			Peak	Non-peak	
125 I-bLPL	95.3 $\pm$ 3.1	1.1 $\pm$ 0.1	0.2 $\pm$ 0.1	0.3 $\pm$ 0.1	3.1 $\pm$ 0.3
125 I-bLPL- α_2 M complex	24.6 $\pm$ 1.7	12.9 $\pm$ 2.1	40.0 $\pm$ 0.3	10.5 $\pm$ 0.9	12.0 $\pm$ 1.7

the gel-permeation chromatography or the plasma incubated with 125 I-bLPL) had been loaded, the column was washed with 0.1 M sodium phosphate/0.8 M NaCl, pH 6.5, until the eluted radioactivity was as low as background (wash I). Then the column was washed with 0.02 M sodium phosphate/0.15 M NaCl, pH 6.0 (wash II). Elution of the α_2 M (+ 125 I-bLPL) was performed with 0.01 M sodium acetate/0.15 M NaCl, pH 5.0 (peak) and the same buffer was used to wash the column after the peak was eluted until radioactivity was as low as background (non-peak). Further elution was then carried out by adding EDTA to a concentration of 20 mM to the sodium acetate buffer (wash III). The Zn^{2+} -chelate chromatography was performed at 4 °C and at a flow rate of 1 ml/min. The procedure was as described for purification of α_2 M [14].

A heparin-Sepharose (2 ml of gel) column was used to bind LPL specifically. A linear gradient (30 ml) from 0 to 1.5 M NaCl in 10 mM Tris/HCl, pH 7.4, buffer containing 20% (v/v)

glycerol and 0.1% (v/v) Triton X-100 was used to elute LPL. The flow rate was 1 ml/min and 1 ml fractions were collected.

E.i.s.a.

Unlabelled α_2 M and LPL were detected by sandwich e.i.s.a. In both cases the microtitre plate was coated with a polyclonal antibody. A rabbit anti- α_2 M IgG (DAKO) was used to capture α_2 M. For LPL, a chicken IgG against bLPL was used [36], which was purified by adsorption to immobilized bLPL (LPL-Sepharose) and eluted with 0.2 M glycine, pH 2.7. After incubation with the polyclonal antibodies (10 μ g/ml in PBS, pH 7.4; 100 μ l/well) for 4 h at 37 °C, the plates were washed with PBS, pH 7.4. Then 100 μ l from each chromatographic fraction was incubated for 12 h at 4 °C. Fractions were diluted with PBS, pH 7.4, when necessary. The unbound material was washed off with PBS (pH 7.4)/0.05% (v/v) Tween 20. LPL was detected

with peroxidase-conjugated 2H10 monoclonal antibody (mAb) diluted 1:5000 in PBS (pH 7.4)/0.2% (v/v) Tween 20. α_2 M was detected with mAb α 1:1 diluted 1:3000 in PBS, which then was detected by a rabbit anti-mouse IgG [diluted 1:5000 in PBS (pH 7.4)/0.2% (v/v) Tween 20], coupled to horseradish peroxidase. mAbs 2H10 and α 1:1 have been characterized and details can be obtained from the authors (E. Vilella, G. Bengtsson-Olivecrona, T. Stigbrand and P. E. Jensen, unpublished work). Plates with conjugated antibodies were incubated for 3 h at room temperature. After four rinses with PBS/0.05% (v/v) Tween 20, development was carried out with *o*-phenylenediamine substrate (0.4 mg/ml, DAKO).

RESULTS AND DISCUSSION

In earlier studies when radiolabelled bLPL was incubated with plasma and then subjected to non-denaturing electrophoresis [31], we observed a labelled band corresponding to a plasma protein with a Stokes' diameter around 15 nm. This could correspond to α_2 M. Therefore purified human α_2 M was examined by non-denaturing electrophoresis under the same conditions as the plasma samples. α_2 M appeared at a similar position to that of the radiolabelled band. *In vitro* experiments were therefore performed to study the binding of LPL to α_2 M. Figure 1 shows the *in vitro* binding of purified 125 I-bLPL to purified α_2 M. Figure 1(a) shows the mobility in a polyacrylamide gel of native, as well as different derivatives of, α_2 M after incubation with LPL. As demonstrated previously [37], native α_2 M (lane 1) has a 'slow' mobility according to the open 'trap' structure of the molecule. On treatment with methylamine, the 'trap' is closed and the mobility becomes 'fast' (lane 2). Treatment of α_2 M with chymotrypsin also produces 'fast' mobility, but because of the 'trapping' and binding of chymotrypsin, the closure of the 'trap' is less pronounced than in methylamine-treated α_2 M [38] (lane 4). In lane 6 both the 'slow' and 'fast' forms are present, as only about half of the α_2 M is converted into the 'fast' form by the addition of half the molar amount of chymotrypsin compared with α_2 M. Incubation of α_2 M with iodoacetamide has no effect on the mobility. This treatment blocks the thiol groups liberated from the thiol esters when α_2 M is treated with methylamine or chymotrypsin. Figure 1(b) shows the autoradiograph of the gel in Figure 1(a). The band at the top of the gel is free LPL which is not expected to enter because of a higher pI value than the pH of the buffer system used in the PAGE [39]. 125 I-bLPL binds to native α_2 M (lane 1), but not to any of the 'fast' forms produced by methylamine or chymotrypsin (lanes 2 and 4). If LPL is present when native α_2 M is incubated with methylamine or chymotrypsin, binding to α_2 M occurs (lanes 3 and 5 respectively). As LPL does not bind to the 'fast' form of α_2 M, the binding must be due to either non-covalent 'trapping' or covalent binding by the γ -glutamyl residue of the thiol group of the activated thiol ester. LPL binds to α_2 M during incubation with methylamine in the presence of iodoacetamide (lane 7). This indicates that LPL is non-covalently 'trapped' in α_2 M during the 'slow' to 'fast' transformation. The samples incubated with iodoacetamide demonstrate weaker radioactive bands than the samples without iodoacetamide (lanes 5 and 8 for chymotrypsin incubation and lanes 3 and 7 for methylamine incubations respectively). This indicates that, in addition to 'trapping', some LPL is bound covalently to α_2 M at the thiol group. This was supported by SDS/PAGE of the same samples, which demonstrated no covalent binding of LPL to α_2 M, except when LPL was incubated together with methylamine or chymotrypsin (not shown). The radiolabelled complexes disappeared when SDS/PAGE was performed on reduced samples.

Binding of LPL to α_2 M was also studied using gel-permeation chromatography. 125 I-bLPL and unlabelled LPL were incubated with native α_2 M and the mixture was applied to the column.

Figure 3 Heparin-Sepharose chromatograms of (a) 125 I-bLPL, (b) 125 I- α_2 M and (c) 125 I-bLPL- α_2 M complex

125 I-bLPL and α_2 M were incubated and subjected to gel-permeation chromatography as described in the Materials and methods section. The peak fractions were pooled and then loaded on a heparin-Sepharose column (for details see the Materials and methods section). After the sample (approx. 4 ml) was loaded, 10 ml (fractions -10 to 0, in the diagrams) of 10 mM Tris/HCl, pH 7.4, buffer was passed through to wash out the unbound material followed by elution with a gradient of NaCl in 10 mM Tris/HCl. For each sample, 15 ml of 0 M NaCl/10 mM Tris buffer and 15 ml of 1.5 M NaCl/10 mM Tris buffer at a flow rate of 1 ml/min were used. Fractions of 1 ml were collected. Radioactivity was measured in each fraction when labelled proteins were used, and the unlabelled α_2 M was detected by e.l.i.s.a. as described in the Materials and methods section. In (a): ●, 125 I-bLPL; ○, [NaCl]. In (b): ●, 125 I- α_2 M; ○, [NaCl]. In (c): □, 125 I-bLPL; ●, α_2 M immunoreactivity; ○, [NaCl].

Figure 4 Non-denaturing PAGE of plasma incubated with ^{125}I -bLPL

Plasma (0.4 ml) from five subjects was incubated with 100 μl (150 ng) of ^{125}I -bLPL at 20 $^{\circ}\text{C}$ for 1 h. Then a 3 μl portion was diluted with sample buffer and loaded on a 4–30% polyacrylamide gradient gel (see the Materials and methods section). (a) Gel stained with Oil Red O; (b) autoradiogram. Lane 1, plasma from subject II; lane 2, plasma from subject III; lane 3, plasma from subject IV; lane 4, plasma from subject I; lane 6, plasma from subject V. Lane 5 contains high-molecular-mass protein standards plus purified $\alpha_2\text{M}$ not stained in this picture for clarity.

Recoveries of radioactivity from the column were 60–70%. Elution of both $\alpha_2\text{M}$ and LPL was detected by sandwich e.l.i.s.a.; the results are shown in Figure 2. The profile for $\alpha_2\text{M}$ when in complex with the lipase was essentially the same as for the protein alone (Figures 2a and 2c). LPL was eluted in fraction 71 (Figure 2b), somewhat retarded in comparison with BSA which was eluted in fraction 53. This retardation of LPL could be due to its interaction with the gel matrix. However, when preincubated with $\alpha_2\text{M}$, LPL was eluted in fraction 38 (Figure 2c). When LPL was preincubated with heparin, only a fraction of the LPL was co-eluted with $\alpha_2\text{M}$ (Figure 2d). This suggests that heparin partly prevents complex-formation between LPL and $\alpha_2\text{M}$. Furthermore, specific binding of LPL to $\alpha_2\text{M}$ was supported by the fact that LPL was not co-eluted with other proteins such as rabbit IgG or albumin (not shown).

To study further the interaction between $\alpha_2\text{M}$ and LPL, samples from the peak fractions of gel-permeation chroma-

tography were applied to a heparin–Sephacrose column and to a chelating Zn^{2+} –Sephacrose 4B column, which are able to bind LPL [32] and $\alpha_2\text{M}$ [14] respectively. As shown in Table 2, free ^{125}I -bLPL did not bind to the Zn^{2+} –Sephacrose. When the putative LPL– $\alpha_2\text{M}$ complex was applied, more than 60% of the LPL radioactivity was bound and 40% of this was eluted together with $\alpha_2\text{M}$. These results further support the hypothesis that the two proteins interact with each other. As seen in Figure 3(a), ^{125}I -bLPL bound to the heparin–Sephacrose column, but ^{125}I - $\alpha_2\text{M}$ did not (Figure 3b). LPL did not bind to heparin–Sephacrose when it was bound to $\alpha_2\text{M}$ (Figure 3c). The lack of binding of the complex (LPL– $\alpha_2\text{M}$) to heparin–Sephacrose and the prevention of the interaction by preincubation of LPL with heparin, as described above, demonstrates that either the lipase was ‘trapped’ inside $\alpha_2\text{M}$ or that the heparin-binding domain of LPL was masked by binding to $\alpha_2\text{M}$. In the gel-filtered complex of LPL and $\alpha_2\text{M}$, the lipase was still catalytically active (as demonstrated by activity measurements according to [40]) and LPL in the complex was still recognized by the antibodies in the sandwich e.l.i.s.a. This suggests an LPL– $\alpha_2\text{M}$ complex, in which at least parts of LPL were not hidden.

To extend the study to more physiological conditions, ^{125}I -bLPL was added to human plasma. The concentration of LPL in human plasma is low (69.8 ± 6.6 ng/ml) and the enzyme is present mainly in its monomeric inactive form [31], assuming that its molecular mass is 55 kDa, it corresponds to 1.3 nM. However, the concentration of $\alpha_2\text{M}$ is about 2.8 μM [41]. Thus, in plasma the ratio of $\alpha_2\text{M}$ to LPL (mol/mol) is about 2000. Consequently, there is $\alpha_2\text{M}$ available for exogenously added LPL. Therefore experiments were carried out in which plasma from five subjects was incubated with labelled ^{125}I -bLPL. The mixture of plasma and labelled bLPL was examined by PAGE and the $\alpha_2\text{M}$ – ^{125}I -bLPL complexes were isolated using the Zn^{2+} –Sephacrose column. Figure 4(a) represents the lipoprotein distribution for the five subjects studied in a 4–30% non-denaturing polyacrylamide gel. The autoradiogram, shown in Figure 4(b), was scanned to quantify the radioactivity in each band and the results demonstrated that about 7–10% of the labelled lipase co-migrated with $\alpha_2\text{M}$, and the rest was associated with lipoproteins (50–58% with VLDL–LDL and 35–40% with large HDL).

The results from Zn^{2+} –Sephacrose chromatography are shown in Table 3. Incubation at 4 $^{\circ}\text{C}$ showed that of the total LPL added to plasma about 2% was eluted with $\alpha_2\text{M}$. However, when incubation was carried out with the same sample but at 20 $^{\circ}\text{C}$, up to 11–17% of the loaded lipase was eluted together with $\alpha_2\text{M}$. To confirm that ^{125}I -bLPL was actually bound to $\alpha_2\text{M}$, eluted

Table 3 Zn^{2+} –Sephacrose chromatography of ^{125}I -bLPL incubated with plasma

Plasma (400 μl) from five subjects (I–V) was incubated with 100 μl (150 ng) of ^{125}I -bLPL for 1 h at 20 $^{\circ}\text{C}$. The mixture of plasma and labelled bLPL was diluted 1:1 with PBS and loaded on a Zn^{2+} –Sephacrose column. Chromatography was performed as described in the Materials and methods section. Plasma from subject I was also incubated with ^{125}I -bLPL at 4 $^{\circ}\text{C}$. Values represent the percentage of the total radioactivity loaded on the column and are means $\pm$ S.D. of triplicate experiments.

Sample	Wash I	Wash II	Elution of $\alpha_2\text{M}$		Wash III
			Peak	Non-peak	
Plasma I + ^{125}I -bLPL (4 $^{\circ}\text{C}$)	94.4 $\pm$ 3.2	0.9 $\pm$ 0.1	1.8 $\pm$ 0.2	0.4 $\pm$ 0.1	2.5 $\pm$ 0.2
Plasma I + ^{125}I -bLPL (20 $^{\circ}\text{C}$)	65.8 $\pm$ 2.9	12.0 $\pm$ 2.7	11.5 $\pm$ 0.1	1.9 $\pm$ 0.3	8.8 $\pm$ 0.4
Plasma II + ^{125}I -bLPL (20 $^{\circ}\text{C}$)	64.5 $\pm$ 3.5	16.9 $\pm$ 0.8	14.0 $\pm$ 0.1	1.8 $\pm$ 0.2	2.8 $\pm$ 0.1
Plasma III + ^{125}I -bLPL (20 $^{\circ}\text{C}$)	61.8 $\pm$ 2.8	15.9 $\pm$ 1.1	11.5 $\pm$ 0.2	1.6 $\pm$ 0.2	9.2 $\pm$ 0.5
Plasma IV + ^{125}I -bLPL (20 $^{\circ}\text{C}$)	51.6 $\pm$ 2.5	16.9 $\pm$ 0.8	13.7 $\pm$ 0.2	1.8 $\pm$ 0.3	16.0 $\pm$ 1.1
Plasma V + ^{125}I -bLPL (20 $^{\circ}\text{C}$)	59.6 $\pm$ 2.2	12.4 $\pm$ 2.0	16.9 $\pm$ 0.2	1.2 $\pm$ 0.2	9.9 $\pm$ 0.6

material was subjected to non-denaturing PAGE (5% gel) and the gel was autoradiographed. Radioactivity was associated with only the α_2 M band, and not with other Zn²⁺-binding plasma proteins. Thus, in the presence of plasma components, a fraction of the LPL was in complex with α_2 M.

Many reports demonstrating non-covalent binding of non-proteolytic proteins to α_2 M refer to basic proteins, e.g. nerve growth factor [7], encephalitogenic basic protein [42], basic fibroblast growth factor [43], cationic aspartate aminotransferase [44], myelin basic protein [45] and eosinophil cationic protein [46]. As LPL is also a basic protein [40], it is possible that all these proteins bind to the same site of α_2 M. Our data indicate that binding of LPL to α_2 M involves the heparin-binding basic region of LPL. It is not known what effects the binding of LPL to α_2 M may have on the physiology of the two proteins in either plasma or tissues. Further experiments are needed to ascertain this.

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