# Acid phosphatases bind to the main high density lipoprotein apolipoprotein A-I

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The serum protein binding secretory prostatic acid phosphatase (PAP) and lysosomal placental acid phosphatase (LAP) was purified using affinity chromatography on gels containing immobilized acid phosphatases. The protein, which could be eluted from these enzyme affinity gels only with 0.05 mol/l HCl (pH 2.0), was shown to be apolipoprotein A-I (apo A-I), the main structural protein of high density lipoprotein (HDL).

*Acid phosphatase* *Apolipoprotein A-I* *HDL* *Affinity chromatography*

## 1. INTRODUCTION

Acid phosphatases (EC 3.1.3.2) are widely distributed in human body fluids and tissues [1]. Prostatic tissue is a rich source of prostatic acid phosphatase (PAP), and the concentration of this isoenzyme is relatively high in hyperplastic prostate, approx. 1 mg/g tissue wet wt [2]. PAP is also secreted into seminal fluid in concentrations of the order of 1 g/l [3], which are 500–1000-fold those measurable in peripheral serum of normal men [4]. In contrast, another acid phosphatase isoenzyme, lysosomal acid phosphatase (LAP), purified from placenta, is present in serum in high concentrations, about 1 g/l (Vihko et al., in preparation). Both these enzymes are inhibited by L-(+)-tartrate, but are immunologically distinct (Vihko et al., in preparation). The physiological roles of these isoenzymes, which hydrolyse a wide range of alkyl, aryl and acyl orthophosphate monoesters and transfer phosphoryl groups, are largely unknown [5].

We have previously shown that serum PAP has two half-lives, of which one was found to be remarkably long, and we suggested that this fraction may represent PAP bound by serum protein/s [4]. The present study was undertaken in order to determine the serum protein which binds PAP and LAP.

## 2. EXPERIMENTAL

Blood was drawn from the antecubital veins of healthy volunteers and patients with carcinoma of the prostate. The serum was collected after centrifugation at 1500 × *g* for 15 min and was used fresh.

### 2.1. *Enzyme purification*

The main isoenzyme of human PAPs, p*I* 4.9, was purified to homogeneity from fresh hyperplastic prostatic tissue, as described [6]. LAP was isolated from full-term human placental tissue by a procedure involving ammonium sulfate fractionation, affinity chromatography on AH-Sepharose 4B with L-(+)-tartrate and Sephadex G-200 gel filtration, essentially as described for the purification of human PAP [6].

### 2.2. *Affinity chromatography*

#### 2.2.1. Preparation of affinity matrices

The ligands were immobilised on CNBr-

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activated Sepharose 4B gel (Pharmacia, Sweden) according to the instructions of the manufacturer. To prepare PAP-Sepharose, 8 mg purified PAP was coupled to 2 g activated Sepharose gel. For the preparation of LAP-Sepharose, 0.8 mg pure LAP was coupled to 0.5 g Sepharose gel.

When the protein that binds to the acid phosphatases was immobilised, 0.3 mg protein and 400 mg gel were used.

2.2.2. Procedure

10 ml serum was incubated with PAP-Sepharose at 4°C for 48 h with mixing. The column was packed and eluted stepwise with 0.2 M PBS (pH 7.4), 0.1 M sodium acetate (pH 4.0) and 0.05 M HCl (pH 2.0). The protein peak eluted with 0.05 M HCl was pooled and used for further studies. In experiments with LAP-Sepharose the volume of serum used was 1.5 ml.

2.3. *High-performance liquid chromatography (HPLC)*

The protein obtained after affinity chromatography was further purified using a Varian model 5000 lipid chromatograph. The sample (250 $\mu$g protein) in 50 mM Tris-acetate (pH 7.0), containing 1 M pyridine and 0.5 M acetic acid, was injected onto a Bondapak C18 column (reverse-phase HPLC, particle size 10 $\mu$m, Waters Associates, USA). The elution buffers were 0.5 M acetic acid (pH 5.4) with 1 M pyridine and 2-propanol.

2.4. *Antisera*

The antisera against human PAP were raised in rabbits as in [7]. The antilysosomal acid phosphatase serum has been described [8]. The monoclonal antisera against apo A-I used have been reported [9]. The antisera against other human serum proteins were commercially available.

2.5. *Radioimmunoassay of the protein bound by acid phosphatases*

The preparation of $^{125}$I-labelled ligand was performed as in [7]. The radioimmunoassay procedure was also performed as described previously for PAP [4] with the following exceptions: the dilution of antiserum was 1:2000; pre-incubation of the specimen and antiserum was for 15 min at 20°C before the addition of approx. 30000 cpm $^{125}$I-labelled bound protein. After overnight incubation at 20°C the antibody-bound $^{125}$I-labelled protein tracer was precipitated with 0.6 ml polyethylene glycol-second antibody solution [10]. The standard curve tubes had the following concentrations of the purified non-labelled protein: 0, 55, 110, 220, 440, 880, 1760 $\mu$g/ml. Both the standards and the samples (1:2000) were diluted with heated (56°C for 30 min) sheep serum diluted with an equal volume of sodium phosphate buffer (50 mM, pH 7.2) containing 20 mmol EDTA and 8 mmol $NaN_3$ per l.

2.6. *Isolation of apo A-I*

Lipoproteins were isolated by sequential ultracentrifugation [11]. Apo A-I was purified as described by Albers and Cheung [12].

2.7. *Isoelectric focusing and immunoblotting*

Isoelectric focusing of purified acid phosphatase-binding protein and serum was carried out as in [13]. Immunoblotting with monoclonal antibodies (2AI) was done as described [9].

2.8. *Other methods*

Protein measurements were performed by the methods of Lowry et al. [14] and by using the Bio-Rad protein measurement kit. Acid phosphatase activity was measured as described in [6]. SDS gel electrophoresis was performed according to Laemmli [15]. Lipoprotein electrophoresis was performed with a Redi-Disc kit (Miles, England).

## 3. RESULTS

We performed studies using affinity chromatography on gels containing covalently linked PAP to see whether the acid phosphatase has affinity for a specific serum protein. The gel was incubated with serum for 48 h and then transferred to a column. The column was washed with PBS until no protein could be demonstrated in the eluate ($A_{280}$). Elution with 0.1 M Na acetate, pH 4.0, did not elute any further protein. With 0.1 M glycine HCl (pH 3.0) about 20% of the bound protein was recovered, whereas the main portion (80%) was eluted with 0.05 M HCl (pH 2.0). The immobilised PAP (8 mg) bound 0.2–1.4% ($n$ = 20 purifications) of the serum protein applied. When the

bound protein (0.7 mg) was reabsorbed on immobilised PAP, 95% of the protein applied was eluted only with 0.05 M HCl. The bound protein represented 5–18% of its total serum concentration. When the protein bound to PAP (PAP-BP) was subjected to HPLC, one main protein peak was evident. This protein was characterised by gel and lipoprotein electrophoreses, immunodiffusion and isoelectric focusing. In SDS-polyacrylamide gel electrophoresis (SDS-PAGE) the eluted protein was homogenous and had an $M_r$ of 27000–30000. In lipoprotein electrophoresis the protein had pre-$\beta$ mobility.

Immunodiffusion using polyclonal monospecific antisera raised against apo AI, AII, AIV, B, E and several serum proteins, $\alpha_1$-antitrypsin, $\alpha_2$-macroglobulin, $C_3$ and $C_4$ complements, $C_1$ inhibitor, acid $\alpha_1$-glycoprotein, ceruloplasmin, hemopexin, transferrin, haptoglobin, IgA, IgD, IgE, IgG and IgM revealed that apo A-I was the only protein that could be demonstrated. Isoelectric focusing followed by immunoblotting with a monoclonal apo A-I antibody verified that the protein was apo A-I (fig.1). It displayed a focusing pattern indistinguishable from that of plasma apo A-I.

An antiserum against the affinity-purified PAP-binding protein was raised in rabbits. In immunodiffusion this antiserum reacted with purified apo A-I but not with apo A-II, A-IV or E. A radioimmunoassay (RIA) was developed using this antiserum as the binder, and affinity-purified PAP-binding protein as the standard, and following iodination, as the label. With this technique purified apo A-I could be efficiently quantified (fig.2). From these data we conclude that the circulating PAP-binding protein is apo A-I.

As acid phosphatase occurs in several iso-forms it was of interest to study whether LAP could also bind to apo A-I. Similar experiments to those described for PAP were therefore performed. Affinity chromatography experiments using columns containing immobilised LAP revealed that LAP was also avidly bound to apo A-I. Immobilized LAP (0.8 mg) could bind 0.6 mg apo A-I from 1.5 ml serum, which was 32% of its total serum concentration. When a gel containing immobilised apo A-I (0.3 mg) was incubated with serum containing a high concentration of LAP (6 g/l) 0.3 mg LAP was bound to the column and could be eluted only with 0.05 M HCl (pH 2.0).

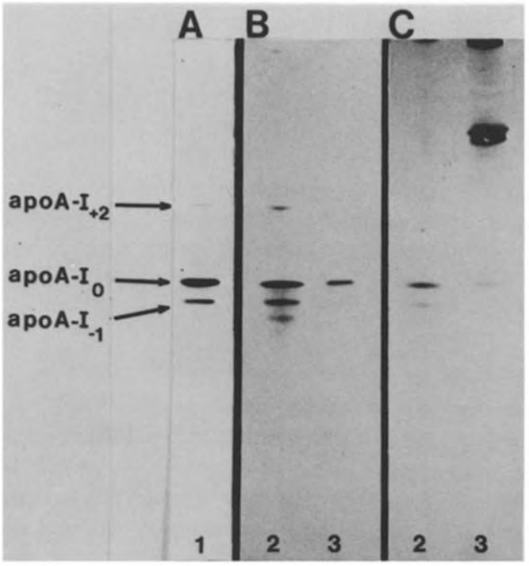


Fig.1. Isoelectric focusing of the serum protein that binds to PAP. (A,B) Isoelectric focusing patterns as visualized by immunoblotting using a monoclonal anti-apo A-I antibody (2AI) of: lane 1, normal human serum (1 $\mu$l); lane 2, protein eluted from the PAP-affinity column at pH 2.0. The faint precipitation line on B, lane 2, below that of apo A-$I_{-1}$ corresponds to apo A-$I_{-2}$. Lane 3, normal human serum (0.5 $\mu$l). (C) Same gel as in B but stained for protein with Coomassie blue.

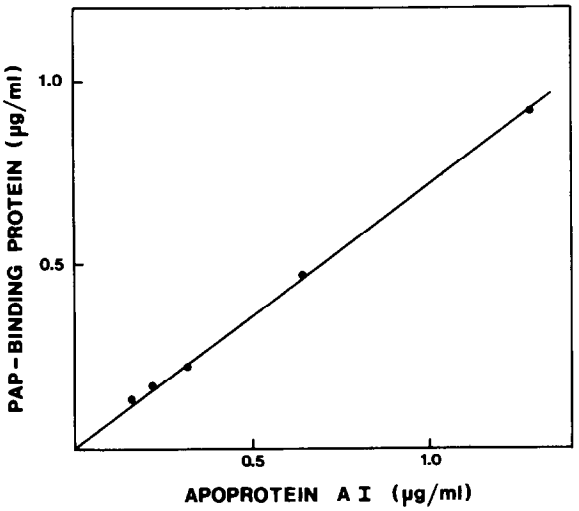


Fig.2. Relationship between the protein that binds PAP as measured by radioimmunoassay, and pure apo A-I.

## 4. DISCUSSION

The high serum concentrations of LAP (1.0 g/l) and the high seminal fluid concentrations of PAP (1 g/l) suggest possible physiological roles for these enzymes. However, these roles remain unknown. This investigation shows that these two isoenzymes are avidly bound to a serum protein, apo A-I, the main protein constituent of HDL.

Much interest has recently been focused on this lipoprotein fraction. Not only is HDL an indicator of the risk of coronary heart disease [16] but it has also been shown to accommodate acute-phase proteins [17] and endotoxins [18]. Evidence has been reported that HDL are involved in the entry of cholesterol to steroidogenic and hepatic cells [19] and in the net movement of cholesterol out of cells [20–22]. Despite the numerous functions that have been assigned to HDL little is known about its metabolism.

Here, we describe a strong and specific binding of acid phosphatases to the main apoprotein of HDL, apo A-I. The physiological meaning of this association, if any, is at present an enigma. We would like to consider some possibilities.

The interaction between apo A-I and the enzyme could be meaningful in the transport of acid phosphatases in plasma or in detoxifying the enzymes as has been shown for endotoxins [18]. Another possibility is that the enzymes take part in the intravascular metabolism of HDL phospholipids, which turn over in HDL at least 5-times faster than the apoproteins [23]. Apo A-I has been shown to be an effective activator of lecithin-cholesteryl acyltransferase (LCAT) [24]. It is not known whether apo A-I can also act as an activator of acid phosphatases in the metabolism of lipoprotein phospholipids.

Finally, acid phosphatase could be involved in the interaction of HDL with cells. Tissues known to be rich in acid phosphatase, such as liver and kidney, have also been shown to take up apo A-I avidly [19,25]. It has also been demonstrated that HDL displays specific binding to placental cells [26] and testis membranes [27] but it is not known whether transfer of apo A-I to these cells occurs. Lysosomes play an important role in receptor-mediated endocytosis and catabolism of LDL [28]. That lysosomes also play an important role in the intracellular metabolism of apo A-I has been shown using inhibitors of lysosomal enzymes [29,30]. In Tangier disease, a disorder characterized by the absence of normal HDL, these lipoproteins are diverted to the lysosomal compartment and degraded [31]. Whether the interaction we have observed between apo A-I and the lysosomal enzyme, acid phosphatase, has any consequences for the intracellular transport of lipoproteins remains to be established. Our results support the finding that there are apo A-I containing lipoproteins with pre-$\beta$ mobility [32].

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