Partial purification of a high density lipoprotein-binding protein from rat liver and kidney membranes

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The existence of a cell receptor which recognises plasma high density lipoprotein (HDL) has been suggested from studies which demonstrate specific binding of HDL_3 to cultured cells derived from various tissues in the body. This study provides evidence of a specific HDL-binding protein in crude plasma membranes prepared from rat kidney and liver. Following separation of solubilised membrane proteins on polyacrylamide gel slabs and 'Western' blotting, one major band was identified which bound HDL_3, or apo AI or apo AII. The protein, which was present in both liver and kidney membranes, was partially purified by repetitive preparative SDS-polyacrylamide gel electrophoresis and although accompanied by considerable loss of binding activity, could still be detected by the ligand-blotting procedure used initially to detect its presence in cell membranes.

HDL-binding protein *(Liver, Kidney)* *Plasma membrane* *Partial purification* *Ligand blotting*

1. INTRODUCTION

Several tissues are implicated in the processing of HDL and numerous laboratories have demonstrated specific binding, internalisation and/or degradation of HDL by cultured cells. Unlike LDL, which primarily donates cholesterol to cells, HDL is probably involved in reverse cholesterol transport (removal of cellular cholesterol) from some tissues [1], delivery of sterol to others (e.g. steroidogenic tissues [2–4]), and possibly both removal from and delivery of cholesterol to the liver. Thus, the processing of HDL is more complex and may involve different mechanisms for different cells. Some insight into these mechanisms may be gained by studies at the molecular level of the ligand-membrane interaction. Towards this aim, the present investigation has provided some clues to the existence and nature of the binding protein in plasma membranes obtained from both rat liver and kidney, two tissues known to be involved in the processing of plasma HDL [5].

2. EXPERIMENTAL

Livers and kidneys were obtained from male Sprague-Dawley rats (180–250 g) and immediately placed in ice-cold phosphate-buffered saline (PBS). The cortex was dissected out from the kidneys, blotted and weighed, and homogenised with 5 vols buffer [20 mM Tris-HCl, 0.15 M NaCl, 1 mM $CaCl_2$, 1 mM phenylmethylsulphonyl fluoride (PMSF), pH 7.5] for 2 × 15 s periods in a Polytron homogeniser. Rat livers were similarly weighed and homogenised. Homogenates of both were centrifuged at 3000 × *g* for 5 min, the supernatants filtered through nylon gauze and recentrifuged at 3000 × *g*. These supernatants were centrifuged at 100000 × *g* for 60 min and the resulting membrane pellet resuspended in 0.25 M Tris-maleate, 1 mM PMSF (pH 6.5) and resuspended by aspiration through a 23-gauge needle. Protein concentrations were adjusted to 10 mg/ml and the membrane suspensions sonicated for two periods of 15–20 s at 4°C. After adjusting the suspensions to 5 mg/ml protein in 0.125 M Tris-maleate,

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0.15 M NaCl, octyl-β-D-glucopyranoside (OG) was added to a final concentration of 40 mM and the mixtures incubated at 4°C for 20 min. The solubilised membrane fraction was obtained by centrifugation at 100000 × *g* for 60 min and the insoluble (pellet) fraction discarded.

HDL-binding proteins were identified on membrane components which had been transferred to and immobilised on nitrocellulose sheets as described [6] for adrenal cortical membranes. Briefly, solubilised membrane proteins were separated by electrophoresis on SDS-polyacrylamide gels (10%) followed by Western blotting [7]. The nitrocellulose strips were incubated with 150 μg/ml HDL_3, or appropriate control solutions for 60 min at room temperature and then washed 4 times with PBS containing 0.1% Tween 20. Bound HDL was detected with anti-apo AI (rabbit) which in turn was identified using biotinylated anti-rabbit Ig and biotinylated streptavidin-HRPO complex (Amersham) as described in [6].

HDL_3 (*d* 1.12–1.21 g/ml) was isolated from normolipemic human serum and washed by ultracentrifugation until devoid of E or B apolipoproteins [9]. AI and AII apolipoproteins were isolated following delipidation of HDL_3 and monospecific antisera to these apolipoproteins were produced as in [10]. Rat HDL_3 was isolated by ultracentrifugation and antisera to rat Apo AI also prepared according to [10].

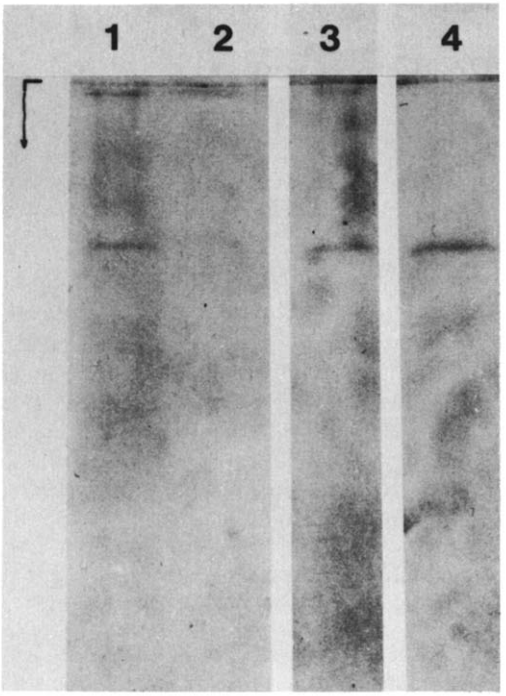

Fig.1. Binding of human HDL_3 and apolipoproteins AI and AII by adrenocortical membrane receptor. Lanes 1, 3 and 4 were loaded with solubilised membranes, lane 2 with albumin. Following SDS-slab gel electrophoresis and transblotting, strips 1 and 2 were incubated with HDL_3, strip 3 with AI and strip 4 with AII apolipoprotein. Binding of proteins was detected after incubation with appropriate antisera as described in section 2.

3. RESULTS

In a previous study, following Western blotting of the solubilised plasma membrane fractions of sheep adrenal cortical membranes, a protein band which recognised human HDL_3 [6] was identified. The same band also weakly bound human LDL. To characterise further the ligand specificity of this membrane protein, strips were incubated with either apo AI or AII, washed and blocked and then incubated with their corresponding antisera. Fig.1 shows that as well as binding intact HDL_3 (lane 1), the same band, with a comparable electrophoretic mobility, also bound apo AI (lane 3) and apo AII (lane 4). Lane 2 was loaded with albumin which did not bind HDL_3. The apparent M_r of the protein is similar (approx. 78000) to that described in [6].

To investigate the distribution of this binding protein in tissues alleged to be involved in in vivo processing of HDL_3 [5] solubilised plasma membrane extracts of rat kidney, and liver were similarly electrophoretically separated, transblotted, and incubated with lipoproteins as described above. Fig.2 shows that a fraction in rat kidney membranes strongly bound human HDL_3 (lanes 1,2) as well as rat HDL_3 (lanes 5,6) and although more weakly expressed, a protein of similar M_r present in rat liver also bound human HDL_3 (lanes 3,4).

To enable characterisation of the HDL_3-binding protein in more detail, rat kidney and liver membrane preparations were separately subjected to repetitive SDS-slab gel electrophoresis, and the position of the HDL_3-binding protein monitored each time by Western blotting. Fig.3 shows that partial purification of the protein was achieved by

this approach, although the binding activity became considerably weaker during the process. The position of the binding protein present in the solubilised membrane preparation (lane 1, Coomassie blue) was identified by ligand blotting a small section of the gel (lane 2), and the corresponding band across the remainder of the gel was eluted and subjected to slab gel electrophoresis.

This process was repeated and the final purification achieved is shown in fig.3, lane 3. A similar purification was achieved with solubilised kidney membranes. Lanes 4 and 5 (partially purified liver HDL receptor) and lanes 6–8 (partially purified kidney HDL receptor) show the result of ligand blotting, indicating that the bottom band (lane 3) has the binding activity. The significance of the two higher M_r bands is unknown, although concentrations of both were considerably reduced following incubation with mercaptoethanol prior to electrophoresis (not shown). Because reduction with thiol reagents appears detrimental to the HDL-binding capacity, ligand blotting of the partially purified binding protein(s) could not be investigated following reduction.

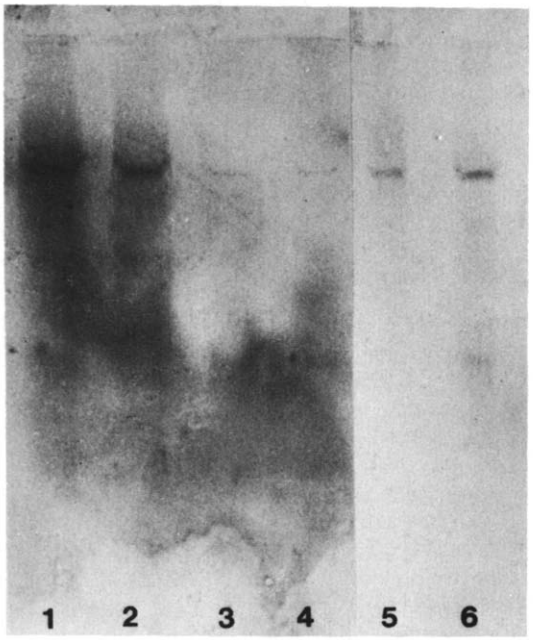

Fig.2. Binding of human HDL_3 by solubilised membrane preparations of rat kidney (1,2) and rat liver (3,4) and of rat HDL by rat kidney membranes (5,6). Following SDS gel electrophoresis and transblotting, nitrocellulose strips were incubated with HDL and processed as described in section 2.

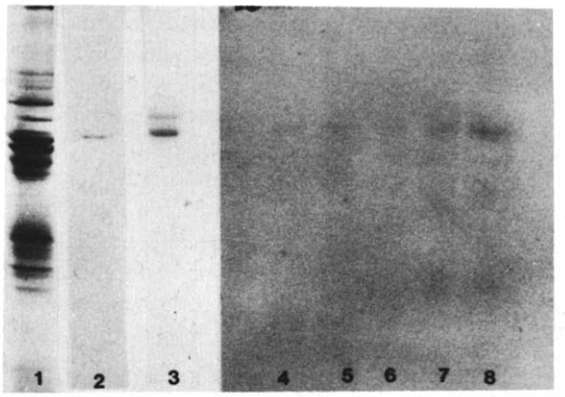

Fig.3. Partial purification of binding protein by repetitive SDS-slab gel electrophoresis. Solubilised liver plasma membrane was electrophoresed (lane 1), Coomassie blue stained gel, and the HDL_3-binding protein, identified by ligand blotting (lane 2) was excised, and re-electrophoresed. The process was repeated twice, resulting in the isolation of a fraction characterised by one major band and two bands of higher M_r (lane 3, Coomassie blue-stained). The same procedure was used to isolate the rat kidney HDL-binding protein. Retention of binding activity, although weakened, was apparent after loading 40 and 80 μg purified liver protein (lanes 4,5) and 20, 40 and 80 μg purified kidney membrane followed by ligand blotting.

4. DISCUSSION

An HDL-binding protein has previously been identified in adrenal cortical plasma membrane [6] and taken together with additional evidence that ACTH stimulates HDL binding in rat adrenals [9] provides strong evidence for the existence of an HDL receptor, at least in rat steroidogenic tissues which depend on HDL to deliver cholesterol as a precursor of steroidogenic hormones. These experiments have now shown that the same membrane protein binds both AI and AII apolipoproteins which strengthens previous observations made using whole cell incubations. In these experiments, specific antibodies (Fab fragments) were used to inhibit binding of HDL to cultured cells and it was found that immunosuppression occurred in the presence of either apo AI or AII antibodies [10].

Additionally, we have now demonstrated that an HDL-binding protein is present in other tissues thought to be involved in the metabolism of plasma HDL. A protein with similar electrophoretic properties has been partially purified from solubilised membranes obtained from both rat livers and kidneys, two organs involved in the processing HDL, although the evidence suggests that apo AI, rather than the intact HDL, may be the major peptide recognised by the kidney [5]. The present ligand-blotting studies suggest that the concentration of the binding protein was strongest in kidney and weakest in liver membranes since more HDL was apparently bound following electrophoresis of similar concentrations of kidney and liver membrane (fig.2). However, the partially purified binding protein isolated from the kidney also appeared to bind more HDL than equivalent amounts of the liver membrane (fig.3) so it is possible that structural differences, affecting HDL binding, exist between the proteins derived from the two sources. One alternative explanation for the weaker interaction of HDL_3 with liver membranes could be the choice of HDL subfraction, since recent evidence [11] suggests that HDL_2 may be the major HDL class responsible for returning cholesterol to the liver from peripheral tissues.

It is of considerable interest that both the putative HDL receptor and the ligand appeared to involve protein components. Recent reports suggest that lipid moieties [12] or unidentified low-M_r, non-peptide components [13] are responsible for the HDL-receptor interaction. These suggestions are not consistent with the present observations which identified the membrane-binding component as a stainable protein band, capable of electrophoretic transfer to nitrocellulose sheets. The ligands of HDL which it bound were delipidated apolipoproteins (AI, AII) as well as intact HDL_3. Therefore, although these results do not exclude lipid (or carbohydrate) from the interaction, they provide strong evidence for the involvement of protein-protein interactions in one form of cellular recognition of plasma HDL_3.

Further purification is hampered by an accompaniment in weakening of binding activity and by the possible formation of aggregates (fig.3, lane 3) which partially at least appear to be disulphide-linked. Work is proceeding to achieve a final purification which will enable a more detailed characterisation of the properties of this new HDL-binding protein.

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