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# Affinity of integrin $\alpha_1\beta_1$ from liver sinusoidal membranes for type IV collagen

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Hepatic sinusoidal membanes isolated from adult rats were extracted with detergent and fractionated on a wheat germ agglutinin affinity column. Bound glycoproteins were eluted with *N*-acetyl glucosamine and chromatographed on a type IV collagen affinity column. Recovery of the bound fraction by EDTA and analysis by SDS-PAGE revealed two glycoproteins with apparent molecular weights of 180 000 and 117 000. These were identified immunologically by Western blotting as the $\alpha$ and $\beta$ subunits of integrin $\alpha_1\beta_1$.

Type IV collagen receptor; Integrin $\alpha_1\beta_1$; Rat liver

## 1. INTRODUCTION

The liver parenchyma is in contact with extracellular matrix components including collagens of various types, proteoglycans, such as heparan sulfate, and non-collagenous glycoproteins such as fibronectin and laminin [1-3]. Matrix macromolecules elicit both adhesive and metabolic responses on parenchymal cells upon contact [4-7], these particular responses being most likely mediated and transmitted by cell-surface receptors for matrix proteins. In liver, integrin $\alpha_5\beta_1$ and the non-integrin glycoprotein AGp110 are fibronectin receptors [8,9], and integrin $\alpha_1\beta_1$ functions as a receptor for both laminin [10] and type I collagen [11].

In this study we report on the isolation from liver parenchymal membranes of a heterodimeric glycoprotein on a type IV collagen affinity column. Immunological comparisons indicate that this protein is integrin $\alpha_1\beta_1$.

## 2. MATERIALS AND METHODS

### 2.1. *Antibodies*

Specific antisera against the $\alpha_5$ and $\beta_1$ subunits were raised in rabbits [8]. The antiserum against the $\alpha_5$ subunit was affinity-purified on a column of $\alpha_5$ conjugated to cyanogen bromide (CNBr)-activated Sepharose 4B. Specific antiserum against the $\alpha_1$ integrin subunit was obtained by immunizing rabbits with protein cut out from an SDS gel of isolated hepatocyte laminin receptor [10]. Goat anti-rabbit IgG conjugated to alkaline phosphatase was purchased from Sigma (Poole, UK).

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### 2.2. *Proteins*

Type IV collagen was isolated from the Engelbreth-Holm-Swarm (EHS) sarcoma as described [12]. Wheat germ agglutinin (WGA) was purchased from Pharmacia (Milton Keynes, UK). Both proteins were conjugated to CNBr-activated Sepharose 4B (Pharmacia) according to the manufacturer's specifications.

### 2.3. *Membrane isolation*

Hepatic sinusoidal plasma membranes were isolated from liver homogenates on sucrose gradients as described [13].

### 2.4. *Electrophoresis*

SDS-polyacrylamide gel electrophoresis was carried out according to Laemmli [14]. Western blotting of proteins on nitrocellulose and detection using antibody probes were as previously described [15].

### 2.5. *WGA affinity chromatography*

A pellet of sinusoidal membranes from 10 adult Sprague-Dawley rats was resuspended in 2 mM $NH_4HCO_3$ pH 8.3, containing 1 mM $CaCl_2$ and 1 M NaCl. The membranes were then spun in a Beckman 42.1 rotor at 40000 rpm for 2 h and extracted in 5 ml of 20 mM CHAPS in HEPES-saline (10 mM HEPES, 150 mM NaCl) pH 7.3, containing 1 mM $CaCl_2$, 1 mM PMSF and homogenized. The homogenate was centrifuged as above and the clear supernatant applied to a 5 ml WGA-Sepharose affinity column (pre-washed with extraction buffer) containing 5 mg of WGA per ml of packed gel. The membrane extract was re-circulated overnight in the cold at a flow rate of 5 ml/h. The column was then washed with HEPES-saline pH 7.3, containing 2 mM $MnCl_2$, 1 mM PMSF and 8 mM CHAPS, brought up to room temperature and eluted with the same buffer as above containing 0.3 M *N*-acetyl-D-glucosamine.

### 2.6. *Type IV collagen affinity chromatography*

The eluate from the WGA column was cooled to 4°C and applied to a type IV collagen-Sepharose affinity column equilibrated in HEPES-saline pH 7.3, 2 mM $MnCl_2$, 1 mM PMSF and 8 mM CHAPS. The column consisted of 2 ml packed Sepharose 4B derivatized with 5 mg of electrophoretically pure type IV collagen. The WGA fraction was recirculated overnight in this column at 4°C, non-bound material was washed away with buffer and proteins bound

to type IV collagen were recovered by elution with HEPES-saline pH 7.3, 1 mM PMSF, 8 mM CHAPS, containing 10 mM EDTA. Protein concentration in the eluate was monitored by absorbance at 280 nm and fractions were run on SDS-PAGE following dialysis and lyophilization.

## 3. RESULTS AND DISCUSSION

Purified hepatic sinusoidal membranes were washed with 1 M NaCl to remove peripheral membrane proteins and then extracted with CHAPS (see section 2). The extracted proteins (Fig. 1a) were fractionated on a WGA column and bound glycoproteins were eluted with 0.3 M *N*-acetyl-glucosamine (Fig. 1b). This fraction was then chromatographed on type IV collagen affinity columns and after exhaustive washing of the column, retained proteins were eluted with EDTA. Examination by SDS-PAGE under reducing (Fig. 1c) and non-reducing conditions (Fig. 1d) revealed two proteins of molecular weights 200 000 and 135 000, or 180 000 and 117 000, respectively. These observed molecular weights are close to those reported for the $\alpha_1$ and $\beta_1$ integrin subunits as isolated on laminin or type I collagen affinity columns [10,11]. An immunological comparison was therefore carried out by Western blotting. EDTA eluates from the type IV collagen affinity column were run on SDS gels under non-reducing conditions, transferred onto nitrocellulose and reacted with antisera specific against $\alpha_1$ (Fig. 1e) or $\beta_1$ (Fig. 1f). Both antisera gave a positive reaction, thus identifying the type IV collagen-bound glycoproteins as integrin $\alpha_1\beta_1$. Antibodies against the $\alpha_5$ subunit of the integrin fibronectin receptor [8] did not recognize the 180 000 protein (not shown). The faint band observed below the reduced $\alpha_1$ subunit (Fig. 1c) is most probably due to proteolytic degradation [10].

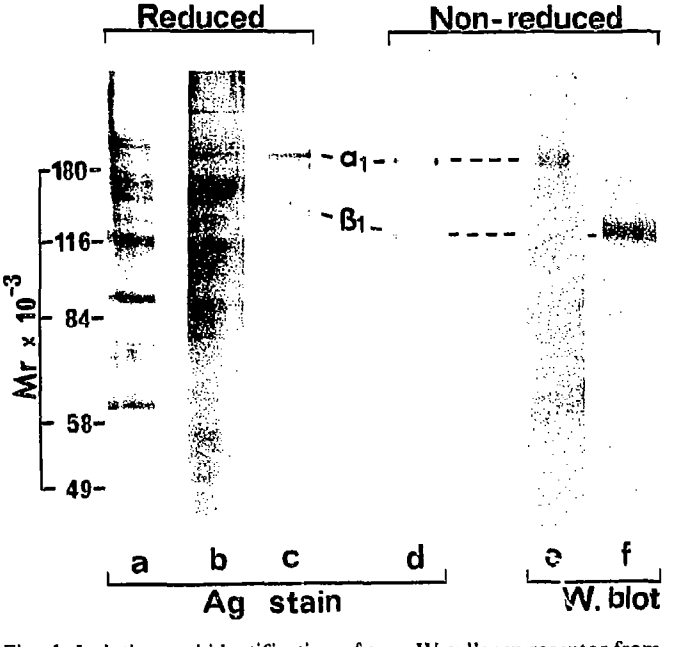


Fig. 1. Isolation and identification of type IV collagen receptor from rat liver. SDS-PAGE of samples run under reducing (a-c) or non-reducing (d-f) conditions. Gels were either silver-stained (a-d) or blotted onto nitrocellulose (e,f). a, detergent extract from hepatic sinusoidal membranes; b, membrane glycoproteins bound to WGA column; c, glycoprotein fraction retained in type IV collagen column and eluted with EDTA; d, fraction as in c, non-reduced; e, fraction as in d, blotted and reacted with serum against $\alpha_1$; f, fraction as in d, blotted and reacted with serum against $\beta_1$.

This report therefore indicates that integrin $\alpha_1\beta_1$ is the putative type IV collagen receptor of liver parenchymal cells. The fact that $\alpha_1\beta_1$ was isolated from sinusoidal membranes correlates with the reported localization of type IV collagen in that membrane domain [1,2]. Use of anti-functional specific antibodies against $\alpha_1$ and localization studies should confirm our report. Unfortunately, our present anti-$\alpha_1$ serum was not effective in adhesion assays and localization studies.

Our finding emphasizes the state of redundancy that exists in cell-extracellular matrix interactions. In the liver parenchyma at least two surface receptors, integrin $\alpha_5\beta_1$ [8], and AGp110 [9] interact with fibronectin and, on the other hand, integrin $\alpha_1\beta_1$ functions as a receptor for three matrix macromolecules: laminin [10], type I collagen [11] and, as shown in this study, type IV collagen. This redundancy may offer flexibility in cell-matrix interactions.

Reported different modes of adhesion of rat hepatocytes on laminin and type IV collagen substrata [4] may be explained by different receptor-ligand affinities of integrin $\alpha_1\beta_1$ for the two matrix components or by the utilization of more than one receptor for each ligand. In the case of hepatocyte reactions with fibronectin for example, a clear synergy has been demonstrated for integrin $\alpha_5\beta_1$ and AGp110 [16].

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