IDENTIFICATION OF THE HUMAN FIBROBLAST SURFACE GLYCOPROTEIN (FSG) AS AMINOPEPTIDASE M

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1. Introduction

In [1,2] a highly antigenic fibroblast surface glycoprotein (FSG) was shown to be an integral membrane protein of M_r 150 000 under denaturating conditions. FSG was not released from the cell surface by trypsin treatment, while papain solubilized a 140 000 M_r hydrophilic part of the molecule, which was also present intracellularly. The antigen was expressed only by a few cultured human cell lines, all of mesenchymal origin [2], but in vivo, in addition to the fibroblasts of most tissues, the brush border of kidney and intestine, the bile canaliculi in liver and the acinar cells of pancreas stained selectively with a monospecific anti-FSG antiserum [3]. Since a variety of hydrolases (aminopeptidase, dissacharidases, alkaline phosphatase and γ-glutamyltransferase), probably involved in the active transport of a number of metabolites, have been localized in these sites [4], we have tested the hypothesis that FSG might be one of these surface enzymes.

From a comparative literature study [5] and from this investigation it can be concluded that the fibroblast surface glycoprotein is an aminopeptidase M.

2. Materials and methods

2.1. *Cell lines and cell culture*

Diploid human skin fibroblasts [6], MG63 cells, derived from a human osteosarcoma [7], CCL30 (squamous cell carcinoma), CCL121 (fibrosarcoma), CCL136 (rhabdomyosarcoma) and CCL138 (pharynx carcinoma), obtained from the American Type Culture Collection (Rockville, MD), and endothelial cells were cultured as in [6]. Peripheral lymphocytes, normal serum and serum from a patient with liver cirrhosis were obtained from the Red Cross Blood Centre, Leuven.

2.2. *Preparation of cell and tissue extracts*

Cell extracts were obtained by sonication of the cell pellet in 75 mM veronal buffer (pH 8.6) containing 2% of the non-ionic detergent Berol (Berol Kemi AB, Sweden). Human kidney and liver were obtained from autopsies performed within 6 h after death, when no primary renal or liver disease was apparent. For kidney, the cortex was separated from the medulla. Homogenization was performed in a Potter-Elvehjem homogenizer with a Teflon pestle in 75 mM veronal (pH 8.6) containing 2% Berol (tissue/buffer, 1:3, w/v), followed by sonication. Unsoluble material was removed by centrifugation.

2.3. *Enzyme assay*

Aminopeptidase activity was determined spectrophotometrically by measuring the rate of liberation of *p*-nitroaniline and β-naphthylamine from amino acid derivatives at 410 nm and 340 nm, respectively. Activities on cell homogenates were determined in 50 mM Tris–HCl (pH 7.3) containing 1 mM substrate at 25°C. In these conditions the rate of substrate hydrolysis increased linearly in function of time and enzyme concentration. Extinction values measured after 30 min were corrected for turbidity and spontaneous substrate hydrolysis. One unit of enzyme activity is defined as the amount of enzyme hydrolyzing 1 μmol substrate/min using the extinction coefficients 8850 $M^{-1} . cm^{-1}$ and 1780 $M^{-1} . cm^{-1}$ for *p*-nitroaniline and β-naphthylamine, respectively.

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Kinetic constants were determined from plots of $1/v$ vs $1/[S]$. Five different substrate concentrations were used in each experiment. All enzyme substrates were purchased from Sigma Chemical Co. (St Louis, MO).

The inhibitory effect of EDTA and 1,10-phenanthroline was assayed by incubating cell homogenates for 20 h at 5°C with different concentrations of the chelator before enzymatic activity was measured. Protein was measured as in [8] using bovine serum albumin as standard.

2.4. *Immunoelectrophoresis*

Crossed and rocket immunoelectrophoresis were done as in [1,9] with monospecific anti-FSG antibodies [2] in the immunogel. Staining of immunoprecipitates for aminopeptidase activity was performed on wet gels by coupling β-naphtylamine, the product of splitting alanine–β-naphthylamide, with fast blue B salt according to [10].

3. Results

3.1. *Immunoprecipitation of aminopeptidase activity with anti-FSG antiserum*

The typical polymorphic FSG immunoprecipitate obtained in crossed immunoelectrophoresis with the monospecific anti-FSG antiserum (fig.1A), exhibited aminopeptidase activity when stained with alanine–β-naphthylamide (fig.1B). To further substantiate that FSG was identical to aminopeptidase, enzymatic activity was measured after double immunoprecipitation of the FSG antigen. Fibroblast homogenates were immunoprecipitated with anti-FSG antiserum followed by swine anti-rabbit IgG (Dakopatts, Sweden). No residual aminopeptidase activity was observed in the supernatant, whether alanine–β-naphthylamide or alanine–*p*-nitroanilide was used as substrate. Control samples receiving non-immune IgG instead of anti-FSG had the same aminopeptidase activity before and after the antibody treatment.

3.2. *Characteristics of fibroblast aminopeptidase*

The fibroblast enzyme showed the highest activity towards alanine–β-naphthylamide (table 1). The values obtained with alanine– and leucine–*p*-nitroanilide were, respectively, 26% and 43% lower. The α-glutamic acid substrate was hydrolyzed at a very slow rate. A Lineweaver-Burk plot of assays performed at pH 7.3 and 25°C using substrate concentrations ranging from 0.08–1 mM indicated an app. K_m value of 0.62 mM for alanine–*p*-nitroanilide. Leucinamide inhibited the activity of the enzyme towards alanine–*p*-nitroanilide competitively with a K_i-value of 3.41 mM indicating that leucinamide was a poor substrate. The enzymatic activity measured in Tris–HCl buffer exhibited an optimum near pH 8.0 with a

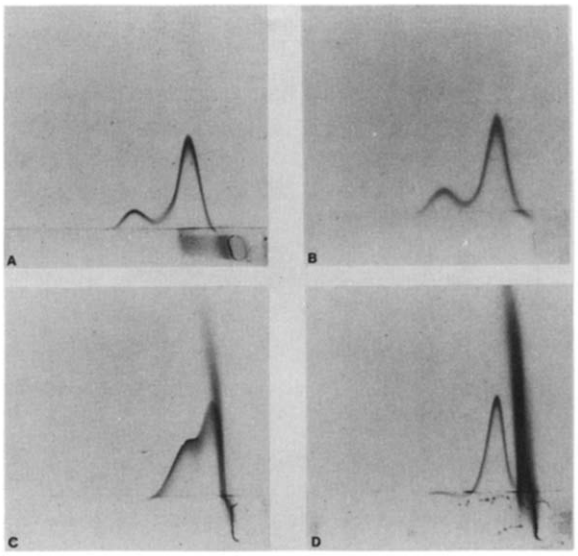

Fig.1. Crossed immunoelectrophoresis of human skin fibroblasts (A,B), human kidney (C) and human liver (D). The immunogel contained anti-FSG antibodies (15 μg IgG/cm²): first dimension, 90 min at 10 V/cm (anode to the left); second dimension, 16 h at 4 V/cm (anode to the top); (A,C,D) the immunoprecipitates were stained with Coomassie brilliant blue; (B) the plate was stained for aminopeptidase activity with alanine–β-naphthylamide as substrate.

Table 1
Aminopeptidase activity in human fibroblasts, measured with different substrates

Substrate	Aminopeptidase activity	
	mU/10⁶ cells	Relative %
L-alanine–β-naphthylamide	66.3	100
L-alanine–*p*-nitroanilide	48.8	74
L-leucine–*p*-nitroanilide	37.6	57
L-α-glutamic acid–*p*-nitroanilide	0.2	0.5

Confluent fibroblast cell layers were dissociated with trypsin and aminopeptidase activity was measured on cell homogenates as in section 2. Each figure represents the average of duplicate assays

Table 2
Quantitative screening of human cells and tissues for the presence of aminopeptidase and FSG

Tissue	Aminopeptidase activity[a]	FSG[b]
Kidney cortex	47.3	23.5
Normal skin fibroblasts (NHF)	41.3	29.5
Fibrosarcoma-derived cells (CCL121)	38.0	24.0
Osteosarcoma-derived cells (MG63)	20.3	15.0
Liver	15.2	14.5
Endothelial cells	13.8	9.0
Embryonal rhabdomyosarcoma-derived cells (CCL136)	7.0	2.5
Pharynx carcinoma-derived cells (CCL138)	6.6	–[c]
Peripheral lymphocytes	5.9	1.5
Squamous cell carcinoma-derived cells (CCL30)	3.6	–[c]
Serum from patient with liver cirrhosis	2.9	1.5
Normal human serum	1.6	–[c]

[a] Aminopeptidase activities towards alanine–*p*-nitroanilide are expressed in mU/mg total protein

[b] Amounts of FSG are expressed as the height (mm) of the rockets obtained in rocket immunoelectrophoresis with the anti-FSG antiserum

[c] Samples with no visible immunoprecipitates formed in rocket immunoelectrophoresis

broad peak extending from pH 7.0–8.5. EDTA and 1,10-phenanthroline, inhibitory only after preincubation, showed maximal inhibition at 0.2 mM EDTA (93%) or 1 mM 1,10-phenanthroline (97%).

3.3. *Screening of different human cell lines, tissues and sera*

The amount of FSG present in human cells of different origin was quantitated both by rocket immunoelectrophoresis and by enzyme activity towards alanine–*p*-nitroanilide. For comparison, kidney cortex and liver, with known high levels of activity, were assayed in the same way. The highest activity was found in kidney cortex, followed by skin fibroblasts and the fibrosarcoma-derived cell line CCL121 (table 2). Peripheral blood lymphocytes and normal serum had low activities. Serum of a patient with liver cirrhosis contained twice as much aminopeptidase activity as normal serum. By rocket immunoelectrophoresis the same general order was obtained. Overall, a good correlation was obtained between the enzymatic activity and the amount of FSG quantitated by rocket immunoelectrophoresis ($r = 0.96$). Although the aminopeptidases from liver and kidney were recognized by the anti-FSG antiserum, their immunoprecipitation patterns in crossed immunoelectrophoresis were not completely identical to the pattern obtained with the fibroblast enzyme (fig.1C,D).

4. Conclusion

Aminopeptidase M (EC 3.4.11.2) is a membrane-associated enzyme that acts upon peptides as well as upon chromogenic substrates. It is distinct from classical leucine aminopeptidase (EC 3.4.11.1) by virtue of differences in solubility, substrate specificity and metal ion requirements [5]. The molecular properties and mode of membrane insertion of aminopeptidase M from intestinal and renal brush border membranes have been well documented [11,12,13].

Here, the 150 000 M_r, highly antigenic surface glycoprotein of human fibroblast [1,2] was shown to exhibit aminopeptidase activity. The enzymatic activity associated with the membrane component acted preferentially on alanine-derivatives of chromogenic substrates indicating that the enzyme is of the microsomal type (aminopeptidase M) [5].

The presence of an antigen on fibroblasts, reacting with anti-intestinal aminopeptidase has been described [14]. Monospecific antibodies prepared against the fibroblast antigen showed cross-reaction with liver and kidney aminopeptidase. The tissue-derived enzymes, however, did not give an identical immunoprecipitation pattern as the fibroblast enzyme in CIE.

In intestine, liver and kidney, high activity of aminopeptidase in the brush border has been correlated with the intense transport which occurs at these sites. For fibroblasts and other mesenchymal cells, a rationale for the presence of large amounts of this enzyme in their plasma membrane remains to be determined.

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