Identification of the 110 000 M_r glycoprotein isolated from rat liver plasma membrane as dipeptidylaminopeptidase IV

Wolfgang Kreisel, Roswitha Heussner, Brigitte Volk, Reinhard Büchsel, Werner Reutter+ and Wolfgang Gerok

Medizinische Klinik, Klinikum der Albert-Ludwigs-Universität, 7800 Freiburg and *+Institut für Molekularbiologie und Biochemie der Freien Universität, 1000 Berlin, Germany*

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1. INTRODUCTION

We have described a dissociated turnover of terminal carbohydrates and protein component of a 110 000 M_r glycoprotein isolated from rat liver plasma membrane: fucose, *N*-acetylneuraminic acid and galactose turn over several times on the intact polypeptide [1], whereas mannose and *N*-acetylglucosamine (core sugars in *N*-glycosidically bound carbohydrate chains) turn over coordinatedly to the protein [2]. Here, we describe the identification of the isolated glycoprotein as the monomer of dipeptidylaminopeptidase IV (EC 3.4.14.X), a dimeric glycoprotein of the plasma membrane. Our results suggest differently glycosylated forms of this enzyme within the cell.

2. MATERIALS AND METHODS

Male Wistar rats (Ivanovas, Kisslegg) weighing 180–200 g were fed on a commercial diet (Altromin®, Altromin GmbH, Lage-Lippe), containing 18–20% of protein, and water ad libitum.

All chemicals were analytical grade, obtained from E. Merck AG (Darmstadt), Serva (Heidelberg) and C. Roth OHG (Karlsruhe). Calibration proteins for electrophoresis, concanavalin (con) A –Sepharose, wheat germ lectin–Sepharose and protein A–Sepharose were from Pharmacia (Freiburg). Glycyl–prolyl–*p*-naphthylamide was obtained from Serva, glycyl–prolyl–*p*-nitroanilide – tosylate from Serva and C. Roth, respectively.

Dipeptidylaminopeptidase IV (DPP IV) was tested as in [3] using a Perkin-Elmer fluorescence photometer. Alternatively, the following method was used (modified according to [4]): 0.05 ml glycyl–prolyl–*p*-nitroanilide – tosylate solution (10 mg/ml H_2O), 0.005–0.1 ml enzyme-containing solution, add 1.0 ml with 0.1 mol/l Tris buffer (pH 8.0). *E* was measured at 405 nm using a Gilford UV/VIS photometer. For routine measurements the latter method was taken.

Protein was determined as in [5]. Rat liver plasma membranes were isolated by the method in [6] with some modifications [7] and checked for purity as described. Fractionation of plasma membrane glycoproteins and isolation of the 110 000 M_r glycoprotein were performed as in [1]. SDS gel electrophoresis was performed as in [8].

Antibodies against the 110 000 M_r glycoprotein were obtained by immunization of rabbits with the purified antigen. The antibodies were enriched from the serum by several ammonium sulfate precipitation steps.

3. RESULTS AND DISCUSSION

Fig.1 shows the elution profile of DPP IV extracted from rat liver plasma membrane (essentially as in [1]) on a con A–Sepharose affinity column: ~95% of the enzyme activity is bound to the lectin and eluted with α-methylmannoside. Most enzyme activity bound to con A is bound to wheat germ lectin and eluted with *N*-acetylglucosamine. The glycoprotein fraction bound to con A and wheat germ lectin thus obtained contains only a few polypeptide bands on SDS gels,

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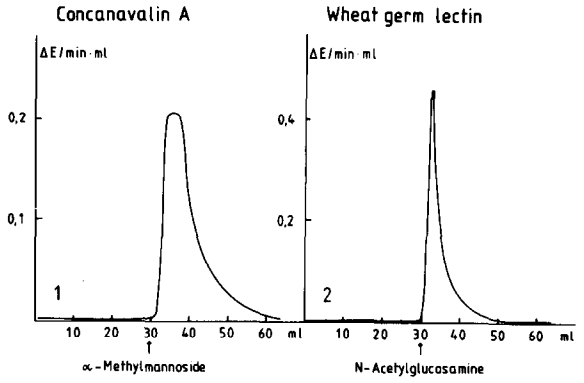

Fig.1. Affinity chromatography of DPP IV on con A–Sepharose and wheat germ lectin–Sepharose: (1) The Triton X-100 extractable proteins of rat liver plasma membrane are fractionated on a con A–Sepharose column. Elution conditions as in [1]; bound glycoproteins are eluted with 4% (w/v) α-methylmannoside. Abscissa, elution volume; ordinate, DPP IV activity measured as E/min · ml at 405 nm; (2) The glycoproteins bound to con A–Sepharose and eluted with α-methylmannoside are fractionated on a wheat germ lectin–Sepharose column. Bound glycoproteins are eluted with 4% (w/v) *N*-acetylglucosamine.

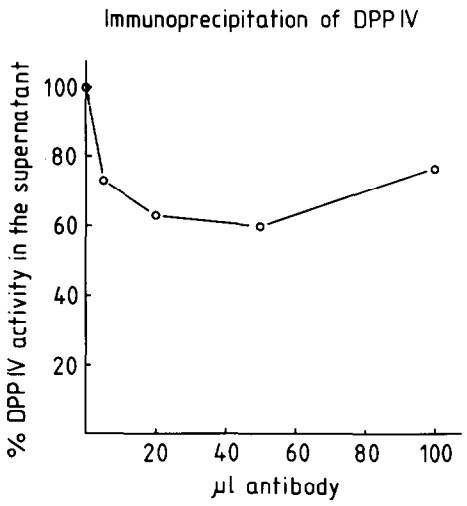

Fig. 2. Immunoprecipitation of DPP IV by antibodies against the 110 000 M_r glycoprotein: 0.5 ml glycoprotein fraction bound to con A and wheat germ lectin and eluted with α-methylmannoside and *N*-acetylglucosamine, respectively, are incubated with increasing amounts of antibody solution for 12 h at room temperature. After addition of protein A–Sepharose (200 μl 50% (wet vol./vol.) solution) and incubation for 1 h at room temperature the bound immunocomplexes are removed by centrifugation. DPP IV is measured in the supernatant: 0.416% Triton X-100 in the incubation mixture; 10 mmol/l Tris; 150 mmol/l NaCl; 1 mmol/l $CaCl_2$; 1 mmol/l $MgCl_2$ (pH 7.20). In control experiments using serum or an immunoglobulin fraction of non-immunized rabbits no precipitation of DPP IV was obtained.

the main band of which is the 110 000 M_r glycoprotein [1].

Using specific antibodies against the 110 000 M_r glycoprotein DPP IV can be immunoprecipitated from the Triton X-100 extract and the glycoprotein fraction bound to con A and wheat germ lectin: after incubation of the respective protein fractions with increasing amounts of antibody the immunocomplexes are removed by the addition of protein A–Sepharose and centrifugation. Up to 40% of the total DPP IV activity can be immunoprecipitated. Our antibodies are directed against the denaturated glycoprotein. This fact may account for the incomplete immunoprecipitation (fig. 2).

If the glycoproteins bound to con A and wheat germ lectin are incubated in the sample buffer for 2 min at room temperature (not boiled!) DPP IV activity is preserved during electrophoresis. After incubation of the gel in 0.1 mol/l Tris buffer (pH 8.0) containing 1 mmol/l glycyl–prolyl–*p*-nitroanilide – tosylate a yellow band arises corresponding to active DPP IV at 220 000 M_r. Its exact localization is achieved by scanning the gel at 405 nm (fig. 3, scan 3). Fig. 3 (scan 2) shows a scan of the same gel at 605 nm after staining the polypeptides with Coomassie blue, indicating a coincidence of the main polypeptide band with DPP IV. At 110 000 and 330 000 M_r minor bands are detectable. If the same glycoprotein fraction is separated on a SDS gel after boiling in sample buffer (our conditions used for the isolation of the 110 000 M_r glycoprotein) the main polypeptide band is shifted to 110 000 M_r (fig. 3, scan 1). At 220 000 and 330 000 M_r minor bands can be detected on this scan. If the active DPP IV localized by a 405 nm scan on the gel is eluted and applied on a second gel after boiling in sample buffer the 110 000 M_r glycoprotein appears and eventually traces of a 220 000 M_r glycoprotein (fig. 3, scan 4).

The findings are interpreted as follows: the active DPP IV consisting of 2 identical subunits

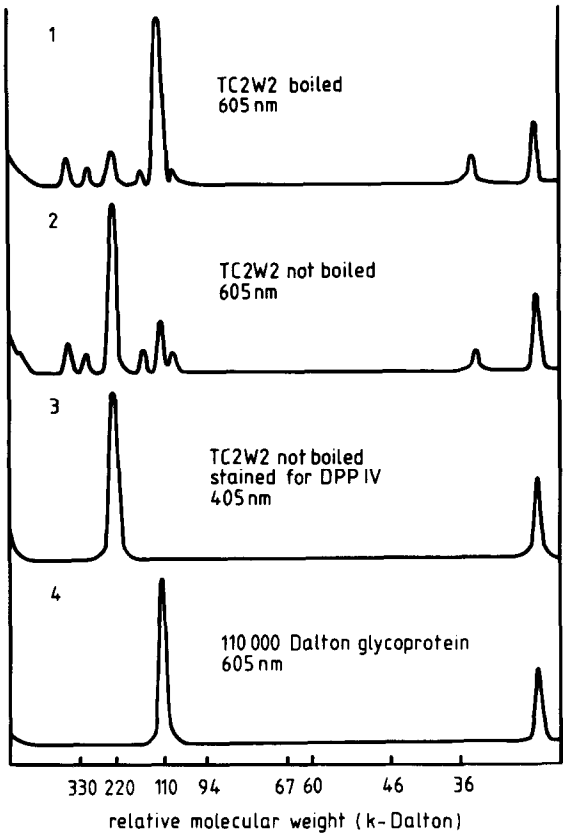

Fig.3. Scans of SDS gels of plasma membrane glycoproteins bound to con A and to wheat germ lectin (=glycoprotein fraction TC2W2 [1]) under different conditions: (1) glycoproteins boiled in sample buffer of electrophoresis (containing SDS and mercaptoethanol), stained with Coomassie brilliant blue, scan at 605 nm; (2) glycoproteins not boiled, stained with Coomassie brilliant blue, scan at 605 nm; (3) glycoproteins not boiled, gel incubated with glycyl–prolyl–*p*-nitroanilide – tosylate. The yellow band corresponds to DPP IV, scan at 405 nm; (4) SDS gel of DPP IV, eluted from gel 3, boiled in sample buffer, stained Coomassie brilliant blue, scan at 605 nm.

[3,9–12] conserves on the gel in scan 2 and 3 mainly its active dimeric form and can be stained specifically using the chromogenic substrate. Only to a minor extent is it converted into the inactive monomer and probably a trimeric or tetrameric aggregate. By boiling in sample buffer containing SDS and mercaptoethanol it is converted into the monomer which we have isolated previously.

An identical 405 nm scan is obtained with a SDS gel of total Triton X-100 extractable glycoproteins under the same conditions as used in fig. 3, scan 2 and 3 (not shown) indicating that only 1 enzyme can convert the chromogenic substrate. This corresponds to the substrate specificity of DPP IV as in [13].

Our results clearly prove an identity of the 110 000 M_r glycoprotein isolated from rat liver plasma membrane with the monomer of dipeptidylaminopeptidase IV. This enzyme is a widespread membrane-bound glycoprotein. So far it has been isolated from kidney [3,9,14,15] and liver [11,12,15]. Furthermore, it occurs in intestinal microvilli [16,17], T-lymphocytes [18], brain [13], bronchopulmonary lavage fluid [19] and serum [20–23]. Its biological function remains to be established. Possibly it is involved in dipeptide transport [24].

The turnover of terminal carbohydrates on the intact polypeptide suggests a process involving partially deglycosylated forms of this glycoprotein, which now has been identified as dipeptidylaminopeptidase IV (EC 3.4.14.X). According to our working hypothesis this effect reflects the action of glycosidases in the lysosomal compartment and glycosyltransferases in the Golgi complex during the recycling of DPP IV rather than a regulatory cycle of the enzymatic activity.

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