Increased activity of dipeptidyl peptidase IV in serum of hepatoma-bearing rats coincides with the loss of the enzyme from the hepatoma plasma membrane

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Dedicated to Professor Georg Hertting on the occasion of his 60th birthday.

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Summary. The specific activity of dipeptidyl peptidase IV (DPPIV E.C. 3.4.14.-) in the plasma membrane of Morris hepatoma 9121 or hepatoma 7777 was 3.5% and 2.9%, respectively, of that in the plasma membrane of rat liver. The enzyme activity in the serum of hepatoma-bearing rats was141% (hepatoma 91219) and 162% (hepatoma 7777) of the normal value. Cytochemical investigation showed that the DPP IV activity was almost completely absent from the hepatoma cell plasma membrane and was not sequestered within these cells. Indirect immunofluorescence staining with a polyclonal antibody directed against DPP IV indicated that the loss of activity was due to the absence of DPP IV molecules in the plasma membrane. The possibility that the enzyme is transferred from the membrane into the serum as a result of structural alterations is discussed.
Key words. Dipeptidyl peptidase IV; hepatoma rats; cancer markers; immunofluorescence; cytochemistry.

Dipeptidyl peptidase IV (DPP IV, E.C.3.4.14.-) is a glycoprotein mainly associated with the brush border membrane of mammalian kidney[1], and the liver plasma membrane[2] as well as with the extracellular matrix of the liver[3], the lung tissue and to a lesser extent with a number of other tissues[4,5]. The DPP IV activity in serum is due to the soluble form of the enzyme. This form is immunologically identical with the membrane-associated one but differs in its carbohydrate composition[6]. It has been proposed that the soluble enzyme is a precursor form of the membrane-associated DPP IV[6]; however, the experimental evidence for this relationship is hitherto lacking.

The DPP IV activity in the human serum was found to be decreased in patients with gastric cancer, pancreatic cancer, acute lymphocytic leukemia, lymphosarcoma and Hodgkin's disease[7,8,9], whereas hepatomas caused a significant increase of enzymatic activity[10]. The cellular mechanism of this alteration in cancer has not been elucidated. The aim of the present study was to compare systematically in rat the DPP IV activity in the normal liver and in hepatomas with the corresponding serum activities. The data presented indicate that the loss of the membrane-associated form from the tumor is concomitant with the increased activity of the enzyme in serum.

Materials and methods. Morris hepatoma 7777, a rapidly growing, poorly differentiated hepatocellular carcinoma, was inoculated into both hind legs of Buffalo rats of both sexes, and was grown for about two weeks. The slower growing hepatoma 9121 was propagated in ACI rats also as a solid tumor. Plasma membranes of the liver and of hepatoma were isolated by zonal centrifugation as described by Pfleger et al.[11] and modified by Büchsel et al.[12]. Protein concentration was determined by the method of Lowry et al.[13]. The DPP IV activity was determined with the substrate Gly-Pro-p-nitroanilide-tosylate (Bachem, Bubendorf, Switzerland) as reported previously[14] and the specific activity was calculated from a standard curve of p-nitroaniline (Serva, Heidelberg, FRG) solution in 0.1 M Tris·HCl buffer, pH 8.0. Plasma membrane 5'-nucleotidase was determined with adenosine-5'-monophosphate as substrate according to Michell and Hawthorne[15].

Cytochemical investigation of DPP IV in 10-µm thick acetone-chloroform pretreated frozen tissue sections was carried out with Gly-Pro-4-methoxy-2-naphthylamine (Bachem) as substrate and Fast Blue B (Serva) for simultaneous coupling as described by Gossrau[16]. 5'-Nucleotidase was visualized in tissues according to Lojda et al.[17]. The antiserum against the plasma membrane fraction enriched in DPP IV was raised in a rabbit[18]. The immunoglobulin fraction was isolated on protein A-Sepharose and characterized as described in 'Results'. Indirect immunofluorescence staining was carried out on 7-µm thick frozen tissue sections. After air drying for 20 min the sections were overlayered with antibody preparation or with preimmune serum, both diluted 1:20 with phosphate buffered saline, pH 7.2. The sections are incubated at room temperature for 1 h and subsequently washed with phosphate buffer. They were then covered with fluorescein-isothiocyanate conjugated to goat anti-rabbit gamma globulin (Nordic-Immunological Laboratories, Tilburg, The Netherlands). After incubation for 1 h at room temperature the slices were thoroughly washed with buffer and placed under coverslips. Photographs were taken immediately afterwards with an automatic camera (Wild MPS 50, Heerbrugg, Switzerland) mounted on a Leitz Dialux 20 microscope. Parallel samples were stained with preimmune serum to assure the specificity of the observed reaction.

Results and discussion. The values of DPP IV activity in rat serum (16.6 ± 2.1 U/l) roughly corresponded to those found by Kato et al.[19] in mice (18.59 ± 0.25 U/l). Specific activity of the enzyme in the isolated liver plasma membrane (620.0 ± 126.3 U/g protein) was higher than that in the plasma membrane preparations of Kreisel et al.[20] (266+56 U/g protein) probably due to a different membrane isolation procedure. As shown in the table, the drastic decrease of the DPP IV activity in the membrane of the tumor was accompanied by an increase of the activity in serum. Assuming that the total protein weight of plasma membrane in the liver (10 g wet wt) was about 10 mg[21], the total activity of the DPP IV in the liver plasma membrane was 6.2 U. At the time of harvesting, the tumors had the approximate weight of 30 g per rat and they gave 45 mg plasma membrane protein[21]. The total DPP IV activity in Morris hepatoma 7777 was about 0.81 U. The corresponding total activities of DPP IV in 7.5 ml rat serum amounted to 0.125 U (normal value) and 0.202 U (in tumor bearing rats).

These data, summarized in figure 1, indicate that both hepatomas, 9121 and 7777, were able to produce only small amounts of DPP IV as compared with the normal liver. By contrast, the membrane associated enzyme 5'-nucleotidase had approximately the same specific activity in the plasma membrane of both liver (0.66 ± 0.23 U/g protein) and hepatoma 7777 (0.85 ± 0.20 U/g protein) (mean ± SD, n = 10). The cytochemical study showed that DPP IV activity was concentrated in bile canalicular areas and to a lower extent in the sinusoidal endothelium of the normal liver. The activity was also present in sparse foci in hepatoma 9121 but was not detectable in hepatoma 7777 (fig. 2). 5'-Nucleotidase staining was similar in the normal liver and in both hepatomas (not shown). The staining for DPPIV indicated that the drastic decrease of the enzyme activity in the tumor plasma membrane was not due to the retention of the enzyme within the cell, which would explain the low activity in the isolated membrane.

However, the question remained whether the activity per enzyme molecule in the tumor was comparable to that of the liver. This could be deduced from the comparison of distribution of the enzymatic activity and the amount of the synthesized DPP IV. To clarify this point, a polyclonal antibody raised against a

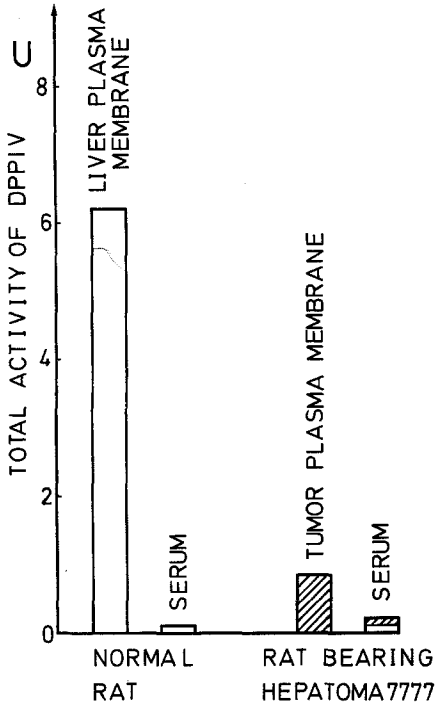

Figure 1. Total DPP IV activity in the plasma membrane of liver and hepatoma 7777 as compared to the total activity in serum of the normal and the tumor bearing rat. One unit (U) was defined as in the table.

plasma membrane fraction enriched in DPP IV activity was applied. The antibody precipitated 90% of DPP IV activity from the solubilized plasma membrane and the precipitate gave a single antigen band in sodium dodecyl-sulphate polyacrylamide gel electrophoresis as reported previously[22]. It reacted also with tumor DPP IV, thus suggesting that tumor and liver enzyme shared antigen determinants (data not shown). The immunofluorescence staining of frozen liver and tumor sections with the antibody showed that the DPP IV in the liver was concentrated mainly in the bile canalicular region in a manner closely paralleling the cytochemical staining. The hepatoma 9121 tissue showed only punctate distribution of DPP IV which might correspond to rudimentary bile canalicular structures, while in the hepatoma 7777 the glycoprotein was practically undetectable. Both tumors contained no demonstrable DPP IV in the cytosol, which corroborated the histochemical finding that the glycoprotein produced in the tumor was not retained within the cell (fig. 3).

The present results show that the transformation of hepatocytes into hepatoma cells is concomitant with a drastic decrease of synthesis of the membrane-bound form of DPP IV. Simultaneously, the serum activity of the enzyme in hepatoma-bearing rats increased by about 40–60%. By contrast, a number of other tumors in man[7,8,9] and in mice[19] caused a reduction of the DPP IV activity in serum. Hepatocellular carcinomas, however, also caused an increase of DPP IV activity in human serum[10]. Whether the DPP IV was lost from the tumor plasma membrane as a result of the disturbance in membrane-assembly or as a result of a controlled secretion process is not clear at present. Fukasawa et al.[6] found that the soluble form of the pig liver DPP IV contained less fucose, mannose and sialic acid and concluded that the soluble form was a precursor of the membrane-associated form. In agreement with this hypothesis is the finding that the glycosyl structure of the membrane-bound DPP IV in liver is of the complex type[23,24]. The structure of the tumor-derived DPP IV is not known and its possible identity with the normal soluble form has yet to be proven. However, the identical isoelectric

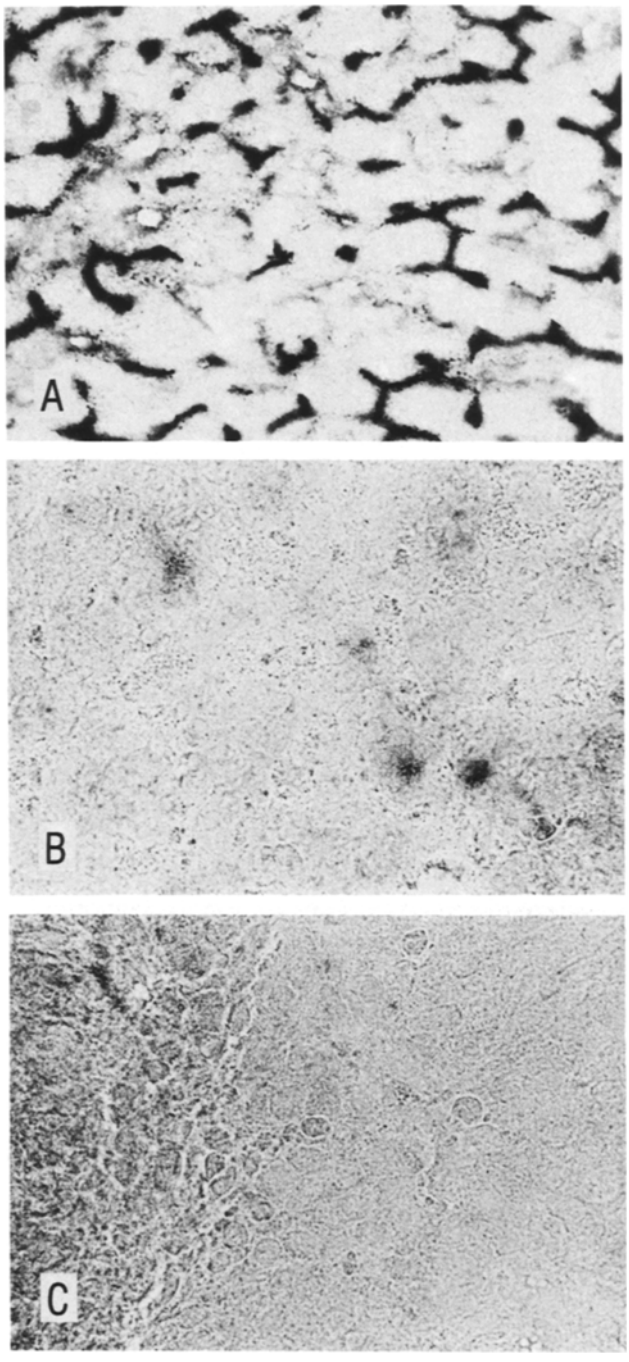

Figure 2. Histochemical staining for DPP IV activity. *A* liver; *B* hepatoma 9121; *C* hepatoma 7777. Dark areas mark the presence of enzymatic activity. Magnification × 600.

points of the tumor-derived DPP IV (pI = 4.33 ± 0.09) and the serum DPP IV in normal rat (pI = 4.4 ± 0.1) (mean of three determinations ± SD: Hanski, Reutter, unpublished) make the identity of the two molecules very likely. The present animal model will make it possible to investigate whether the loss of DPP IV from the tumor plasma membrane was due to the difference in the carbohydrate composition between the soluble and the membrane-associated form of the enzyme.

Note added in proof: Recently it has been reported that DPP IV activity in detergent extracts of hepatocellular carcinomas was significantly lower

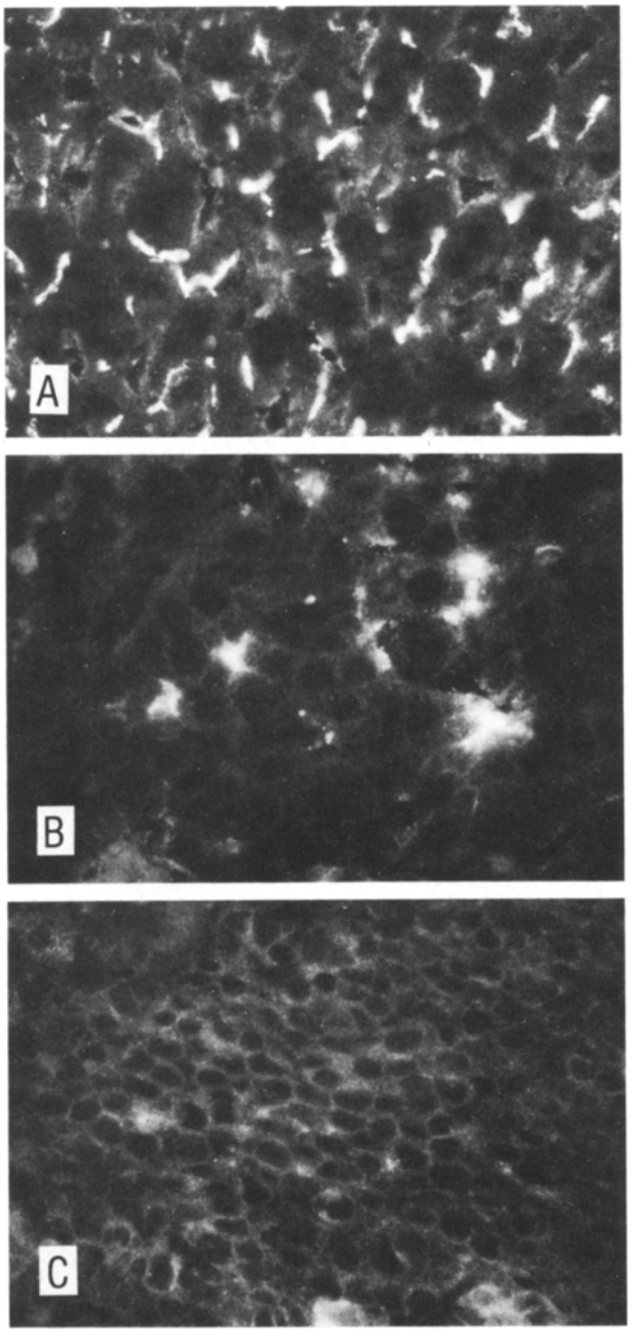

Figure 3. Indirect immunofluorescence staining with a polyclonal antibody directed against DPP IV. *A* liver; *B* hepatoma 9121; *C* hepatoma 7777. Bright areas mark the presence of enzyme molecules. Magnification × 600.

than in extracts of hepatocytes. (Walborg, E.F., Tsuchida, S., Allison, J.P., DeLourdes Ponce, M., Barrick, A., Weeden, D.S., Thomas, M.W., and Hixson, D.C., in: Cell Membranes and Cancer, p. 25. Ed. T. Galeotti. Elsevier, Amsterdam 1985.

DPP IV activity in the plasma membrane of liver and hepatoma and the corresponding serum values. Plasma membrane values are means (± SD) of four preparations for which 15 rats were used each time. Serum values (± SD) were obtained from six rats. The DPP IV activity was determined as described in 'Materials and methods'. One unit was defined as the amount of enzyme catalyzing the formation of 1 μmol p-nitroaniline/min at 37 °C

	Plasma membrane U/g	% of control	Serum U/l	% of control
Normal liver	620.0 ± 126.3	100	16.6 ± 2.1	100
Hepatoma 9121	21.9 ± 2.6	3.5	23.4 ± 3.9	141
Hepatoma 7777	18.0 ± 2.4	2.9	26.9 ± 6.6	162

Acknowledgments. The authors thank Prof. Dr. C. Bauer for valuable discussions. This project has been supported by the Deutsche Forschungsgemeinschaft, Fonds der Chemischen Industrie and the Hermann und Lilly Schilling-Stiftung.

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0014-4754/86/070826-03$1.50 + 0.20/0