Alteration in Glycosidases from Well-Differentiated Colorectal Adenocarcinoma of Rat

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KOSAKAI, M., ISEMURA, M., MUNAKATA, H. and YOZISAWA, Z. Alteration in Glycosidases from Well-Differentiated Colorectal Adenocarcinoma of Rat. Tohoku J. exp. Med., 1985, 146 (3), 303-311 —— The activities of six glycosidases in a rat colorectal adenocarcinoma were measured and compared with those of normal colonic mucosa. The specific activities of β-galactosidase (EC 3.2.1.23) and β-glucuronidase (EC 3.2.1.31) in the adenocarcinoma were similar to those of the corresponding ones in the normal mucosa, whereas those of β-N-acetylglucosaminidase (EC 3.2.1.30), α-L-fucosidase (EC 3.2.1.51), α-galactosidase (EC 3.2.1.22) and β-glucosidase (EC 3.2.1.21) were reduced in the former as compared with those in the latter. In the case of α-L-fucosidase, two forms were newly detected in the tumor. The relative abundance of three forms of β-N-acetylglucosaminidase was quite different between the adenocarcinoma and the normal mucosa, and the level of the intermediate form in the tumor was markedly reduced. However, thermostability and Km values of two forms A and B in the tumor were not different from those of the corresponding ones in the normal tissue. —— glycosidases; colonic mucosa; colorectal adenocarcinoma

Many reports have documented changes of glycoproteins and glycolipids in association with tumorigenesis and metastasis (Warren et al. 1973; Bramwell and Harris 1978; Koyama et al. 1979; Takasaki et al. 1980; Hakomori 1981). The differences in glycosidase levels and their isozyme patterns have also been frequently reported for various tumors (Brattain et al. 1979; Mian et al. 1979; Okochi et al. 1979; Varani et al. 1979; Dobrossy et al. 1981), and Bosmann and Hall (1974) have suggested the possibility that the lysosomal enzymes may modify the cell surface components.

In a previous paper, we reported alterations in the carbohydrate composition and the blood group activities of sialoglycopeptides derived from a rat colorectal adenocarcinoma as compared with those from the normal colonic mucosa (Isemura et al. 1983). In the present study, we examined the several glycosidase activities in this experimental tumor in comparison with those in the normal colonic...
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Materials and Methods

Chemicals. 4-Methylumbelliferyl (4MU)-glycosides of N-acetyl-β-D-glucosamine, β-D-glucuronic acid and α-L-fucose, and p-nitrophenyl-N-acetyl-β-D-glucosaminide were obtained from Nakarai Chemicals and those of α-D-galactose, β-D-galactose and β-D-glucose from Sigma Chemical Co. DEAE-cellulose was obtained from Brown Co.

Tissue homogenates. The tissues of a transplantable colorectal adenocarcinoma and the normal colonic mucosa of ACI/N rats were prepared as described previously (Goto et al. 1975; Isemura et al. 1983). The tumor tissue and the colonic mucosal scrapings were separately homogenized with three volumes of 0.05 M citrate-phosphate buffer (pH 4.5) containing 0.1% Triton X-100. The homogenate was centrifuged at 30,000 × g for 20 min. Aliquots of the supernatant were analyzed for the glycosidase activities and the protein content as described below. The remaining portions of the supernatant were dialyzed against 0.01 M phosphate buffer (pH 6.0) for 12 hr. These procedures were carried out at 4 °C unless otherwise indicated.

Enzyme assay. The activities of glycosidases were measured with 2.4 mM 4MU-N-acetyl-β-glucosaminide at pH 4.5, 1.2 mM 4MU-β-galactoside at pH 4.5, 2.4 mM 4MU-β-glucuronide at pH 4.5, 1.2 mM 4MU-α-L-fucoside at pH 5.5, 0.5 mM 4MU-α-D-galactoside at pH 4.5 and 1.2 mM 4MU-β-glucoside at pH 6.0 for respective enzymes. The activity for p-nitrophenyl-N-acetyl-β-D-glucosaminide of β-N-acetylglycosaminidase was measured by the method of Mian et al. (1979). The activities of β-N-acetylglycosaminidase, β-galactosidase, β-glucuronidase and α-L-fucosidase in the fractions obtained by DEAE-cellulose column chromatography were separately determined with 1.2 mM of the relevant substrates. For the kinetic study on three forms of β-N-acetylglycosaminidase, 0.1-2.0 mM of the substrate was used.

To 20 μl of each sample was added 100 μl of the appropriate substrate in 0.05 M citrate-phosphate buffer and the mixture was incubated at 37°C. The reaction was terminated by cooling the test tubes in ice-water, followed by the addition of 3 ml of 0.1 M carbonate-bicarbonate buffer (pH 10.2). The fluorescence of released 4-methylumbelliferylone was measured at 448 nm (emission) with excitation at 365 nm in a Shimadzu model RF-510 spectrofluorophotometer. Protein was determined by the method of Lowry et al. (1951) with bovine serum albumin as a standard.

Results

The specific activity of glycosidases

In order to compare the efficiency of enzyme solubilization with the previous data, the supernatant of tissue homogenate was analyzed for the specific activity of β-N-acetylglycosaminidase. Since no data with 4MU-N-acetyl-β-glucosaminide have been reported, p-nitrophenyl-N-acetyl-β-glucosaminide was used as a substrate. The specific activity determined was 3.58 μmol/hr/mg of protein. The value was much higher than that (0.011 μmol/hr/mg of protein) of rat colonic mucosa of a different strain reported by Mian et al. (1979), but was comparable with that (1.95 μmol/hr/mg of protein) of human colonic mucosa (Tsao et al. 1979). These data suggested that the enzyme solubilization was achieved to an extent which is comparable with or much higher than that reported previously.

The specific activities of glycosidases in the rat colorectal adenocarcinoma mucosa.
Glycosidases in Colorectal Adenocarcinoma

In both tissues the specific activity of β-N-acetylglucosaminidase was the highest among glycosidases studied, while only trace activity of β-glucosidase was detected. The specific activities of β-galactosidase and β-glucuronidase in the tumor tissue were slightly higher than those of the normal tissues, whereas those of other glycosidases in the former were lower than those in the latter. Especially that of β-N-acetylglucosaminidase in the former was only 28% of that in the latter.

The specific activities of glycosidases in the normal rat plasma were less than 1.5% of those in the colonic mucosa (Table 1). These data suggest that the contribution of the plasma enzymes to the tissue homogenates was negligible.

**DEAE-cellulose column chromatography**

The elution patterns of four glycosidases in the colorectal adenocarcinoma were compared with those in the colonic mucosa by DEAE-cellulose column chromatography (Fig. 1). No significant difference was detected in the chromatographic patterns of β-glucuronidase between the tumor and the normal mucosa (Fig. 1a and b). The ratio of the basic form to the acidic ones of β-galactosidase from the tumor was elevated as compared with that from the normal mucosa (Fig. 1c and d). In addition, the main peak of the acidic forms from the former was eluted at higher NaCl concentration than those from the latter, indicating a certain altered composition of this enzyme in the adenocarcinoma.

In the case of α-L-fucosidase, the adenocarcinoma contained three forms, two of which were not detected in the normal mucosa (Fig. 1e and f).

The most remarkable difference in the chromotographic pattern was observed in β-N-acetylglucosaminidase (Fig. 1g and h). The normal mucosa contained similar amounts of three forms which were separable by DEAE-cellulose column chromatography (Fig. 1h and Table 2). In the order of elution from the column, they were identified as the B, intermediate, and A forms according to the designation by Mian et al. (1979). In contrast, the tumor contained the intermediate form representing only 7% of the total activity (Fig. 1g and Table 2).
In order to study on the thermal stability of three forms of \( \beta \)-N-acetylglucosaminidase, sample solutions were heated at 50°C and then the...
glycosidase activity was determined. The samples of tube numbers of 8, 39 and 47 were used as test solutions of the B, intermediate and A forms, respectively (Fig. 1g and 1h). In a preliminary experiment, it was found that the degree of the thermal stability depended on the protein concentration of the enzyme.

Table 2. Proportions* of three forms of β-N-acetylglucosaminidase from the adenocarcinoma and the normal colonic mucosa

<table>
<thead>
<tr>
<th>Enzyme form</th>
<th>Adenocarcinoma</th>
<th>Colonic mucosa</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>65</td>
<td>31</td>
</tr>
<tr>
<td>Intermediate</td>
<td>7</td>
<td>34</td>
</tr>
<tr>
<td>B</td>
<td>28</td>
<td>35</td>
</tr>
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</table>

* Expressed as percent of the total enzyme activity.

Fig. 2. Thermal stability of three forms of β-N-acetylglucosaminidase at 50°C. Enzyme solutions from the normal colonic mucosa (a) or from the adenocarcinoma (b) were diluted with citrate-phosphate buffer (pH 4.5) to the same protein concentration and then heated at 50°C for scheduled times. An aliquot (20 μl) was withdrawn and the enzyme activities were measured. The activities are expressed as percentages of the activity of the control which had been kept at 0°C. •, A form; □, intermediate form; ○, B form.
solution to be tested. Therefore the enzyme solutions were adjusted to the same protein concentration by appropriate dilution.

The results indicated that the A form from the normal mucosa was inactivated rapidly at 50°C, while the B form was the most thermostable among three forms (Fig. 2a). The intermediate form was more stable than the A form.

The similar patterns of the thermostability were observed for the corresponding forms from the adenocarcinoma (Fig. 2b).

Kinetics

Table 3 lists the Km values for hydrolysis of 4MU-N-acetyl-β-glucosaminide by three forms of β-N-acetylglucosaminidase from the tumor and the normal mucosa. The Km value was the largest for the A form, and the smallest for the B form. No difference was observed between the A and B forms from these two tissues.

DISCUSSION

The rat colorectal adenocarcinoma examined in the present study is well-differentiated, papillary mucinous infiltrative and transplantable, and has very low potential of metastasis (Goto et al. 1975). This tumor tissue exhibited the reduced specific activities in four glycosidase activities, although those of β-galactosidase and β-glucuronidase were similar or slightly elevated as compared with the normal tissue.

Although the specific activity of α-L-fucosidase was reduced in the adenocarcinoma as compared with that in the normal tissue, two additional forms were newly detected in the former. This may be analogous to the phenomenon observed by Broadhead et al. (1981). These authors showed the abnormal profile of this enzyme in the acute lymphoblastic leukemia by isoelectric focusing.

The specific activity of β-N-acetylglucosaminidase in the rat colorectal adenocarcinoma was the highest among those of glycosidases studied. A number of investigators have reported the increased activity of this enzyme in association with malignant transformation (Bosmann and Hall 1974; Mian and Cowen 1974; Whitehurst et al. 1982) and the enhanced metastatic ability of tumors (Varani et

Table 3. Michaelis constants* for three forms of β-N-acetylglucosaminidase from the adenocarcinoma and the normal colonic mucosa

<table>
<thead>
<tr>
<th>Enzyme form</th>
<th>Adenocarcinoma</th>
<th>Colonic mucosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.82</td>
<td>0.86</td>
</tr>
<tr>
<td>Intermediate</td>
<td>—†</td>
<td>0.60</td>
</tr>
<tr>
<td>B</td>
<td>0.33</td>
<td>0.31</td>
</tr>
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* Expressed as mM with 4-MU-N-acetyl-β-glucosaminide as the substrate.
† Not determined.

On the other hand, no significant difference in this enzyme level was observed in renal and colonic carcinoma (Kim and Isaacs 1975; Brattain et al. 1977; Okochi et al. 1979; Tsao et al. 1979) and the decrease in its specific activity was reported in fast growing hepatoma and chronic lymphocytic leukemia (Weber et al. 1973; Dempse et al. 1980).

In many normal tissues, β-N-acetylhexosaminidase has been known to have two major isozymes, A and B, the former of which is predominant. In contrast, tumors have frequently a higher proportion of the B isozyme relative to the A isozyme as compared with that found in the matched control tissues, and the increased activity of the B isozyme has been ascribed to the elevation in this proportion (Okochi et al. 1979; Tsao et al. 1979).

On the other hand, no significant differences in this proportion were shown in rat colonic adenocarcinoma and human prostatic carcinoma (Mian et al. 1979; Whitehurst et al. 1982). In the present study, two components of β-N-acetylglucosaminidase corresponding chromatographically to the A and B isozymes were termed the A and B forms, respectively, according to the designation by Mian et al. (1979). The proportion of the B form to the A form in the tumor was revealed to decrease by half of that in the normal colonic mucosa (Table 2), indicating the reduction of the former was greater than that of the latter in the tumor.

Brattain et al. (1979) found the thermolabile B isozyme in the rat colonic carcinoma. The similar change in the B isozyme has also been reported for colonic carcinoma and liver metastatic tumor (Kimball et al. 1981; Alhadeff and Holzinger 1982). On the other hand, Mian et al. (1979) found a different change of the B form in a well-differentiated colonic adenocarcinoma of rat. This unusual B form was shown to be more thermostable and to have a smaller Km value as compared with the normal B form. In the present case, however, we could not detect any significant difference in the thermostability and the Km value of this form between the tumor and normal. The different observations are probably due to the difference in the tumor tissues used, which were induced with the different chemical carcinogens.

The normal colonic mucosa of rat was found to contain a third form of β-N-acetylglucosaminidase in comparable amount to other two forms. The component eluted between the B and the A forms had the intermediate properties in both thermostability and the affinity for the substrate. Therefore, this appears to be the same component as that termed as the I form by Mian et al. (1979) for a rat enzyme. They showed the complete disappearance of the I form in the rat colonic adenocarcinoma. We also found the striking, though not complete, reduction of this form in the tumor. Thus, there is the possibility that the reduction of this form is the general phenomenon in the rat colonic adenocarcinoma. It should be noted that the properties of the I form of rat
β-N-acetylglucosaminidase differed from those of human I form characterized by Price and Dance (1972).

Although the present study revealed some alterations in glycosidases of the rat colorectal adenocarcinoma, further studies will be necessary to clarify the relationship between the properties of this tumor and the observed changes in these glycosidases.

Acknowledgments

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References


14) Keyama, K., Nudelman, E., Fukuda, M. & Hakomori, S. (1979) Correlation of glycosylation in a membrane protein with a molecular weight of 150,000 with tumor-