Blood Group Activity of Human Sucrase from Intestinal Metaplasia*

MARIKO KURISU, NORIKO NUMANYU, TAKASHI KAWACHI, AND TAKASHI Sugimura
From the Biochemistry Division, National Cancer Center Research Institute, Tsukiji, Chuo-Ku, Tokyo, Japan

Sucrases were purified from human small intestine and from areas of intestinal metaplasia of the stomach mucosa surrounding stomach cancers. The kinetic constants and pH activity profiles of enzyme preparations from the two sources were similar. No blood group activity of sucrase was detectable in preparations from three cases of intestinal metaplasia, but preparations from two other cases showed activity like that of the small intestine. These results indicate that sucrase from areas of intestinal metaplasia has similar enzymatic properties to those of enzyme from the small intestine, but that the antigenic sugar moiety of the enzyme associated with blood group activity varies.

In humans, intestinal metaplasia results from chronic gastritis and seems to be due to abnormal differentiation of the stomach mucosa characterized by the appearance of intestinal type epithelial cells. Enzymological studies have shown that areas of intestinal metaplasia contain enzymes specific to the small intestine, including disaccharidases and alkaline phosphatase (1). Human stomach cancer often arises from areas of intestinal metaplasia, so this abnormality has been studied mainly in relation to carcinogenesis of the stomach (1-4).

In this work, we compared sucrase (EC 3.2.1.26) in areas of intestinal metaplasia with that of normal small intestine. Human intestinal sucrase is reported to have ABO blood group activity in its sugar moiety (5). Changes in blood group glycoconjugates in human cancer tissues (6-10). Therefore, we are interested in the ABO blood group activity of sucrase from areas of intestinal metaplasia. As reported in this paper we found that ABO blood group activity of sucrase from areas of intestinal metaplasia was present in some cases but reduced or absent in others.

EXPERIMENTAL PROCEDURES

Preparation of Sucrase - Stomach with areas of intestinal metaplasia were obtained at operation from five cases of gastric cancer. The ages, sexes, and blood group types of these patients were as follows: T.S. 66, male, type A; S.K. 67, male, type A; F.A. 61, female, type B; I.Y. 61, male, type B; and M.I. 62, male, type B. Sections of the small intestine from the duodenum to the jejunum were obtained at operation from a case of gastric cancer (F.A. 61, female, type B) and from two cases of colon cancer (C.S. 71, female, type A; M.K. 56, female, type O). The area of intestinal metaplasia was determined by the "Tes-Tape" method for detection of sucrase as described previously (1). The mucous membrane of the area of intestinal metaplasia was separated from the submucosa and stored at -70°. Sucrase was purified by a modification of the method of Cogoli et al. (11), involving salinisolation with papain, ammonium sulfate fractionation, gel filtration on Bio-Gel P-300 and chromatography on DEAE-cellulose, and finally electrophoresis on polyacrylamide gel to separate contaminated minor blood group substances(s).

Sucrase Assay - Sucrase activity was measured by the method of Dahlqvist (12, 13) with 0.1 M sucrone as substrate. The glucose produced was usually assayed by a one-step micromethod with a phosphate-glucose oxidase reagent (12), but in experiments on the effect of pH a two-step method with a Tris/glucose oxidase reagent (13) was used instead, and the buffers of various pH values all contained 0.1 M sodium ions (14). For kinetic studies, seven different substrate concentrations were used and the Michaelis constants were determined by the method of Lineweaver and Burk (15).

Protein was determined by the method of Lowry et al. (16).

Polyacrylamide Gel Electrophoresis - Polyacrylamide gel electrophoresis was carried out as described by Ornstein (17) and Davie (18) using 5% acrylamide as separating gel. The buffers for the separating gel, stacking gel, and reservoir were of pH 8.9, 6.7, and 8.3, respectively. After electrophoresis, the gels were stained for protein with Coomassie brilliant blue or for sugar by the periodic acid-Schiff method (19, 20). Some gels were also cut into transverse sections and slitted overnight in phosphate-buffered saline to measure sucrase activity and blood group activity.

Blood Group Activity - ABO blood group activity was measured by the hemagglutination inhibition test using a microtitration system (21). The wells contained 25 μl of antigen diluted serially with phosphate-buffered saline containing 0.05% gelatin and 25 μl of antiserum of twice the concentration necessary to agglutinate the red cells completely. The mixtures were incubated at 37° for 2 h, and then 25 μl of glutaraldehyde-fixed red blood cells were added. The end point of the inhibition titer was taken as the dilution which inhibited half the hemagglutination.

Chemicals - Papain and o-dianisidine were purchased from Sigma Chemical Co. (St. Louis, Mo.). Glucose oxidase was a product of Nagase Sangyo Co. (Osaka, Japan). Peroxidase was a product of Boehringer Mannheim, GmbH (Mannheim, Germany). Bio-Gel P-300 was obtained from Bio-Rad (Richmond, Calif.) and DEAE-cellulose (DE52) from Whatman (Kent, United Kingdom). Anti-A and anti-B antibodies were purchased from Ortho Pharmaceutical Corp. (Baristan, N.J.). All other chemicals were of the highest grade available commercially.

RESULTS

Purification of Sucrase - Sucrases were purified from areas of intestinal metaplasia and from the small intestine, each example of which is given in Table I (S.K.) and Table II (F.A.), respectively. Further chromatography of the preparations on DEAE-cellulose resulted in increase in the specific activity to
18 to 23 units/mg of protein. On polyacrylamide gel electrophoresis the purified enzymes from the area of metaplasia and the intestine both gave a single band of glycoprotein which coincided in position with sucrase activity in the lower part of gel but on the upper part of gel showed very minor contaminated glycoprotein (Figs. 1 and 2). Gel filtration on Bio-Gel P-300 was effective for purification of the enzyme, especially that from the area of intestinal metaplasia, and was used instead of the affinity chromatography on Sephadex G-200 used previously (11), because human sucrase showed low affinity for the latter at 4°C.

Sucrase from the area of intestinal metaplasia was eluted in the same position as that from the small intestine on both Bio-Gel P-300 and DEAE-cellulose chromatography.

Kinetics and pH Activity Profiles—With sucrase as substrate the Michaelis constants for the purified enzymes from small intestine (21 mM, M.K.) and an area of intestinal metaplasia (18 mM, T.S.) were similar. Their Vmax values were also similar, being 25.6 units/mg of protein (M.K.) and 22.2 units/mg of protein (T.S.), respectively. The pH activity profiles of the two enzymes were also essentially the same (Fig. 3).

Blood Group Activity of Sucrase—Blood group activity was detected in two preparations of sucrase from the small intestine as reported previously by Kelly and Alpers (5). Namely, as shown in Table III, the enzyme from a patient of blood group type A (C.S.) showed hemagglutination inhibition of blood group type A only, that from a patient of type B (F.A.) showed only type B activity. The enzyme from a patient of type O (M.K.), as expected, showed neither type A nor type B activity.

Table III also shows the blood group activities of five enzyme preparations from areas of intestinal metaplasia. The preparations from two patients of blood group type A (T.S. and S.K.) showed no type A activity (and no type B activity). One of the three preparations from patients of type B showed neither type B nor type A activity (I.Y.), whereas the other two preparations showed type B activity but not type A activity (F.A. and M.I.).

Table I

| Fraction                | Total activity | Total protein | Specific activity |
|------------------------|----------------|---------------|------------------|
| Papain-solubilized supernatant | 25.6 | 880 | 0.029 |
| Ammonium sulfate, 60-70% saturation | 17.3 | 41.5 | 0.42 |
| Bio-Gel P-300 | 15.1 | 2.00 | 7.55 |
| DEAE-cellulose | 10.7 | 0.75 | 14.3 |

Table II

| Fraction                | Total activity | Total protein | Specific activity |
|------------------------|----------------|---------------|------------------|
| Papain-solubilized supernatant | 217 | 1190 | 0.182 |
| Ammonium sulfate, 60-70% saturation | 89.7 | 76.3 | 1.18 |
| Bio-Gel P-300 | 84.5 | 19.1 | 4.42 |
| DEAE-cellulose | 60.4 | 3.85 | 15.7 |

Fig. 3. pH activity profiles of purified sucrases from the small intestine (M.K.) and from an area of intestinal metaplasia (T.S.). Of each of the following buffers, 0.1 M was added to reaction mixtures: glycine-HCl containing 0.1 M NaCl, pH 2.5 to 3.5; sodium acetate, pH 4.0 to 5.5; sodium malate, pH 5.5 to 6.5; sodium phosphate, pH 6.0 to 8.0; sodium barbital, pH 8.5 to 9.0. - - - , small intestine; O--O, intestinal metaplasia.

Table III

| Patient | Age | Sex | Blood group type | Secretor or nonsecretor | Inhibition of type A cell agglutination | Inhibition of type B cell agglutination |
|---------|-----|-----|------------------|-------------------------|----------------------------------------|----------------------------------------|
| C.S.    | 71  | F   | A                | Secretor                | 1.4                                    | None                                   |
| M.K.    | 56  | F   | O                | Secretor                | None                                   | 2.8                                    |
| T.S.    | 86  | M   | A                | Secretor                | None                                   | None                                   |
| S.K.    | 67  | M   | A                | Secretor                | None                                   | None                                   |
| F.A.    | 61  | F   | B                | Secretor                | None                                   | 2.1                                    |
| I.Y.    | 61  | M   | B                | Secretor                | None                                   | None                                   |
| M.I.    | 62  | M   | B                | Secretor                | None                                   | None                                   |

Small intestine: None means that no inhibition was observed with the undiluted sample at the enzyme concentration indicated in the text.

* Not determined.
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M.I.). These results were confirmed as follows: four preparations from areas of intestinal metaplasia (S.K., F.A., I.Y., and M.I.) and one preparation from the small intestine (F.A.) were subjected to electrophoresis on polyacrylamide gel. Then the sucrase activity and blood group activity in each gel were examined. Fig. 4 shows the distributions of the two activities in the gels. The preparation from the intestine of the patient of blood group type B showed blood group type B activity coinciding with sucrase activity (a). Two preparations of types A and B, respectively, from areas of intestinal metaplasia showed no blood group activity coinciding with sucrase activity (b and d), while the other two preparations from cases of type B showed blood group type B activity (c and e). Fig. 4 also shows that blood group activity was found at the top of the polyacrylamide gel. This might be due to a blood group substance contaminating the sucrase fraction during its purification.

The blood group activities of the sucrase preparations after electrophoresis on polyacrylamide gel are summarized in Table III. All patients of blood group type A or B were found to secrete material with blood group activity in their saliva. Two of the five enzyme preparations from areas of intestinal metaplasia had similar level of blood group activity to those of preparations from the small intestine, namely the end point of hemagglutination inhibition was 2.1 milliunits/ml for the former, comparable to 1.4 and 2.8 milliunits/ml of the latter. The other three preparations showed no blood group activity even with undiluted specimens of which the enzyme concentrations were 60, 740, and 7.0 milliunits/ml for T.S., S.K., and I.Y., respectively.

DISCUSSION

In this work, three of five preparations of sucrase from areas of intestinal metaplasia were found to have no blood group activity, but they had similar enzymatic properties to those of sucrase from the small intestine. The patients from whom the preparations were obtained were all found to be secretors, as shown in Table III, so this phenomenon was not due to the fact that the cases were nonsecretors.

Blood group activity was always found at the top of the polyacrylamide gel when the sucrase was electrophoresed (Fig. 4), and at this position a very minor protein band and sugar band was detected (Figs. 1 and 2). It might be due to a small amount of blood group-active glycoproteins or glycolipids, for recently Slomiany et al. (22, 23) reported that glycolipids as well as glycoproteins were found in blood group substances from gastric mucosa. If so, these glycoproteins or glycolipids seem to have interacted with glycoprotein sucrase in the preparations, and to be separated from it by electrophoresis.

ABO blood group antigenicity is determined by a terminal portion of its sugar chain, and it is normally present in the stomach, colon, and many other organs besides erythrocytes (24). Recently many reports (6–10) have shown that blood group activity is deleted in stomach and colon tumors and that in these tumors new antigens appear which are deficient in blood group sugar chains. Some changes of membrane glycoproteins and glycolipids associated with malignant transformation have also been observed to be due to incomplete sugar chains and sometimes accompanied with low activity of a particular glycosyltransferase (10). The absence of blood group activity which we observed in some preparations of sucrase from areas of intestinal metaplasia may be also due to incomplete sugar chains.

Tumor-specific antigens, carcinoembryonic antigen, and α-fetoprotein, have been found in gastric carcinoma and they have also been detected in areas of intestinal metaplasia (25–27). These findings together with the present results suggest that intestinal metaplasia is closely related to development of stomach cancer. Further biochemical studies are required on intestinal metaplasia and a method is needed for inducing intestinalization in animals.

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