On Proteins of Gastric Cancer

II. Further Physical and Chemical Properties of the Protein Fractions*

By

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In continuation of the former report,¹⁾ the results of electrophoresis of the protein fractions of gastric cancers and gastric mucosa, paper partition chromatography of their components and else will be described here.

EXPERIMENTS AND COMMENTS

Electrophoresis

Preps. II's and III's obtained from the cancers and mucosa in the former report¹⁾ which were the fractions insoluble in glacial acetic acid and non-precipitable from water by acid and the ones soluble in glacial

Fig. 1. Electrophoretic pattern of Preps. II's. A: pattern of a 1.2% solution of Prep. II from gastric cancer of persons belonging to Group A; 15°C; current 8 ma; exposure 30 min. B: pattern of a 1% solution of Prep. II from gastric mucosa of persons belonging to the same blood group as above; 14°C; current 8 ma; exposure 45 min. Ascending limbs to the left.

* The 7th Report of Masamune and coworkers' "On Proteins and Amino Acids."

Fig. 2. Electrophoretic pattern of a 36/27% solution of Preps. III's. A: pattern of Prep. III from gastric cancer of persons belonging to Group O; 14°C; current 9 ma; exposure 30 min. B: pattern of Prep. III from gastric mucosa of persons belonging to Group A; 15°C; current 9 ma; exposure 30 min. Ascending limbs to the left.

acetic acid respectively, were examined in a Tiselius electrophoresis apparatus, employing a phosphate buffer of pH 7.8 and I 0.29 (Figs. 1-2).

The pattern of the pair of Preps. III's are similar to each other, showing two similar corresponding boundaries. But that of the pair of Preps. II's both with three boundaries are dissimilar, the relative velocity of the chief component among the others in one preparation being not in accord with that in the other preparation.

Paper Chromatography

Amino acids. Preps. II's and III's were examined. Hydrolysis was effected by heating at 100°C with 6 *N* HCl (under reflux). The hydrolysates were removed from HCl by repeated vacuum-distillations with water. The pH of the solutions sampled for irrigation were 2.0-3.0. Solvents: 1) butanol-acetic acid-water (4:1:5)²⁾ and 2) phenol-water (4:1). Two-dimension chromatography was processed by using these solvents in the arranged or inverted order. Indicator, 0.2% ninhydrin in butanol. Besides, chromatograms run in parallel by Solvent 1 alone were developed by means of not only ninhydrin but also platinum iodide and Pauli reagent to more strictly identify methionine, histidine and tyrosine (S. Fig. 4). Figs. 3-5 are the chromatograms of Preps. II's and Figs. 6-7 those of Preps. III's. The former Figs. show that Prep. II of gastric cancers (Group A) and that of gastric mucosa (Group A) both contained aspartic and glutamic acid, serine, glycine, threonine, alanine, methionine and/or valine, leucine and/or isoleucine, phenylalanine, tyrosine, lysine, histidine, arginine and probable underglutamic acid. All the chromatograms showed one unidentified spot X (a probable peptide)

Fig. 3. Diagrammatical reproduction of a butanol-acetic acid-water/phenol-water-ammonia chromatogram (A) and a chromatogram irrigated in the inverted order (B) of Prep. II from a gastric cancer of persons belonging to Group A. 28 mg. of the preparation were heated with 5 cc. of 6 *N* HCl for 27 2/3 hrs. The solution prepared for irrigation (pH 2.8) was 0.6 cc., of which 0.03 and 0.02 cc. portions were sampled in A and B respectively. Temperature of test $19^{\circ} \pm 1^{\circ}\text{C}$. Indicator ninhydrin.

1: aspartic acid, 2: glutamic acid, 3: serine, 4: glycine, 5: threonine, 6: alanine, 7: proline, 8: tyrosine, 9: valine and/or methionine, 10: phenylalanine, 11: leucine and/or isoleucine, 12: hexosamine, 13: lysine, 14: histidine, 15: arginine, 16: hydroxyproline (?), X, Y, Z: not exactly identified. (Some of the spots are not seen here).

Fig. 5

Fig. 4. Diagrammatical reproduction of butanol-acetic acid-water chromatograms of the hydrolysate solution used for the chromatogram in Fig. 3. 0.05 cc. portions were sampled. Paper strips 30 cm. $\times$ 2.2 cm. to 4.4 cm. (trapezoids). Temperature of test, $19^{\circ} \pm 1^{\circ} \text{C}$. a, b and c were developed with ninhydrin, platinum iodide and Pauli reagent respectively.

Ciphers express the same as in Fig. 3.

Fig. 5. Diagrammatical reproduction of a butanol-acetic acid-water/phenol-water-ammonia chromatogram and a chromatogram irrigated in the inverted order of Prep. II from normal gastric mucosa of persons belonging to Group A. 33.5 mg. of the preparation were heated with 6 cc. of 6 N HCl for 29 $\frac{2}{3}$ hrs. The solution prepared for irrigation (pH 2.0) was 0.8 cc., of which 0.02 cc. and 0.03 cc. portions were sampled in A and B. Temperature of test $19^{\circ} \pm 1^{\circ} \text{C}$. Indicator, ninhydrin.

The ciphers and characters signify the same as in Fig. 3.

Fig. 6. Diagrammatic reproduction of a butanol-acetic acid-water/phenol-water-ammonia chromatogram (A) and a chromatogram irrigated in the inverted order (B) of Prep. III from a gastric cancer of persons belonging to Group O. 30 mg. of the preparation were heated with 6 cc. of 6 *N* HCl for 22 3/4 hrs. The solution prepared for irrigation (pH 2.6) was 0.6 cc., of which 0.01 and 0.012 cc. portions were sampled in A and B respectively. Temperature of test $19^{\circ} \pm 1^{\circ}\text{C}$. Indicator, ninhydrin.

The ciphers and characters signify the same as in Fig. 3.

besides. In Fig. 3 there are seen three spots marked with ?, which, however, are difficult to judge if they represent any amino acids or are to be ascribed to technical fault. Spot 16 in Fig. 5, a reproduction of the chromatogram of Prep. II of the mucosa, falls approximately on the

Fig. 7. Diagrammatical reproduction of a butanol-acetic acid-water/phenol-water-ammonia chromatogram (A) and a chromatogram irrigated in the inverted order (B) of Prep. III of gastric mucosa of persons belonging to Group A. 30 mg. of the preparation were heated with 6 cc. of 6 N HCl for 22 3/4 hrs. The solution prepared for irrigation (pH 2.6) was 0.6 cc., of which 0.01 and 0.012 cc. portions were sampled in A and B respectively. Temperature of test $19^{\circ} \pm 1^{\circ}\text{C}$. Indicator, ninhydrin.

Ciphers and characters signify the same as in Fig. 3.

position of hydroxyproline, whose corresponding spot was not detected in the chromatograms of the corresponding preparation of the gastric cancer from persons of the same blood group (Fig. 3). Figs. 6-7 demon-

strate that Preps. III's from gastric cancers (Group O) and mucosa (Group A) contained aspartic and glutamic acid, serine, glycine, threonine, alanine, proline, valine and/or methionine, leucine and/or isoleucine, lysine, histidine, arginine, probable underglutamic acid (Y), a probable peptide (X) and an unidentified amino acid (Z). The hydrolysate of the preparation from the mucosa gave additional spots corresponding to positions of phenylalanine and tyrosine though very weak and another unidentified spot which was seemingly produced by technical fault (the spot with ? in Fig. 7). By sampling a larger amount of the hydrolysate of the cancer substance and irrigation in the second way, phenylalanine could be demonstrated, but tyrosine could not at all. The cancer and mucosa preparations employed here had been obtained from tissue materials from persons belonging to different blood groups. But the possibility that the tyrosine deficiency of the cancer substance may be due to its being an O substance might be precluded.

In the corresponding chromatograms of any pair of the preparations from the cancers and mucosa, some of the corresponding amino acid-spots were so divergent in colour strength that their different contents must be assumed according to the source of the substances.

Sugars. Prep. III of Group A gastric cancer and mucosa were formerly examined and reported³⁾ to contain chondrosamine, glucosamine, galactose and fucose. Processed in a similar manner (kindly tested by Dr. Yosizawa), Prep.-II's from the gastric cancer of Group A and gastric mucosa of the same blood group showed spots at positions of the sugars above, and, in addition, a weak spot of mannose (Fig. 8).

Optical Rotation of Hydrolysates

Prep. II from the gastric cancer of Group A persons was hydrolyzed as for amino acid chromatography (duration of hydrolysis 13 2/3 hrs.). The hydrolysate was freed from HCl as far as possible by repeated vacuum-distillations with water. The final still residue was taken up in a little water and filtered, and the filtrate and washing were evaporated together on a boiling water-bath. The remainder here was dried to constant and 7.1 mg. of it were taken up in 2.1 cc. of water (conc. 0.338%, pH 4.3). The rotation was measured after 24 hrs. from dissolution:

$$[\alpha]_D^{20} = \frac{-0.183 \times 100}{0.338 \times 1} = -54.1^\circ.$$

The corresponding preparation from the gastric mucosa of persons of the same blood group was hydrolyzed with HCl and the insoluble humin eliminated. 17.6 mg. of the final evaporated residue were yielded from 29.6 mg. of the mucosa substance, and a 0.562% solution (pH 4.2) was prepared to determine the rotation after 24 hrs.:

Fig. 8. Diagrammatical reproduction of a *n*-butylacetate-acetic acid-water (3:2:1) chromatogram of Preps. II's from the gastric cancer of Group A persons and gastric mucosa of persons of the same blood group. 50 mg. of each of the substances were hydrolyzed in an ampoule with 5 cc. of 1 *N* H₂SO₄ by heating at 100°C for 5 hrs. The solution of the hydrolysates prepared for irrigation were both 0.5 cc., of each of which a 0.015 cc. portion was sampled. Duration and temperature of test, about 15 hrs. and 20°±1°C. Indicator, aniline hydrogen phthalate of Partridge.

a and b: standards. c: Prep. II from gastric cancers. d: Prep. II from gastric mucosa. 1: chondrosamine sulfate, 2: glucosamine sulfate, 3: galactose, 4: mannose, 5: rhamnose.

$$[\alpha]_D^{20} = \frac{-0.109 \times 100}{0.562 \times 1} = -19.4^\circ.$$

Thus the rotation of the hydrolysates were found incoincident, in harmony with the consequences of chromatography suggesting the different amino acid contents of the preparations.

SUMMARY

The electrophoretic patterns of Prep. III of the gastric cancers and mucosa resembled each other. But, those of Preps. II's differed from each other, although they both demonstrated three boundaries. Concerning the amino acid composition, Preps. II's and III's were somewhat different qualitatively as well as quantitatively from each other in

respective pair of preparations from the two sources. The optical rotations of a hydrolysate of the Preps. II's supported this fact. Combined with the analytical results in the former report, the present findings indicate that the corresponding proteins of gastric cancer and the mucosa are not identical despite of their resemblance in rough composition.

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References and Notes

- 1) Masukawa, Tohoku J. Exp. Med., this volume, p. 251.
 - 2) The upper layer of the equilibrated mixture was taken for the purpose of irrigation.
 - 3) Masamune, Yosizawa, Oh-Uti, Matuda & Masukawa, Tohoku J. Exp. Med., 1952, 56, 37.
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