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Interaction of Urinary Glycoproteins of Cancer-Bearing and Normal Rats with Frog Egg Agglutinins[1)]

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AH109A tumor cell agglutinations caused by *Rana catesbeiana* agglutinin and *Rana japonica* agglutinin were inhibited by urinary glycoproteins and glycopeptides from cancer-bearing and normal rats. Fractions of a series of substances (molecular weight above 10000) from cancer-bearing rat urine (10T) and from normal rat urine (N10T) inhibited cytoagglutination caused by *Rana catesbeiana* agglutinin and by *Rana japonica* agglutinin, while other fractions (ranging in molecular weight from 1000 to 10000) from cancer-bearing rat urine (01T) and from normal rat urine (N01T) only inhibited that caused by *Rana catesbeiana* agglutinin. It is probable that these two agglutinins recognize different AH109A cell surface sialoglycoconjugates. Since the content of sialic acid of each fraction is independent of its inhibitory capacity, these agglutinins seem to recognize a certain sialoglycoconjugate, regardless of the content of sialosyl residues.

Keywords——urinary glycoprotein; sialoglycoconjugate; frog egg agglutinin; *Rana catesbeiana*; *Rana japonica*; AH109A tumor cell

Lectins are widely distributed in plants. Many of them combine specifically with carbohydrate structures, and some lectins have been found to differentially agglutinate normal and transformed cells.[2)]

We have demonstrated that eggs of various frogs contain various kinds of lectins. Eggs of *Rana catesbeiana, Rana japonica* and *Rana nigromaculata* contain agglutinins that showed a preferential and strong agglutination of various tumor cells.[3)] The tumor cell agglutination was not inhibited by various monosaccharides and oligosaccharides. However, the ganglioside fraction of human erythrocyte membranes and the glycoprotein isolated from Ehrlich ascites tumor cell membranes completely inhibited the agglutination of Ehrlich ascites tumor cells caused by these agglutinins.[3)]

Comparative studies on glycoproteins of body fluids of cancer-bearing individuals and those from normals have already been reported by Hakomori *et al.*[4,5)] To detect qualitative or quantitative changes of glycoprotein from rat urine during the development of cancer, we have initiated a study based on the application of these agglutinins as a diagnostic tool.

This paper describes the interactions of urinary glycoproteins of cancer-bearing and normal rats with these agglutinins.

Experimental

Urine of 50 Donryu rats were collected under toluene. The rats were inoculated intraperitoneally with AH109A cells (5×10^6/rat), and then every day pooled urine was homogenized with 10 volumes of ice-cold acetone. Next, 30 g of AH109A-bearing rat urine acetone-dried powder (combined from the 3rd day to the 8th day after inoculation) or that from normal rats was homogenized with 900 ml of ice-cold PBS (0.01 M, pH 7.4) and centrifuged at $15000 \times g$ for 30 min.

Separation of Urinary Glycoproteins and Glycopeptides——Concentrates obtained by successive ultrafiltrations of the clear supernatant fluids obtained above with Diafilter (Ulvac Service Corp., Bio-Engineering) G-10T and Diafilter G-01T filters under N_2 gas at 4 atmospheric pressure were termed 10T and 01T from cancer-bearing rat

urine and N10T and N01T from normal rat urine, respectively. Lyophilized 10T was dissolved in 0.1 M NaCl and the solution was applied to a column of Sephadex G-100 equilibrated with 0.1 M NaCl. Fractions of 5 ml each were collected with an automatic fraction collector and the absorbancy at 280 nm was determined on each fraction. The eluates in each peak were pooled, concentrated to small volume, dialyzed against distilled water, and lyophilized. Lyophilized N10T was treated in a similar manner. Lyophilized 01T was dissolved in 0.1 M NaCl and the solution was applied to a column of Sephadex G-50 equilibrated with 0.1 M NaCl followed by desalting on Sephadex G-10. Fractions of 5 ml each were collected and the absorbancy at 230 nm was determined on each fraction. The eluates in each peak were pooled and lyophilized. N01T was treated in a similar manner.

Contents of Sialic Acid and Total Hexoses——Colorimetric methods were used for determination of sialic acid[6] and total hexoses.[7]

Agglutination Inhibition Assay——Agglutination inhibition assay was carried out as described previously.[3a]

Results and Discussion

Chart 1 summarizes the fractionation of urinary glycoproteins and glycopeptides. Inhibitions of *RC* agglutinin- and *RJ* agglutinin-induced AH109A cell agglutination by urinary glycoproteins are shown in Table I. The order of decreasing inhibitory capacity for

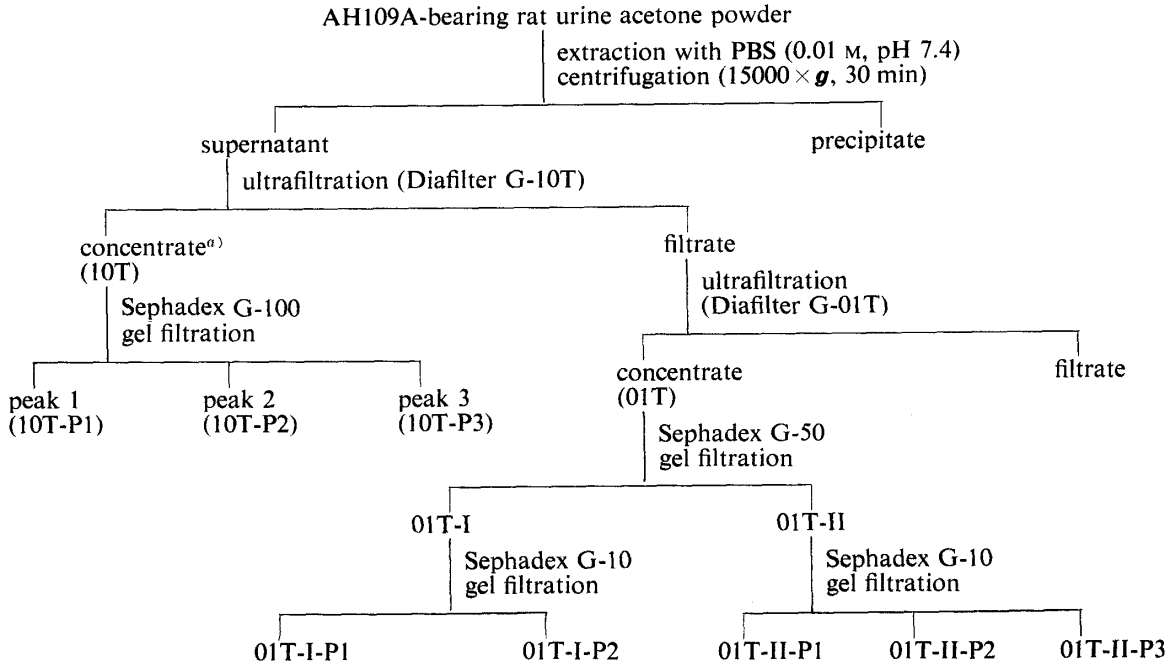

Chart 1. Fractionation of AH109A-bearing Rat Urinary Glycoproteins and Glycopeptides

a) Normal rat urine glycoproteins and glycopeptides were fractionated by the same procedure, and the corresponding fractions are indicated by N.

AH109A cell agglutination induced by *RC* agglutinin was 01T ≧ N01T > 10T > N10T, and the order for that induced by *RJ* agglutinin was 10T > N10T; however, in the case of *RJ* agglutinin, no inhibitory activity was exhibited by 01T or N10T. Inhibition of Ehrlich cell agglutination by these fractions was similar to that described above for AH109A cell agglutination (data not shown). Since inhibitory activity of 01T and N01T upon *RC* agglutinin-induced agglutination is distinct from that upon *RJ* agglutinin-induced agglutination, it is suggested that these two agglutinins may recognize different cell surface glycoconjugates.

The elution patterns of 10T and N10T through gel filtration on Sephadex G-100 are shown in Fig. 1. A comparison of Fig. 1A with Fig. 1B indicates remarkable changes in P2 and P3 (see Fig. 1). The inhibitory activity of each fraction is summarized in Table II. The

Table I. Reactivities of *RC* Agglutinin and *RJ* Agglutinin with Urinary Glycoproteins and Glycopeptides

Fraction	The concentration of the fraction that inhibited cytoagglutination of AH109A cell suspension caused by 3 agglutination doses of													
	RC agglutinin							*RJ* agglutinin						
	2000[b]	1000	500	250	125	62	31	2000	1000	500	250	125	62	31
10T	+[a]	+	±	−	−	−	−	+	+	+	+	±	−	−
01T	+	+	+	+	+	±	−	−	−	−	−	−	−	−
N10T	+	−	−	−	−	−	−	+	+	+	±	−	−	−
N01T	+	+	+	+	+	−	−	−	−	−	−	−	−	−

a) + or −; The presence or absence of inhibitory activity.
b) μg protein of the fraction/0.1 ml.

Table II. Inhibition of *RC* Agglutinin-induced AH109A Cell Agglutination by Each Fraction

Fraction	The concentration of the fraction that inhibited cytoagglutination by 3 agglutination doses of *RC* agglutinin								
	2000[b]	1000	500	250	125	62	31	15	7.5
10T	+[a]	+	±	−	−	−	−	−	−
10T-P1			+	+	+	±	−	−	−
10T-P2			+	+	±	−	−	−	−
10T-P3			−	−	−	−	−	−	−
N10T	+	−	−	−	−	−	−	−	−
N10T-P1			+	+	+	+	−	−	−
N10T-P2		±	−	−	−	−	−	−	−
N10T-P3			−	−	−	−	−	−	−
01T	+	+	+	+	+	±	−	−	−
01T-I-P1			+	+	+	±	−	−	−
01T-I-P2			+	+	+	+	+	±	−
01T-II-P1			−	−	−	−	−	−	−
01T-II-P2			−	−	−	−	−	−	−
01T-II-P3			−	−	−	−	−	−	−
N01T	+	+	+	+	±	−	−	−	−
N01T-I-P1			+	+	+	±	−	−	−
N01T-I-P2			+	+	+	+	±	−	−
N01T-II-P1			−	−	−	−	−	−	−
N01T-II-P2			−	−	−	−	−	−	−
N01T-II-P3			−	−	−	−	−	−	−

a) + or −; The presence or absence of inhibitory activity.
b) μg protein of the fraction/0.1 ml.

order of decreasing inhibitory activity was N10T-P1 > 10T-P1 > 10T-P2, but N10T-P2, 10T-P3 and N10T-P3 were inactive. Since it is well documented that increased proteolytic enzyme activity is often associated with the presence of cancerous growth,[8] the results that N10T-P1 was more active than 10T-P1 and inhibitory activity was exhibited by 10T-P2 but not by N10T-P2 suggest that high molecular weight normal rat urinary glycoprotein which seems to react with *RC* agglutinin may be degraded to low molecular weight glycoprotein by proteo-

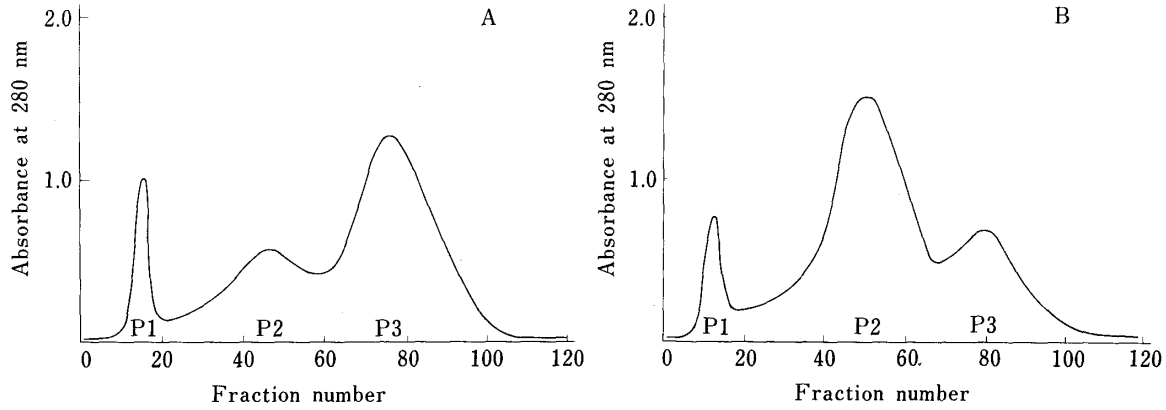

Fig. 1. Chromatographic Patterns of 10T and N10T on Sephadex G-100

A, gel filtration pattern of 10T on Sephadex G-100 (column size, 4.0 × 40 cm) in 0.1 M NaCl. The flow rate was 80 ml/h and the fraction size was 5 ml. B, gel filtration pattern of N10T. The chromatography was carried out according to the same procedure as described in A.

TABLE III. Chemical Composition of Fractions of 10T and N10T

Fraction	Sialic acid (%)	Total hexoses (%)
10T-P1	1.0	7.2
10T-P2	1.5	4.3
10T-P3	5.0	8.0
N10T-P1	1.8	8.6
N10T-P2	4.4	3.3
N10T-P3	5.6	9.7

lytic enzyme(s) in cancer-bearing rats. There are only small differences in chromatographic patterns between fractionations of a series of 01T and those of N01T (data not shown), and the inhibitory activities of 01T-I-P1 and N01T-I-P1, and of 01T-I-P2 and N01T-I-P2 are similar (see Table II).

As shown in Table III, content of sialic acid is not correlated to that of total hexoses. Since sialosyl residues of glycoconjugates are involved in these agglutinin receptor sites[3] and the content of sialic acid of each fraction is independent of its inhibitory capacity, these agglutinins seem to recognize a certain specific sialoglycoconjugate, regardless of the content of sialosyl residues.

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References and Notes

1) The abbreviations used are: *RC, Rana catesbeiana*; *RJ, Rana japonica*; PBS, phosphate-buffered saline.
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