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Localization of binding sites of *Ulex europaeus* I,
*Helix pomatia* and *Griffonia simplicifolia* I-B₄ lectins and analysis
of their backbone structures by several glycosidases and
poly-N-acetyllactosamine-specific lectins in human breast carcinomas

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Abstract Several studies have shown the deletion of
blood group A or B antigens and the accumulation of H
antigens in human breast carcinomas. Other studies have
independently demonstrated that the binding sites of lec-
tins such as *Helix pomatia* agglutinin (HPA) and
*Griffonia simplicifolia* agglutinin I-B₄ (GSAI-B₄) are
highly expressed in these cells. In order to clarify the
molecular mechanisms of malignant transformation and
metastasis of carcinoma cells, it is important to under-
stand the relationship between such phenotypically dis-
tinct events. For this purpose, we examined whether the
binding sites of these lectins and *Ulex europaeus* aggluti-
nin I (UEA-I) are expressed concomitantly in the same
carcinoma cells and analyzed their backbone structures.
The expression of the binding sites of these lectins was
observed independently of the blood group (ABO) of the
patients and was not affected by the histological type of
the carcinomas. Observation of serial sections stained
with these lectins revealed that the distribution of HPA
binding sites was almost identical to that of GSAI-B₄ in
most cases. Furthermore, in some cases, UEA-I binding
patterns were similar to those of HPA and GSAI-B₄ but
in other cases, mosaic staining patterns with these lectins
were also observed, i.e., some cell clusters were stained
with both HPA and GSAI-B₄ but not with UEA-I and ad-
jacent cell clusters were stained only with UEA-I. Diges-
tion with endo-1,3-galactosidase or N-glycosidase F mark-
edly reduced the staining intensity of these lectins.
Together with the reduction of staining by these lectins, re-
activity with *Griffonia simplicifolia* agglutinin II ap-
peared in carcinoma cells following endo-β-galactosi-
dase digestion. Among the lectins specific to poly-N-ac-
tyllactosamine, *Lycopersicon esculentum* agglutinin
(LEA) most vividly and consistently stained the cancer
cells. Next to LEA, pokeweed mitogen agglutinin was
also effective in staining these cells. Carcinoma cells re-
active with these lectins corresponded well to those
stained with both HPA and GSAI-B₄, and in some cases,
with UEA-I. These results demonstrate that the binding
sites of UEA-I, HPA, and GSAI-B₄ are expressed con-
comitantly in the same carcinoma cells and all carry lin-
ear and branched poly-N-acetyllactosamine on N-gly-
cans, suggesting that the synthesis of this complex car-
bohydrate is one of the most important and basic pro-
cesses leading to the malignant transformation of cells,
invasion, and metastasis of carcinoma cells.

Introduction
Numerous biochemical, immunochemical, and histo-
chemical studies have been carried out on the altered ex-
pression of blood group-related antigens in tumors (Feizi
breast carcinomas, it has been demonstrated that the
blood group antigens expressed in normal mammary
glands such as A, B or Lewis b antigens are lost, while
their precursors, H antigens and T or Tn antigens, accu-
mulate (Gupta and Schuster 1973; Springer 1984, 1989;
Lee et al. 1985; Vowden et al. 1986; Ildiko and Marinck-
avel 1991; Nakagoe et al. 1991; Ura et al. 1992; Reed et
al. 1994).

Many studies using labeled lectins have also been
conducted to elucidate the changes in profiles of carbo-
ydrate antigens and to identify a specific marker for the
prognosis of breast carcinoma (Alroy et al. 1988). Among
the lectins examined, *Helix pomatia* agglutinin
(HPA) has been one of the most extensively examined
Table 1 Lectins used in this study, their monosaccharide, poly-N-acetyllactosamine, and blood group specificities and inhibitory reagents used for control experiments. UEA-I Ulex europaeus agglutinin I, HPA Helix pomatia agglutinin, GSAI-B4 Griffonia simplicifolia agglutinin I-B4, GSA-II G. simplicifolia agglutinin II, DSA Datura stramonium agglutinin, Suc-WGA succinyl wheat germ agglutinin, LEA Lycopersicon esculentum agglutinin, PWM pokeweed mitogen agglutinin, Fuc L-fucose, GalNAc N-acetyl-D-galactosamine, Gal D-galactose, GlcNAc N-acetyl-D-glucosamine, Man D-mannose

<table>
<thead>
<tr>
<th>Lectin</th>
<th>Specificity</th>
<th>Inhibitory reagents</th>
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<tbody>
<tr>
<td>UEA-I</td>
<td>Fuc; H and Le antigen</td>
<td>100 mM Fuc</td>
</tr>
<tr>
<td>HPA</td>
<td>GalNAc; A, Tn, and Forssman antigen</td>
<td>100 mM GalNAc</td>
</tr>
<tr>
<td>GSAI-B4</td>
<td>Gal; Gal-α-1-3Gal; B antigen</td>
<td>100 mM Gal</td>
</tr>
<tr>
<td>GSA-II</td>
<td>GlcNAc</td>
<td>100 mM GlcNAc</td>
</tr>
<tr>
<td>DSA</td>
<td>(Galβ1-4GlcNAcβ1-3)1-2 β1-6 Man</td>
<td>150 mM N-acetyllactosamine</td>
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<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suc-WGA</td>
<td></td>
<td>50 mM N,N,N'-triacetyl-chitotriose</td>
</tr>
<tr>
<td>LEA</td>
<td>(Galβ1-4GlcNAcβ1-3)2-3 β1-6</td>
<td>150 mM N-acetyllactosamine</td>
</tr>
<tr>
<td>PWM</td>
<td>[Galβ1-4GlcNAcβ1-3]3-6</td>
<td>150 mM N-acetyllactosamine</td>
</tr>
</tbody>
</table>

a Specificity for poly N-acetyllactosamine was not determined

Materials and methods

Tissues of human breast carcinomas (five cases of papillo tubular carcinoma, six cases of solid-tubular carcinoma and six cases of scirrhous carcinoma) were obtained from biopsies. The histological classification of breast carcinoma, which is fundamentally the same as the World Health Organisation classification, has been adopted by the Japan Mammary Cancer Society (Sakamoto 1987). Tissue specimens were fixed in 10% formalin and embedded in paraffin, and serial sections were cut at a thickness of 4 µm. The blood group (ABO) of the patients was determined by a conventional hemagglutination method.

Monoclonal anti-A (mAb-A) antibody A005 and monoclonal anti-B (mAb-B) antibody H079 were purchased from BioCarb Chemicals (Lund, Sweden) and Knickerbocer, (Barcelona, Spain). Biotin-labeled Datura stramonium agglutinin (DSA), Lycopersicon esculentum agglutinin (LEA), succinyl wheat germ agglutinin (Suc-WGA), peroxidase-labeled G. simplicifolia agglutinin II (GSA-II), peroxidase-labeled GSAI-B4, and peroxidase-labeled UEA-I were purchased from EY Laboratories (San Mateo, Calif., U.S.A.). Biotin-labeled pokeweed mitogen agglutinin (PWM) and peroxidase-labeled HPA were from Sigma Chemical (St Louis, Mo., USA). Endo-β-D-galactosidase (Escherichia freundii), α-N-acetylgalactosaminidase (Acremonium sp.) and β-N-acetylhexasaminidase (Jack bean) were obtained from Seikagaku Kogyo (Tokyo, Japan). Recombinant peptide-N-glycosidase F (N-glycosidase F), α-galactosidase (Green coffee beans) were purchased from Boehringer Mannheim Biochemica (Mannheim, Germany). Saccharide and blood group specificities of lectins and inhibitory reagents for control experiments are illustrated in Table 1. Digestion with endo-β-D-galactosidase was performed by incubating tissue sections in a solution (0.1 M sodium acetate buffer, pH 5.8 containing 0.07 M NaCl) containing the enzyme at a concentration of 0.2 U/ml for 24 h at 37°C (Ito et al. 1994a). Digestion with N-glycosidase F was carried out by incubating tissue sections in a solution (0.1 M sodium phosphate buffer, pH 7.2 containing 25 mM EDTA and 1% Triton X-100) containing the enzyme at a concentration of 10 U/ml for 24 h at 37°C. Digestion with α-galactosidase, α-N-acetylgalactosaminidase and β-N-acetylhexasaminidase was performed as reported previously (Ito et al. 1987, 1988; Ito and Hirota 1992).

Details of immunostaining with mAbs and the labeled lectin staining method have been reported previously (Ito et al. 1987, 1995). In brief, sections were incubated for 4 h at 4°C in a solution of mAb (1:50) or of lectin conjugates (20 µg/ml for GSA-II,