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Note

Experimental modification of *N*-linked sugars of membrane proteins in a lymphoma cell line affects the binding of soybean agglutinin but not of several other lectins*†

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In studying the biological role of cell-surface carbohydrates in cellular interactions, inhibitors of *N*-linked glycosylation or processing can modify cell-surface glycoproteins. When processing only was interfered with, most cellular functions tested so far were not impaired. However, the blood-borne arrest pattern of lymphoma cells was modified[1,2]. In an attempt to correlate biological results with the structure of cell-surface glycoconjugates, biochemical characterisation of the carbohydrate is required. In a first step, lymphoma cells have been labelled with fluoresceinated lectins (Table I) after culture in the presence of various inhibitors of *N*-linked glycosylation or processing.

Table II gives the effect of treatment with neuraminidase (Nase) and of culture in the presence of inhibitors of *N*-linked glycosylation or processing. Results from a typical experiment with SBA (see Table I for abbreviations of lectins used) are illustrated in Figs. 1 and 2. Treatment with Nase increased the intensity of staining with all of the lectins used except WGA. Lectin binding was indeed stronger after removal of sialic acid, except for WGA (which binds to 2-acetamido-2-deoxy-D-glucose and sialic acid[3]). Culture in the presence of inhibitors strongly reduced the binding of SBA but had no effect on staining with other lectins, nor on labelling of the anti-Thy-1.2 antigen. Thy 1.2 is a glycoprotein, but it is known that the alloantibody recognises a polypeptidic (and not a carbohydrate) epitope[4]. This finding indicates that, for the Thy-1 surface-glycoprotein at least, our treatments did not reduce the expression of the protein backbone. It has been shown that Thy-1 antigen is modified after culture in the presence of swainsonine[1] (SW). It was

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†Membrane Carbohydrates of Lymphoid Cells, Part II. For Part I, see ref. 2

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TABLE I

LECTINS USED IN THIS STUDY

Origin	*Abbreviation*	*Specificity*	*Sugar used for inhibition*	*Concentration (μg/mL)*
Arachis hypogea (Peanut)	PNA	β-D-Gal-(1→3)-β-D-GalNAc	D-Galactose	50
Canavalia ensiformis (Jack bean)	Con A	α-D-Man, α-D-Glc	Methyl α-D-mannopyranoside	25
Glycine max. (Soybean)	SBA	α- or β-D-GalNAc	D-GalNAc	60
Trichosanthes kinlowii (Tianhuafen)	TKA	D-Gal	D-Galactose	60
Triticum vulgaris (Wheat germ)	WGA	$[(1\rightarrow4)$-β-D-GlcNAc$]_2$, sialic acid	Chitobiose	15

TABLE II

RELATIVE SURFACE BINDING (±S.D.) OF FLUORESCEINATED LECTINS AND ANTI-Thy-1.2 ANTIBODIES TO BL/VL_3 CELLS WITH MODIFIED CELL-SURFACE SUGARS IN PERCENT OF MEDIAN FLUORESCENCE-INTENSITY OF UNTREATED CELLS[a]

VL_3	*SBA*	*ConA*	*PNA*	*TKA*	*WGA*	*Anti-Thy-1.2[d]*
Neuraminidase	220 ±83.7 (3)[b]	145 ±27 (3)	435 ±246.8 (3)	151 ±69 (3)	78 ±17.3 (3)	N.d.[e]
1-Deoxynojirimycin 24 h	54 ±10.6[c] (7)	93.6 ±31.6 (7)	102.8 ±8.2 (5)	77.2 ±13.7 (4)	78.2 ±37.8 (5)	85–104
1-Deoxynojirimycin 48 h	62.7 ±12.4 (3)	85 ±47.7 (6)	88.4 ±31.8 (5)	110.5 ±67 (4)	96.2 ±41.2 (4)	92–130
Swainsonine 24 h	59 ±7.8[c] (3)	109.6 ±32.9 (5)	105.7 ±59.6 (4)	97.2 ±15.4 (4)	115.7 ±5.8 (3)	100–104
Swainsonine 48 h	52.7 ±4.5[c] (3)	120 ±46.6 (7)	87.4 ±49.2 (5)	93.2 ±14.5 (5)	111.7 ±14 (3)	115
2-Deoxyglucose 24 h	67.6 ±6.9[c] (5)	94.2 ±36.7 (4)	87 ±25 (5)	96.7 ±26 (4)	102 ±4 (5)	85–95
Tunicamycin 24 h	62.8 ±8.6[c] (5)	109 ±29.8 (7)	129.2 ±24.5 (4)	95.5 ±10.3 (4)	122.6 ±17 (3)	95–107

[a]Stained after culture in regular medium (arbitrarily taken as 100%) or in the presence of various compounds. [b]Number of experiments. [c]Significantly ($p < 0.001$) different from control, untreated cells. [d]Individual values are given whenever 1 or 2 experiments only were done. [e]Not determined.

expected that inhibition of *N*-linked glycosylation or processing would modify the pattern of binding of Con A. For instance, treatment with tuncamycin (TM) or 2-deoxy-D-*arabino*-hexose (DOG), which prevent the synthesis of *N*-linked sugars, could strongly decrease the binding of Con A. In other systems[5], treatment with SW resulted in increased binding of Con A. Glycoproteins, synthesised in the presence of 1-deoxynojirimycin (dNM) should also bind more Con A than their normal counterparts, since *N*-linked oligosaccharides have terminal mannosyl and glucosyl groups[6]. Our results can best be explained if it is assumed that (*a*) SBA binds to *N*-linked residues probably through galactose residues [the "SBA-receptor(s)" have a rapid turn-over], (*b*) Con A, WGA, PNA, and TKA mainly bind to glycolipids or proteins with *O*-linked sugars or to glycoproteins with *N*-linked sugars having a slower turn-over.

Although SBA has a higher affinity for GalNAc (which is not found in *N*-linked sugars), it also binds to galactose[3]. The accessibility of galactose in *N*-linked sugars of glycoproteins is increased after treatment with Nase, whereas other treatments should decrease the amount of galactose of glycoproteins. The half-life of surface glycoproteins is very variable in lymphoma cells and lymphocytes[7–9]. Unexpected results with lectin binding after interfering with glycosylation, similar to those noted here, have been reported recently[10].

Since different compounds which interfere with *N*-linked glycosylation had

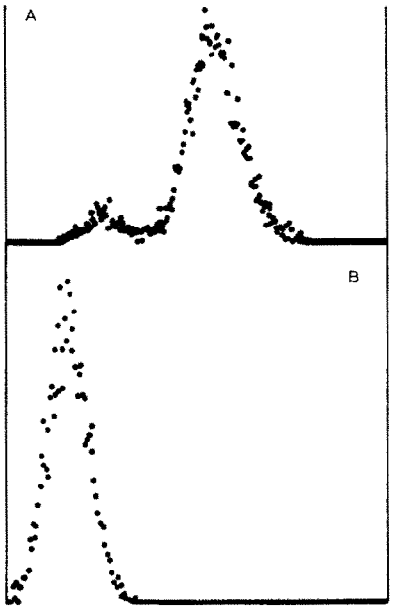

Fig. 1. Relative fluorescence intensity distribution obtained with fluorescein-conjugated SBA on BL/VL_3 cells: A, absence of sugar; B, presence of 0.2M GalNAc. Logarithmic scale from channel 1 (left) to channel 255 (right).

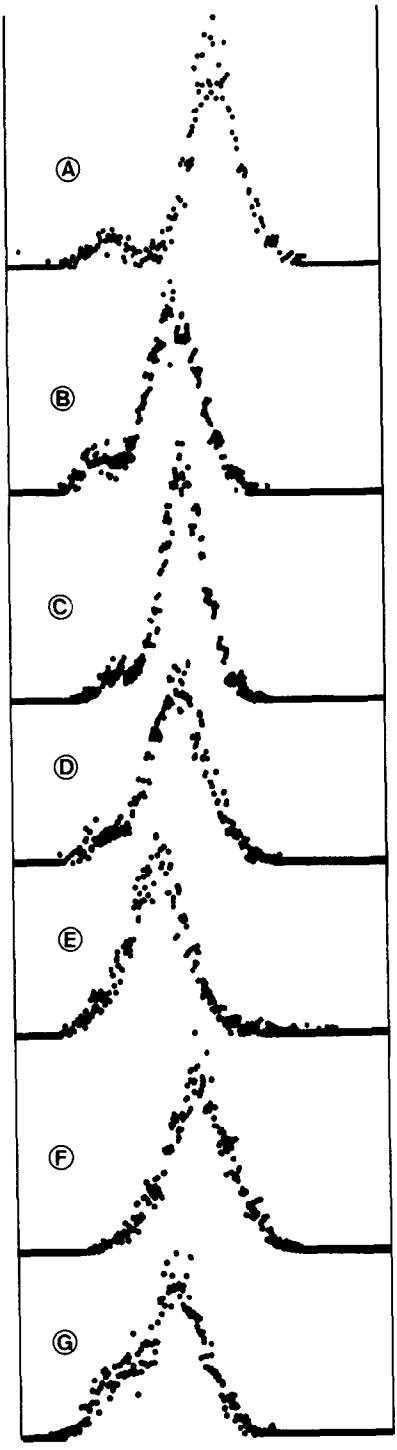

Fig. 2. As in Fig. 1, but the cells had been cultivated in the presence of various inhibitors: A, none; B, swainsonine (24 h, 0.2 μg/mL); C, swainsonine (48 h, 0.2 μg/mL); D, 1-deoxynojirimycin (24 h, 10mM); E, 1-deoxynojirimycin (48 h, 10mM); F, 2-deoxyglucose (24 h, 20mM); G, tunicamycin (24 h, 50 μg/mL).

similar effects on lectin binding, but different biological properties[1,2], a more-detailed analysis of modified glycoconjugates will be required. Treatment of cells with inhibitors of glycosylation or processing as described here introduces only limited modification (as judged by lectin binding and electrophoresis of membrane proteins[1,2]) and, accordingly, constitutes a suitable approach for evaluating the contribution of sugars to membrane function.

EXPERIMENTAL

Materials. — Fluoresceinated lectins, purchased from E-Y through Sanbio (Nistelrode, The Netherlands), are listed in Table I, with their nominal sugar specificity, the concentration used, and the sugars used for inhibition. The murine lymphoma line BL/VL_3, derived from a Radiation Leukemia Virus-induced thymic lymphoma of a C57BL/Ka mouse, was initially obtained from M. Lieberman[11]. Hybridoma Ho-13-4-9 was provided by the Salk Institute (San Diego, U.S.A.). Fluoresceinated goat anti-mouse IgM (Fc) was purchased from Nordic Immunologicals. Swainsonine (SW) was a gift from P. Dorling (School of Veterinary Studies, Murdoch, W. Australia) and 1-deoxynojirimycin (dNM) was a gift from E. Truscheit and D. Schmidt (Bayer, Wuppertal, Germany). Tunicamycin (TM), 2-deoxy-D-*arabino*-hexose (2-deoxyglucose, DOG), D-galactose, methyl α-D-mannopyranoside, 2-acetamido-2-deoxy-D-galactose (GalNAc), and chitobiose were commercial products.

Treatment of cells. — The cells were cultivated as recommended[11]. Inhibitors were added to exponentially growing cells 24 or 48 h before harvest. For neuraminidase (Nase) treatment, 10^7 cells (washed twice in Hanks medium) were resuspended in 1 mL of Hanks medium and incubated with Nase (0.02 U) from *Vibrio cholerae* (Behringwerke) for 45 min at 37°.

Labelling. — After washing twice in cold Hanks medium and once in cold phosphate-buffered saline (PBS; 20mM sodium phosphate, pH 7.4; NaCl, 150mM), cells were counted. Fluoresceinated lectins (with or without 0.2M competing sugar) were reacted with 2×10^6 cells in 200 μL of a solution (pH 7.0) containing 150mM NaCl, 50mM Tris, 10mM $CaCl_2$, 0.1mM $MnCl_2$, and 0.1mM $MgCl_2$, for 1 h at 4°. After three washings in PBS, the cells were fixed for 1 h in cold paraformaldehyde (1% in PBS), washed twice with cold PBS, resuspended in PBS (200 μL), and examined the next day. For anti-Thy-1.2 labelling, washed cells were incubated for 30 min at 4° with supernatant solution from hybridoma Ho-13-4-9, diluted 1:100 in PBS, washed three times with PBS, incubated for another 30 min at 4° with the goat anti-mouse IgM (Fc) fluoresceinated antibodies (diluted 1:40 in PBS), and then washed and processed as described for lectin staining.

Flow cytometry[12–15]. — For each condition, 5,000 cells were examined with a velocity of 900–1100 cells/s, using a FACS IV cell sorter equipped with a 70-μm nozzle (Becton Dickinson) and an argon ion laser emitting a 200-mW beam at 488 nm (type 164-05, Spectra Physics). On the basis of the gated forward-light-scatter

(2–15°) emission signal, the fluorescence emission of the cells of interest was collected by using a 520-nm long-pass dielectric filter and a 530-nm long-pass glass filter in front of a photomultiplier (750 V, model 9524 A QL-30, Emi-Gencom Inc.) equipped with a three-decade logarithmic amplifier. Data were recorded on histograms with 256-channel resolution. To compare the relative fluorescence intensities, calibration curves relating logarithmic and linear fluorescence intensity were determined[12]. For each condition, the percentage of positive cells and the median intensity of staining were recorded. The different cell populations were best characterised by the percentage of positive cells. For a given cell type, different experimental treatments are best evaluated by comparing the median intensity of fluorescence.

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