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INTERACTIONS OF LECTINS WITH NORMAL, SWAINSONINE-TREATED AND RICIN-RESISTANT BABY HAMSTER KIDNEY BHK CELLS*

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ABSTRACT

The aggregation of single-cell suspensions of normal and four ricin-resistant cell lines of baby hamster kidney (BHK) cells by several lectins has been studied by particle counting. Normal BHK cells were aggregated by concanavalin A, *Ricinus communis* agglutinin and ricin, *Abrus precatorius* agglutinin, wheat germ agglutinin, and *Erythrina cristagalli* and *Erythrina corallodendron* agglutinins. Neuraminidase treatment increased 4–13 fold the aggregation of BHK cells by the latter two lectins, as reported earlier for ricin (see ref. 13). After long-term culture of normal BHK cells with swainsonine, an inhibitor of complex N-glycan assembly, the aggregation of cells by each lectin except concanavalin A was much decreased or totally abolished. The lectin-induced aggregation of ricin-resistant cell lines Ric^R 14, 15, 19, and 21 was very similar to swainsonine treated BHK cells. Aggregation of Ric^R 15, 19, and 21 cells by *Erythrina* lectins was increased markedly by neuraminidase treatment of the cells. A smaller effect was obtained with Ric 14 cells. The data reported are consistent with similar hybrid N-glycans being present in swainsonine-treated BHK cells and the ricin-resistant cells. The hybrid structures bind lectins of *Ricinus, Abrus*, and *Erythrina* species after desialylation.

INTRODUCTION

Ricin, a glycoprotein isolated from seeds of *Ricinus communis*, is a highly toxic and specific inhibitor of eukaryotic protein synthesis[1]. Ricin consists of two polypeptide chains, one of which (β-subunit) is responsible for the binding of ricin to the cell surface, an essential first step in cytotoxicity. The preferred receptors for ricin are branched, complex N-glycans containing terminal galactose residues[2]. Selection of ricin-resistant mutants of normally sensitive cells leads to altered patterns of glycosylation and a decrease in the content of branched, complex N-glycans in cellular glycoproteins, including those expressed at the cell surface[3]. Recently, the N-glycan structures present in several ricin-resistant mutants of baby

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TABLE I

N-GLYCAN COMPOSITION OF BHK CELLS AND RICIN-RESISTANT MUTANTS

Cell-line	*% of total*[a]			
	Tri/tetraantennary	*Bi-antennary*	*Hybrid*[b]	*High mannose*
BHK	41	16	2	41
Ric 14			4	96
Ric 15			14	86
Ric 19			32	68
Ric 21			41 simple 22 branched	37[c]

[a]Estimated from recovery of radioactivity in purified glycopeptide fractions prepared from [^{3}H]mannose-labelled cells. See refs. 4–7. [b]Structures of the simple and branched hybrid N-glycans of Ric 21 cells have been described[7]. The predominant hybrid of Ric 15 and 19 has a structure similar to the simple hybrid of Ric 21 cells, but containing two additional mannosyl residues linked 1→3 and 1→6 to the (1→6)-linked mannose unit of the core sequence. The structures of the hybrid N-glycans present as minor components in BHK cells and Ric^R 14 cells have not been determined. [c]Predominant species: $(Man)_5(GlcNAc)_2Asn$ and $(Man)_6(GlcNAc)_2Asn$.

hamster kidney cells[4–7] have been determined (Table I). One mutant, Ric^R 14, contains high-mannose N-glycans almost exclusively, because of a profoundly decreased activity[8] of *N*-acetylglucosaminyl transferase I, a key enzyme in the conversion of high-mannose N-glycans into branched complex chains[9], Three other mutants, Ric^R 15, 19, and 21, synthesize, in addition to high-mannose N-glycans, hybrid structures containing three (Ric^R 21) mannose residues[7] or five (Ric^R 15 and 19) mannose residues (unpublished results), respectively.

Hybrid N-glycans identical to those present in Ric^R 15 and 19 cells accumulate in normal BHK cells treated with swainsonine[10], an alkaloid inhibitor of the processing glycosidase[9], α-mannosidase II. Swainsonine-treated BHK cells show a greatly increased resistance to ricin cytotoxicity; after removal of the alkaloid from cell cultures and re-growth, the cells return to a sensitive state[10].

In order to characterize more fully the similarities between swainsonine-treated BHK cells and ricin-resistant mutants, we have studied their interactions with several lectins. We have used a quantitative agglutination-assay to monitor the binding of the lectins to the cells.

EXPERIMENTAL

Lectins, enzymes, and sugars. — Concanavalin A was obtained as a three-times crystallised, freeze-dried powder from Miles–Yeda, U.K. *Ricinus communis* agglutinin (mol. wt. 120,000) and ricin (mol. wt. 60,000) were from Sigma, U.K. *Abrus precatorius* agglutinin was a gift from S. Olsnes, Oslo, Norway. Wheat germ agglutinin was from Miles-Yeda and *Vicia villosa* agglutinin was from E-Y Laboratories, U.S.A. *Erythrina cristagalli* and *Erythrina corallodendron* aggluti-

nins were gifts from N. Sharon, Rehovot, Israel. *Vibrio cholera* neuraminidase (E.C. 3.2.1.18) was obtained from Behringwerk–Hoechst, London, U.K. as a solution of 1 IU/mL (1000 units/mL) in 0.05M sodium acetate buffer, pH 5.5, containing 9 mg/mL NaCl and 1 mg/mL $CaCl_2$. Bovine pancreatic trypsin (EC 3.4.21.4.), TPCK treated, and deoxyribonuclease I (EC 3.1.21.1.) were from Sigma, U.K.

Neuraminyl-lactose was obtained from Behringwerk–Hoechst. *N*-Acetyl-lactosamine was a synthetic product obtained from M. C. Glick, Philadelphia. O-Glycans of known structure were obtained by published methods from fetuin[11] by P. Gleeson and G. Mills. Their structures were verified by 500-MHz n.m.r. analysis. All other sugars were of highest available purity from Sigma.

Cells. — Baby hamster kidney cells (BHK21/C13) were grown in 175-cm^2 plastic flasks in modified Eagle's medium containing 10% fetal calf serum in monolayer cultures at 37°. Ricin-resistant mutants (Ric^R 14, Ric^R 15, Ric^R 19, and Ri^R 21) of BHK cells[12] were grown similarly. In some experiments, the culture medium also contained swainsonine at 2.5 μg/mL final concentration. Swainsonine was a gift from B. Winchester and P. Dorling.

Neuraminidase treatment of cells was performed[13] by first washing nearly confluent monolayer cultures with phosphate-buffered saline (10 mL each time) followed by incubation with neuraminidase (50 units/mL, finally) in 50mM sodium acetate buffer (pH 5.5) containing 9 mg/mL of NaCl and 1 mg/mL of $CaCl_2$ for 30 min at 37°. The cells were again washed. with serum-free culture medium (3 times, 10 mL each time) and used immediately for agglutination assays.

Agglutination assay. — Monolayer culture growing in serum-supplemented medium were washed once with serum-free culture medium (10 mL) and incubated at 37° for ~2–3 min with 10 mL of sterile suspension medium: trypsin (0.2 mg/mL) and deoxyribonuclease I (10 μg/mL) in serum-free medium[14]. The cells were readily detached from the growth surface by using this procedure as single cells with little or no cell lysis. The action of trypsin was stopped after 2–3 min by addition of fetal calf serum (1 mL). The cells were pelleted at 3000*g* for 3 min, washed at 37° twice in serum-free medium, once with phosphate-buffered saline pH 7, and were then finally suspended in serum-free medium. An aliquot was used to determine cell density as described later, and the suspension was adjusted to 1.5×10^7 cells/mL by addition of serum-free medium.

Aliquots (0.2 mL) of a cell suspension were mixed in Eppendorf tubes with lectin solutions (0.2 mL) of known concentration. The mixtures were incubated and rotated continuously at room temperature for 90 min. Samples (0.2 mL) of each mixture were withdrawn, mixed with 10 mL of Isoton II (Coulter Electronics), and analyzed on a Coulter Model ZB1 Particle counter (Coulter Electronics) using a 100-μm probe[15]. The agglutination data are expressed as the fraction of the total particles in mixtures containing a lectin compared with control mixtures containing no lectin.

Hapten-inhibition studies adopted essentially the same procedure as the

foregoing. Aliquots of a single-cell suspension (0.2 mL) were mixed with sugar solutions (0.1 mL) of known concentration. Then 0.1 mL of a lectin solution was added to give a final concentration of 50 μg/mL in each tube. The mixtures were incubated for 90 min at room temperature and the particle numbers (A,B,C) were then determined in the Coulter counter. The hapten inhibitory activity is expressed by the quotient:

$$\% \text{ inhibition} = \left[1 - \frac{(A - C)}{(A - B)}\right] \times 100$$

where A refers to incubation mixtures containing cells alone, B is cells plus lectin, and C is the complete mixture with hapten inhibitor.

RESULTS

Agglutination of normal BHK cells. — Single-cell suspensions were mixed with various lectin concentrations and incubated at 37°. Agglutination was followed by particle counting. Preliminary experiments showed that maximal agglutination at a given lectin concentration was reached after 1 h of incubation. Routinely the mixtures were incubated for 90 min before analysis.

Normal BHK cells were agglutinated by seven lectins examined: concanavalin A, wheat germ agglutinin, ricin, and the agglutinin of *Ricinus communis*, the agglutinin of *Abrus precatorius*, and *Erythrina* agglutinins (Figs. 1 and 2). The concentrations of each lectin required to agglutinate BHK cells at 50% of maximal agglutination are shown in Table II. These data, together with the known sugar specificities of the lectins, indicate the presence in the glycoproteins expressed at the BHK cells-surface of glycans containing mannose residues (concanavalin A) in biantennary, hybrid, or high-mannose chains, galactose, or *N*-acetylgalactosamine residues (*Ricinus* and *Abrus* lectins) and sialic acid (wheat germ agglutinin). Experiments using *Vicia villosa* lectin (Fig. 2) showed that BHK cells were not agglutinated by concentrations up to 300 μg/mL. Recent studies[16] have identified the dominant sugar specificity of this lectin as *N*-acetyl-β-galactosamine residues present in O-glycans of glycoproteins. The failure of *Vicia villosa* lectin to agglutinate BHK cells agrees with direct structural analysis of the O-glycans present in the glycoproteins of these cells[17]. The O-glycans appear to be based on the disaccharide β-D-galactosyl-(1→3)-*N*-acetylgalactosamine, predominantly mono- and di-sialylated derivatives similar to the O-glycans of fetuin[11] and other glycoproteins.

The agglutination of BHK cells by the lectins of *E. cristagalli* and *E. corallodendron* is of special interest. Recent studies have identified the binding specificity of *E. cristagalli* lectin by inhibition of haemagglutination using defined hapten sugars[18,19] or fluorescent-ligand techniques[20,21]. These data indicated a preferred binding to β-galactosyl-(1→4) terminal residues. This conclusion was extended by hapten inhibition of BHK cell-agglutination mediated by the *Erythrina* lectins (Fig.

TABLE II

AGGLUTINATION OF BHK CELL-LINES BY VARIOUS LECTINS

Cell-line	*Treatment of cells*	*Lectin concentration (μg/mL) for 50% agglutination*[a]						
		Con A	*Ricin*	Ricinus *agglutinin*	Abrus *agglutinin*	E. cris *agglutinin*	E. corall *agglutinin*	*WGA*
BHK	None	32	50	30	26	8	55	17
	Neuraminidase					2	4	
	Swainsonine	6	NA	NA	SA	316	NA	
Ric 14	None	6	NA	NA	NA	SA	NA	11
	Neuraminidase					13	SA	
Ric 15	None	8	SA	230	96	190	NA	9
	Neuraminidase					5	4	
Ric 19	None	12	SA	SA	23	SA	NA	14
	Neuraminidase					4	5	
Ric 21	None	14	NA	SA	SA	SA	NA	21
	Neuraminidase					2	63	

[a]Abbreviations: (blank) not determined in this study; NA, no agglutination at any concentration; SA, some agglutination, <50% at the highest concentration; WGA, wheat germ agglutinin.

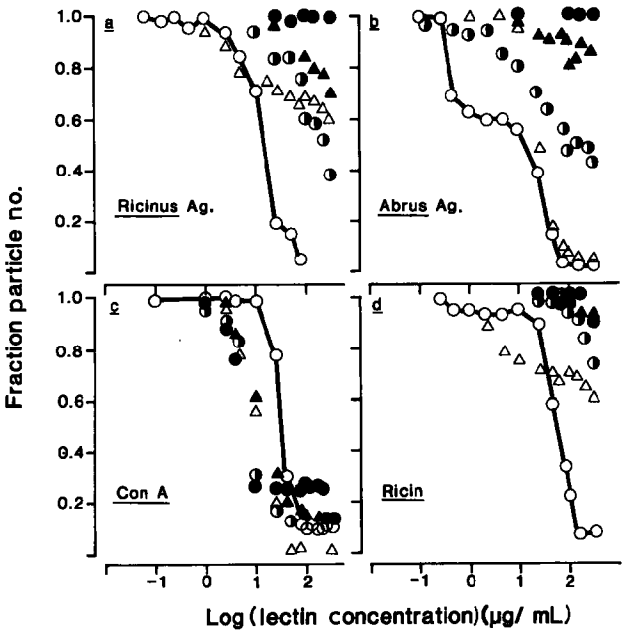

Fig. 1. Agglutination of BHK cell lines by *Ricinus* agglutinin (*a*), *Abrus* agglutinin (*b*), concanavalin A (*c*), and ricin (*d*). Key: BHK cells (○), Ric^R 14 cells (●), Ric^R 15 cells (◑), Ric^R 19 cells (△), and Ric^R 21 cells (▲).

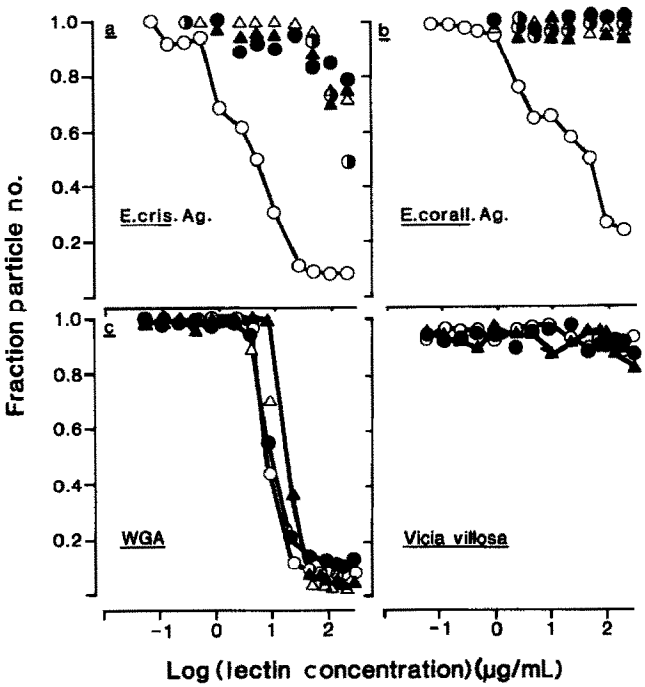

Fig. 2. Agglutination of BHK cell lines by *Erythrina cristagalli* (*a*) or *Erythrina corallodendron* (*b*) agglutinins, wheat germ agglutinin (*c*), and *Vicia villosa* agglutinin (*d*). See Fig. 1 for other details.

3). Agglutination was inhibited effectively (Table III) by D-galactose and by the α- and β-glycosides of galactose. Disaccharides containing, such nonreducing galactose residues as lactose or *N*-acetyllactosamine, were even better inhibitors of agglutination. The inhibitory activity of the latter disaccharide is interesting as the sequence involved, β-D-galactosyl-(1→4)-*N*-acetylglucosamine, is characteristic of complex N-glycans of glycoproteins. Substitution of the nonreducing galactosyl residue by sialic acid in α-(2→3) linkage abolished inhibitory activity (Table III).

N-Acetylgalactosamine, a common constituent of O-glycan of glycoproteins, including those of BHK cells[17], was also an effective inhibitor of agglutination mediated by *Erythrina* lectins. Similarly the reduced disaccharide, β-D-galactosyl-(1→4)-*N*-acetylgalactosaminitol, was also an effective inhibitor (Table III). Significantly, the mono- or di-sialylated derivatives of this disaccharide were very poor inhibitors of agglutination. Other evidence has shown that the O-glycans of BHK cells consist of sialylated derivatives of β-D-galactosyl-(1→4)-*N*-acetylgalactosamine, and no significant amounts of the nonsialylated disaccharide were detected[17]. Hence the agglutinatin of BHK cells by *Erythrina* lectins may be ascribed to binding to N-glycan sugar sequences. The conclusion was confirmed and extended by studies using ricin-resistant mutants of BHK cells.

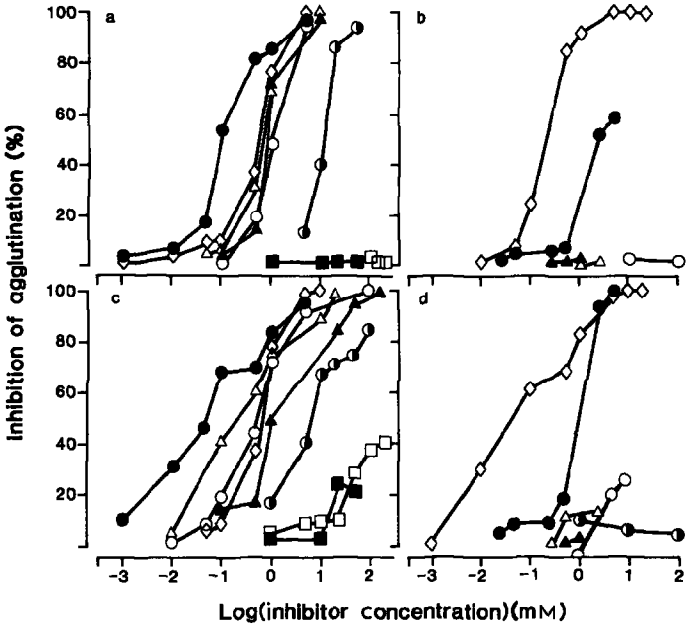

Fig. 3. Hapten inhibition of agglutination of BHK cells by *Erythrina cristagalli* (*a*, *b*) or *Erythrina corallodendron* (*c*, *d*) agglutinins. Key: *a* and *c*. Galactose (◇), D-fucose (◑), *N*-acetylglucosamine (□), *N*-acetylgalactosamine (○), *N*-acetylneuraminic acid (■), methyl α-D-galactoside (△), methyl β-D-galactoside (▲), and β-D-galactosyl(1→4)-*N*-acetylglucosamine (●). *b* and *d*. L-Fucose (◑), lactose (◇), α-NeuAc-(2→3)-β-Gal-(1→4)-Glc (○), β-D-galactosyl-(1→3)-*N*-acetylgalactosaminitol (●), α-NeuAc-(2→3)-β-Gal-(1→3)-GalNAc-ol (△), and α-NeuAc-(2→3)-Gal-(1→3)-[α-NeuAc-(2→6)]-GalNAc-ol (▲).

TABLE III

HAPTEN INHIBITION OF AGGLUTINATION OF BHK CELLS BY *Erythrina lectins*

Inhibitor	*Conc. (mM) for 50% inhibition*[a]	
	E. cristagalli	E. coralledendron
D-Galactose	0.7	0.6
D-Fucose	11	6.3
L-Fucose		
N-Acetyl-D-glucosamine		
N-Acetyl-D-galactosamine	1.4	0.6
N-Acetylneuraminic acid		
Methyl α-D-galactoside	0.7	0.2
Methyl β-D-galactoside	0.8	1
β-Gal-(1→4)-Glc	0.19	0.04
β-Gal-(1→4)-GlcNAc	0.1	0.06
α-NeuAc-(2→3)-β-Gal-(1→4)-Glc		
β-Gal-(1→3)-GalNAc-ol	0.45	1
α-NeuAc-(2→3)-β-Gal-(1→3)-GalNAc-ol		
α-NeuAc-(2→3)-β-Gal-(1→3)-α-[NeuAc-(2→6)]-GalNAc-ol		

[a](Blank) Inhibition of agglutination did not reach 50% at the highest concentration of sugar tested.

Agglutination of ricin-resistant BHK cells. — Each of the mutant cell-lines was agglutinated very poorly by ricin, the selective lectin used in isolation of these cell-lines (Fig. 1). Mutants Ric 14, 15, and 21 bind ricin <10% of the binding to normal BHK cells[12,13] and no agglutination was obtained at concentrations up to at least 100 μg/mL (Fig. 1, Table II). Mutant Ric 19, which retains some binding of ricin[12,13], was agglutinated to some extent at lower concentrations of ricin, but agglutination never reached 50% (Fig. 1, Table II). Similar results were obtained for *Ricinus* agglutinin. Interestingly, the *Abrus* agglutinin agglutinated both Ric^R 15 and Ric^R 19 cells, in the latter case to a similar extent as normal BHK cells (Fig. 1, Table II). These results indicate differences in the sugar sequences recognized by *Ricinus* and *Abrus* agglutinins.

The mutants were agglutinated as well as normal BHK cells by wheat germ agglutinin (Fig. 2, Table II) indicating, in agreement with an earlier study[22], the presence of sialic acid in surface glycoproteins of each cell line. Presumably, sialic acid residues present in O-glycans and hybrid N-glycans in the mutants are responsible for agglutination, whereas, in normal BHK cells, the sialic acid residues of branched complex N-glycans as well as O-glycans are recognized by the lectin. In addition, the chitobiose sequence of the core region of N-glycans may contribute to wheat-germ agglutinin binding[22]. As expected from the N-glycan composition of the various cell-lines, concanavalin A agglutinated the mutants more effectively than normal cells. This result is due presumably to the increased expression of high-mannose and hybrid N-glycans in the mutants[5–7] and is consistent with the greater proportion of N-glycans binding to concanavalin A–Sepharose compared with normal BHK cell glycopeptides[4].

The clearest difference between normal BHK cells and the mutants was obtained in agglutination assays using *Erythrina* lectins (Fig. 2, Table II). The agglutinin of *E. corallodendron* did not agglutinate the mutant cells at any concentration up to 300 μg/mL. *E. cristagalli* agglutinin, which produced a more effective agglutination of normal BHK cells than *E. corallodendron* agglutinin, also caused a small amount of agglutination of the ricin-resistant mutants, but only at very high concentrations.

Effects of neuraminidase. — We have shown previously that neuraminidase treatment of Ric^R 21 cells increased binding of ricin to the cells and induced a greatly increased sensitivity to ricin cytotoxicity[13]. Neuraminidase-treated normal BHK cells also were found to bind increased amounts of ricin and became more sensitive to the toxin. Similar results were obtained for the *Erythrina* lectins (Table II). The agglutination of normal BHK cells was increased 4–10 fold by neuraminidase treatment of the cells. The effect of neuraminidase on the mutants was even more marked. The neuraminidase-treated mutants were agglutinated by *E. cristagalli* lectin at concentrations similar to those required for the agglutination of normal untreated or neuraminidase-treated BHK cells. Similar findings for *E. corallodendron* lectin were obtained after neuraminidase treatment of Ric^R 15, 19, and 21 mutants. However, Ric^R 14 cells were poorly agglutinated by this lectin,

even after neuraminidase treatment. Presumably this is because of the relative amounts of reactive sugar sequences, β-D-galactosyl-(1→4)-*N*-acetylglucosamine or β-D-galactosyl-(1→3)-*N*-acetylgalactosamine, exposed by neuraminidase treatment of Ric^R 14 cells compared with the three other mutants. As the level of O-glycans is similar in each cell-line, the major species contributing to binding of *E. corallodendron* would appear to be desialylated hybrid N-glycans in the Ric^R 15, 19, and 21 mutants.

Effects of swainsonine. — Swainsonine is an inhibitor of α-D-mannosidase II, a glycosidase involved in the processing of nascent chains during N-glycan assembly[23]. BHK cells treated with swainsonine produce no branched complex N-glycans, but instead synthesize hybrid N-glycans identical to those identified in Ric^R 15 and 19 mutants[10].

The interactions of swainsonine-treated BHK cells with lectins are collected in Fig. 4 and the concentrations required for 50% agglutination are shown in Table II. Swainsonine-treated cells are agglutinated more effectively than are untreated cells by concanavalin A. By contrast, the agglutination by ricin, *Ricinus* agglutinin, *Abrus* agglutinin, and *Erythrina* lectins is virtually abolished after swainsonine treatment. Therefore, the behaviour of swainsonine-treated cells follows very closely that of the mutants carrying hybrid N-glycans. The effect on lectin agglutina-

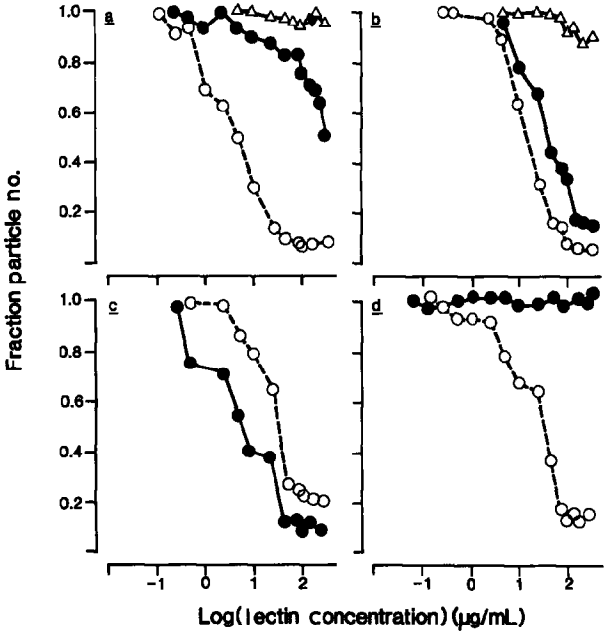

Fig. 4. Agglutination of swainsonine-treated BHK cells by lectins. *a, Erythrina cristagalli* (●) or *Erythrina corallodendron* (△) agglutinin. *b, Ricinus* agglutinin. The agglutination of cells exposed to swainsonine for one day (●) or 4 days (△) is shown. *c*, Concanavalin A. *d*, Ricin. The agglutination of untreated BHK cxells (○) by each lectin is shown by the *broken lines*.

tion by swainsonine required prolonged exposure of the cells to the alkaloid. Cells treated for only one day with swainsonine were agglutinated by *Ricinus* agglutinin to an extent similar to untreated BHK cells (Fig. 4b), whereas no agglutination was obtained using cells treated for 4 days. Presumably a considerable exposure to swainsonine is required to ensure complete replacement of cell-surface glycoproteins carrying a normal glycosylation pattern with glycoproteins containing hybrid N-glycans.

DISCUSSION

The results discussed for normal BHK cells and ricin-resistant mutants are fully consistent with the known N-glycan composition of these cell-lines (Table I) and the sugar specificities of the lectins. Presumably, the greatly reduced agglutination of each mutant by ricin, the selective lectin, is related to the greatly increased resistance of the cells to ricin. Previously it was shown that, in general, the ricin-resistant mutants contain a decreased amount of ricin receptors at the cell surface, although some residual binding was retained[12]. The Ric^R 14 cell-line, for example, bound ricin to ~10% of normal, sensitive BHK cells. By contrast, the resistance to ricin cytotoxicity was increased at least 100 fold[12]. Therefore, the small amounts of residual ricin receptors shown to be present on the surface of the mutant cells appear to mediate neither agglutination by ricin nor its cytotoxicity at concentrations that are capable of both agglutination and killing of normal, sensitive BHK cells. Previously we have shown that ricin bound at the surface of the resistant mutants from solutions containing a ricin concentration sufficient for killing normal BHK cells is not taken up into the mutant cells[24]. This suggests that the internalization of ricin and the agglutination of cells by ricin may share some common mechanistic steps. Lectin-induced cell agglutination has been correlated with several factors, including a lateral diffusion within the surface membrane of glycoproteins engaged in lectin binding[25].

The properties of the *Erythrina* lectins described here are similar to those inferred from haemagglutination data obtained previously[18,19]. *N*-acetyl-lactosamine is the best inhibitor of agglutination of BHK cells as well as haemagglutination. However, lactose is an almost equally effective inhibitor of BHK cell-agglutination mediated by *Erythrina* lectins, whereas it was five times less effective as an inhibitor of haemagglutination. As described[18,19] by others using an haemagglutination assay, sialylation of the galactose residue of the disaccharide abolished inhibitor activity. The derivative used was the α-(2→3) derivative: it remains to be determined if a 6-*O*-substituted galactose retains affinity for the lectins. The sugar specificity of *Erythrina* lectins is similar to the *Ricinus communis* lectins and is consistent with the resistance to ricin of Ric^R 15, 19, and 21 containing 3-*O*-substituted galactose residues in hybrid N-glycans.

N-Acetylgalactosamine is an inhibitor of *Erythrina* lectins, as assayed by agglutination of BHK cells or haemagglutination. As BHK cells were not aggluti-

nated by the *Vicia villosa* lectin, the glycoproteins of these cells do not contain β-linked *N*-acetylgalactosamine residues[16]. These cells do contain O-glycans, however, amounting to approximately 20–25% of total protein-linked carbohydrate[17]. The disaccharide β-Gal-(1→3)-GalNAc was a good inhibitor of BHK cell-agglutination by *Erythrina* lectins. Again sialylation of this sequence abolished inhibitory activity. As no significant amount of non-sialylated O-glycans was detected in BHK cells[17], the major receptors for the lectins are N-glycans. After desialylation, however, these O-glycans do function as receptors for *Erythrina* lectins. This is shown by the agglutination, after neuraminidase treatment, of Ric^R 14 cells by *Erythrina cristogalli* agglutinin. These cells contain almost exclusively high-mannose N-glycans and only trace amounts of hybrid N-glycans (unpublished results, Table II).

We conclude that growth of normal BHK cells in swainsonine-containing medium causes a phenotypic change that is closely correlated with ricin-resistant mutants expressing hybrid N-glycans. Recently we have shown that the N-glycans present in swainsonine-treated BHK cells contain five mannose residues, as predicted, and a galactose residue 3-*O*-substituted by sialic acid[10]. This structure is identical with (Ric 15 and 19) or closely similar to (Ric 21) the basic sequence of the N-glycans of ricin-resistant mutants. Like these mutants, normal BHK cells exposed for prolonged periods to swainsonine acquire resistance to ricin and exhibit a similar rounded morphology, with cells poorly attached to the growth substratum[10]. However, swainsonine treatment does not induce loss of a pericellular fibronectin matrix, whereas the mutants fail to retain a fibronectin matrix (unpublished results). This result shows that not all of the pleiotropic effects expressed in ricin-resistant BHK cells are necessarily a consequence of the substitution of normal branched, complex N-glycans by hybrid structures; additional, as yet unidentified factors, are involved.

REFERENCES

1 S. Olsnes and A. Pihl, in P. Cohen and S. van Heyningen (Eds.), *Molecular Action of Toxins and Viruses*, Elsevier, Amsterdam, 1982, pp. 51–105.
2 J. U. Baenziger and D. Fiete, *J. Biol. Chem.*, 254 (1979) 9795–9799.
3 E. B. Briles, *Int. Rev. Cytol.*, 75 (1982) 101–165.
4 R. C. Hughes and G. Mills, *Biochem. J.* 211 (1983) 575–587.
5 R. C. Hughes, G. Mills, and D. Stojanovic, *Carbohydr. Res.*, 120 (1983) 215–234.
6 R. C. Hughes and G. Mills, *Biochem. J.*, 226 (1985) 487–498.
7 P. A. Gleeson, J. Feeney, and R. C. Hughes, *Biochemistry*, 24 (1985) 493–503.
8 P. Vischer and R. C. Hughes, *Eur. J. Biochem.*, 117 (1981) 275–284.
9 H. Schachter, S. Narasimhan, P. A. Gleeson, and G. Vella, *Can. J. Biochem. Cell Biol.*, 61 (1983) 1049–1066.
10 L. Foddy, J. Feeney, and R. C. Hughes, *Biochem. J.*, 233 (1986) 697–706.
11 B. Nilsson, N. E. Nurdon, and S. Svensson, *J. Biol. Chem.*, 254 (1979) 4545–4553.
12 A. Meager, A. Ungkitchanukit, and R. C. Hughes, *Biochem. J.*, 154 (1976) 113–124.
13 S. Rosen and R. C. Hughes, *Biochemistry*, 16 (1977) 4908–4915.
14 J. G. Edwards, J. A. Campbell, P. J. Robson, and M. B. Vicker, *J. Cell Sci.*, 19 (1975) 653–667.
15 J. Edwards, in R. H. Pain and S. J. Smith (Eds.), *New Techniques in Biophysical and Cell Biology*, Vol. 1, Wiley, London, 1973, pp. 1–127.
16 A. Conzelman and S. Kornfeld, *J. Biol. Chem.*, 259 (1984) 12528–12535.

17 D. STOJANOVIC, Ph.D. Thesis (1983), University of London.
18 J. L. IGLESIAS, H. LIS, AND N. SHARON, *Eur. J. Biochem.*, 123 (1982) 247–252.
19 P. M. KALADAS, E. A. KABAT, J. L. IGLESIAS, H. LIS, AND N. SHARON, *Arch. Biochem. Biophys.*, 217 (1982) 624–637.
20 H. DE BOECK, F. G. LOONTIENS, H. LIS, AND N. SHARON, *Arch. Biochem. Biophys.*, 234 (1984) 297–304.
21 H. DE BOECK, R. B. MACGREGOR, R. M. CLEGG, N. SHARON, AND R. C. HUGHES, *Eur. J. Biochem.*, 149 (1985) 141–145.
22 A. OBRENOVITCH, C. SENE, A. C. ROCHE, M. MONSIGNY, P. VISCHER, AND R. C. HUGHES, *Biochimie*, 3 (1981) 169–175.
23 D. R. P. TULSIANI, T. M. HARRIS, AND O. TOUSTER, *J. Biol. Chem.*, 257 (1982) 7936–7939.
24 P. A. GLEESON AND R. C. HUGHES, *J. Cell. Sci.*, 76 (1985) 283-301.
25 G. NICOLSON AND G. POSTE, *Biochim. Biophys. Acta*, 554 (1979) 520–531.