Volume 289, number 2, 145–147 FEBS 10119 September 1991
© 1991 Federation of European Biochemical Societies 00145793/91/$3.50
ADONIS 001457939100834T

Cell adhesive activity of two animal lectins through different recognition mechanisms

Yasuhiro Ozeki, Taei Matsui and Koiti Titani

Institute for Comprehensive Medical Science, Fujita Health University School of Medicine, Toyoake, Aichi 470-11, Japan

Received 28 June 1991; revised version received 1 July 1991

Cell adhesive activity of two animal lectins, frog (*Rana catesbeiana*) S-type 14K lectin and echinoidin (a C-type lectin from sea urchin plasma), was studied with human rhabdomyosarcoma (RD) cells. RD cells attached to and spread on plastic plates coated with each lectin. Cell adhesion by the frog lectin was completely inhibited by the addition of lactose or asialofetuin glycopeptide. Echinoidin-induced cell adhesion was only inhibited by peptide GRGDS. Since echinoidin is known to contain an RGD-sequence, our results clearly indicate that this sequence is active as the cell adhesive signal. These results suggest that some of the animal lectins may function as a cell adhesive molecule rather than using the carbohydrate-recognition mechanism.

β-Galactoside binding lectin; Echinoidin; Cell adhesion; Integrin; Human rhabdomyosarcoma cell

1. INTRODUCTION

Animal lectins are known to be ubiquitous. They have been classified into two major groups, S-type (SH-reagent dependent) and C-type (Ca^{2+}-dependent) lectins, according to their properties and sequence homologies [1]. The biological function of these lectins is still unclear, but it is suggested that they function in several cellular recognitions [2,3].

Recently, we purified a β-galactoside-binding lectin from frog (*Rana catesbeiana*) eggs and identified it as a S-type lectin according to the partial amino acid sequence [4]. Echinoidin is a C-type lectin purified from the coelomic fluid of the sea urchin (*Anthocidaris crassispina*), and it recognizes mucin-type oligosaccharides [5]. Echinoidin has an Arg-Gly-Asp (RGD) sequence [6] which is known to be an active signal in cell adhesive molecules including fibronectin, vitronectin and the von Willebrand factor, but the cell adhesive activity of echinoidin has not yet been demonstrated.

In the present study, we examined the cell adhesive activity of two animal lectins, frog 14K-lectin and sea urchin echinoidin towards human cancer cells.

2. MATERIALS AND METHODS

2.1. *Lectins*

The frog 14K-lectin was prepared as previously described [4]. Echinoidin was purified from the coelomic fluid of sea urchin (*Anthocidaris crassispina*) according as described [5]. Each of the purified lectins gave a single band at a relative molecular mass of 14 kDa by SDS-PAGE in the presence of β-mercaptoethanol [7]. N-terminal sequence analysis was performed on a Protein Sequencer [4].

Correspondence address: K. Titani, Institute for Comprehensive Medical Science, Fujita Health University School of Medicine, Toyoake, Aichi 470-11, Japan

2.2. *Preparation of glycopeptides from asialofetuin by digestion with actinase*

Two hundred mg of asialofetuin prepared by the method of Roff and Wang [8] was digested with actinase (Kaken-Kagaku, Japan) as described [9]. The digest was fractionated by gel-filtration on a Sephadex G-50 column (1.5 × 50 cm). Sugar-rich fractions were detected by the method of Dubois et al. [10].

2.3. *Hemagglutination activity assay*

Lectin-induced hemagglutination was estimated by incubation of a lectin (30 μg/ml) with human type-A erythrocytes (0.25% v/v), trypsinized and fixed on a glass plate.

2.4. *Cell attachment assay*

A cell binding assay was performed as described by Maeda et al. [10]. Twenty-four- or ninety-six-well bacteriological plates (Petray SH-T24FS, Terumo, Japan) were coated with a lectin dissolved in 100 mM $NaHCO_3$, pH 9.5, for 4 h at room temperature and then blocked with 2% bovine serum albumin (BSA) for 3 h. Human rhabdomyosarcoma (RD) cells were dissociated from the culture dish with 0.25% trypsin and 0.02% EDTA. After washing with serum-free Dulbecco's modified Eagle's medium (adhesion medium), the cells were incubated on the coated plates at 1×10^5 cells/well in the presence or absence of sugar (100 mM lactose, 100 mM glucose or 500 μg/ml of glycopeptide from asialofetuin) or synthetic peptide (100 μM GRGDS or GRGES) for 60 min at 37°C and gently washed three times with the adhesion medium. The attached cells were fixed with 4% formaldehyde, containing 4% glucose, and photographed using a phase-contrast microscope.

3. RESULTS AND DISCUSSION

Since the amino-terminal 10-residue sequence of echinoidin purified in this study was identical to the one previously described [6] (data not shown), the purified lectin was assumed to be echinoidin. Sugar specificity of the frog 14K-lectin and echinoidin was compared by a hemagglutination assay (Table I). Hemagglutination

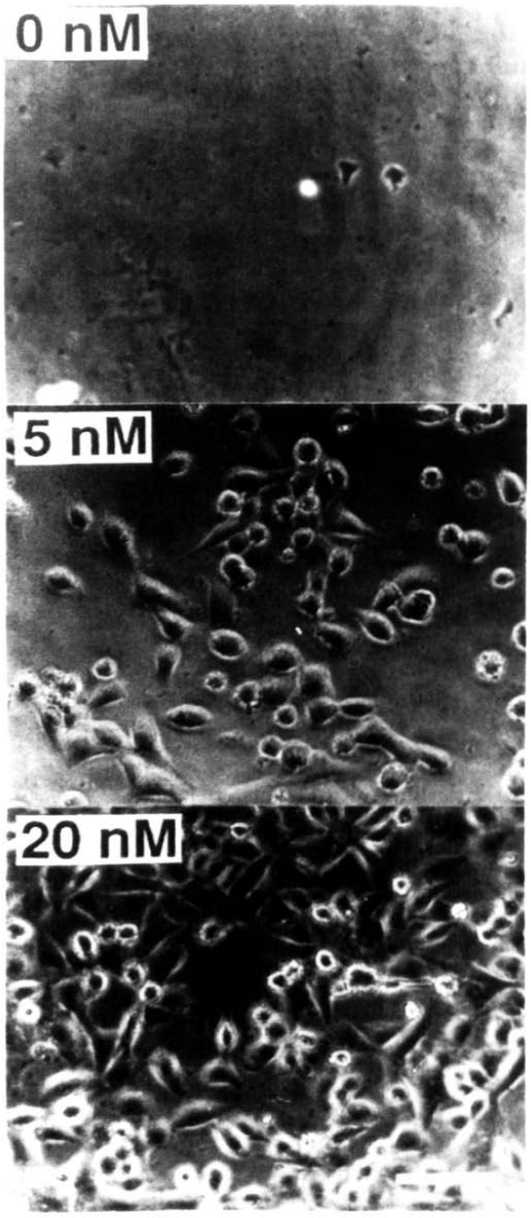

Fig. 1. Cell adhesive activity of animal lectin. Human RD cells (1×10^5cells/ml) were incubated on plastic plates coated with 0 nM, 5 nM or 20 nM of frog 14K-lectin for 1 h at 37°C. After washing, the plates were observed and photographed using a phase contrast microscope. Bar=100 μm.

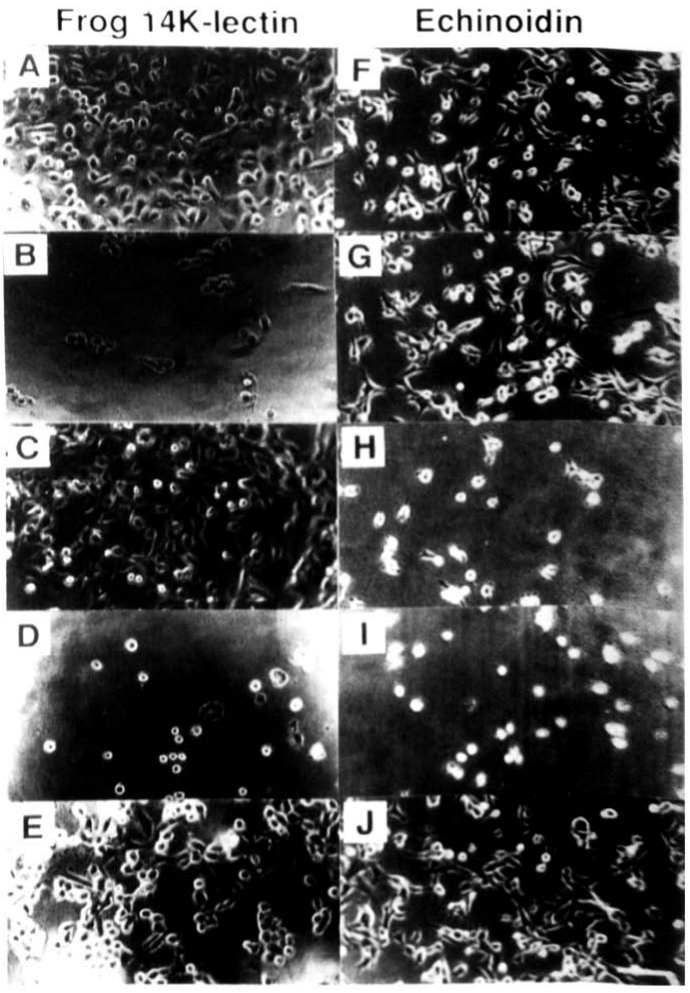

Fig. 2. Effects of sugars and peptides on the lectin-mediated cell adhesion. Human RD cells were incubated on plastic plates coated with 20 nM of the frog 14K-lectin (A to E) or echinoidin (F to J) in the presence of the following reagents; none (A,F), 500 μg/ml asialofetuinglycopeptide (B,G), 100 μM GRGDS peptide (C,H), 100 mM lactose (D), 100 mM glucose (E), 500 μg/ml asialoglycopeptide and 100 μM GRGDS peptide (I), 100 μM GRGES peptide (J). Bar=100 μm.

induced by each lectin was inhibited by the addition of asialofetuin glycopeptide. Lactose also inhibited the hemagglutinating activity of the frog 14K-lectin, but it did not inhibit the echinoidin-induced hemagglutination. Together with the previous report [5] that echinoidin has an affinity to (NeuAcα2→3)Galβ1→3GalNAc structure, our results indicated that echinoidin may recognize Galβ1→3GalNAc residue of the asialoglycopeptide.

Human RD cells adhered to and spread on both the lectin-coated plates depending on the concentration of lectin, though the cells did not adhere to the BSA-coated plastic plate (Fig. 1). The cell adhesion to the plate coated with the frog 14K-lectin was blocked by asialoglycopeptide (500 μg/ml) or lactose (100 mM) (Fig.

Table I

Effects of saccharides on lectin-induced hemagglutination.

Additions	Hemagglutination*	
	Frog 14K-lectin	Echinoidin**
None	+	+
100 mM lactose	−	+
100 mM glucose	+	+
500 μg/ml asialoglycopeptide	−	−

*Trypsinized and fixed human type-A erythrocytes were used for assay.

** Assay was performed in the presence of 2 mM $CaCl_2$.

2B,D), but not with glucose (100 mM) or GRGDS peptide (100 μM) (Fig. 2C,E). The cell adhesive activity of echinoidin was not inhibited by asialoglycopeptide (Fig. 2G), but inhibited by pentapeptide GRGDS (Fig. 2H). This inhibition was RGD-dependent, because GRGES, an inactive homologue of GRGDS, showed no effect on the cell adhesion induced with echinoidin (Fig. 2J). A mixture of asialoglycopeptide and GRGDS peptide did not enhance the inhibitory effect (Fig. 2E), suggesting that the cells adhered to the echinoidin-coated substrate only through the RGD sequence. Although intact fetuin has been reported to have a potential cell adhesive activity [12], the glycopeptides from asialofetuin showed no such activity in our study (data not shown). Both peptides, GRGDS and GRGES, had no effect on the frog 14K-lectin-induced cell adhesion.

These results, taken together, clearly indicate that these two animal lectins have an ability to promote cell adhesion and spreading via different adhesion mechanisms. The frog 14K-lectin exerts its cell adhesive activity through the lectin–sugar chain interaction, suggesting that the glycoconjugate receptor for the lectin is present on the surface of RD cells. 14K-Lectins are widely distributed among vertebrates and known to have similar primary structures and sugar specificities. Raz and Lotan [13] showed a significant correlation between the content of a β-galactoside binding lectin and the metastatic activity of mouse melanoma cells. Oda et al. [14] indicated that a 14K-lectin is localized in the extracellular matrix of chick skin and the amount of lectin changes during differentiation. These results suggest that widely distributed 14K-lectins may function as cell adhesive molecules in the extracellular matrix. The lectin-mediated cellular interaction may be regulated by the amount of lectin or the cell surface ligand.

The cell adhesive activity of echinoidin was mediated by the RGD sequence within the lectin, but not by the carbohydrate recognition domain. It has been shown that GRGDS peptide binds to RD cells through the receptor (integrin) [15]. Our results indicate that the RGD-sequence of echinoidin is recognized by the integrin molecule on RD cells. Springer et al. [16] showed that a lectin from the slime mold *Dictyostrlium discoideums*, named discoidin I, has an ability to mediate cell–cell adhesion via the inner RGD sequence, directing the cellular migration and morphogenesis of the slime mold. Three other rat C-type lectins, mannose-binding protein [17], Kupffer cell lectin [18] and macrophage lectin [19] have also been shown to contain the RGD sequence. It would be interesting to examine whether these lectins also have an RGD-dependent cell adhesive activity. It is possible that some of these lectins may mediate interaction as a bifunctional molecule between glucoconjugates and integrins.

REFERENCES

[1] Drickamer, K. (1988) J. Biol. Chem. 263, 9557–9560.
[2] Sharon, N. and Lis, H. (1989) Science 246, 227–234.
[3] Barondes, S.H. (1988) Trends Biochem. Sci. 13, 480–482.
[4] Ozeki, Y., Matsui, T., Nitta, K., Kawauchi, H., Takayanagi, Y. and Titani, K. (1991) Biochem. Biophys. Res. Commun., in press.
[5] Giga, Y., Sutoh, K. and Ikai, A. (1985) Biochemistry 24, 4416–4467.
[6] Giga, Y., Ikai, A. and Takahashi, K. (1987) J. Biol. Chem. 262, 6197–6203.
[7] Laemmli, U.K. (1970) Nature 227, 680–685.
[8] Roff, C.F. and Wang, J.L. (1983) J. Biol. Chem. 258, 10657–10663.
[9] Matsui, T. (1984) Biol. Bull. 166, 178–188.
[10] Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F. (1956) Anal. Chem. 28, 350–356.
[11] Maeda, T., Oyama, R., Ichihara-Tanaka, K., Kimizuka, F., Kato, I., Titani, K. and Sekiguchi, K. (1989) J. Biol. Chem. 264, 15165–15168.
[12] Rizzino, A. and Sato, G. (1978) Proc. Natl. Acad. Sci. USA 75, 1844–1848.
[13] Raz, A., Meromsky, L., Zvibel, I. and Lotan, R. (1987) Int. J. Cancer 39, 353–360.
[14] Oda, Y., Ohyama, Y., Obinata, A. and Kasai, K. (1989) Exp. Cell Res. 182, 33–43.
[15] Hynes, R.D. (1987) Cell 48, 549–554.
[16] Springer, W.R., Douglas, N., Cooper, W. and Barondes, S.H. (1984) Cell 39, 557–564.
[17] Drickamer, K., Kordal, M.S. and Reynolds, L. (1986) J. Biol. Chem. 261, 6878–6887.
[18] Hoyle, G.W. and Hill, R.L. (1988) J. Biol. Chem. 263, 7487–7492.
[19] Ii, M., Kurata, H., Itoh, N., Yamashina, I. and Kawasaki, T. (1990) J. Biol. Chem. 265, 11295–11298.