Studies on three kinds of lectins from *Xenopus laevis* skin[1]

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Summary. Among skin extracts of various frogs, lectin activity was found only in fractions prepared from *Xenopus laevis* skin. 3 skin lectins have been separated. Among these, the lectin designated S2-Dlc was isolated in a homogeneous state and showed a preferential agglutination of Ehrlich and S-180 ascites tumor cells; other tumor cells and human erythrocytes were not agglutinated. The lectin-dependent agglutination was inhibited by D-fucose, L-arabinose, D-galactose, lactose and lactulose. The sugar specificity of this lectin is similar to that of the lectin from *Xenopus laevis* eggs which agglutinates Ehrlich and S-180 ascites cells.

In recent years, there has been increasing interest in lectins from animal cells and tissues[2,3]. Various lectins with different specificities have been isolated from eggs of various frogs[4-10]. Recently, 3 agglutinins from *Rana catesbeiana* eggs have been described[4,5]. One of these agglutinins agglutinates Ehrlich ascites tumor cells, and the others, which seem to recognize the trisaccharide residue GalNAcα1→3Galβ1→4GlcNAc, agglutinate human A-erythrocytes[4].

In the present study, we demonstrate reactivities of 3 lectins extracted from *Xenopus laevis* skin to various cells, and describe the isolation of the lectin which showed a preferential agglutination of some tumor cells but not other type of cells, and its sugar specificity.

Materials and methods. Frog skins were stripped off, rinsed with physiological saline, homogenized with ice-cold physiological saline and acetone (1:10), and dried. The powder was homogenized with 20 volumes of physiological saline and centrifuged at 15,000 × g for 30 min, and the supernatant was dialyzed against distilled water and then lyophilized. The lyophilized powder (1.0 g) was dissolved in 0.1 M NaCl (20 ml) and applied to a column of Sephadex G-75 which was equilibrated with 0.1 M NaCl (fig. 1A). The agglutinating activity was found only in fractions of skin from *Xenopus laevis* (table 1). Agglutination and inhibition assays for tumor cells were performed essentially as described[6]. Hemagglutination assays were performed in microtiter plates[4]. The fraction which was extracted from *Xenopus laevis* skin and agglutinated Ehrlich ascites cells and S-180 ascites cells (S2) was further separated by chromatography on a column of DEAE-cellulose (fig. 1B).

Results and discussion. Although skin extracts from *Rana japonica, Rana catesbeiana, Rana nigromaculata* and *Bufo vulgaris*

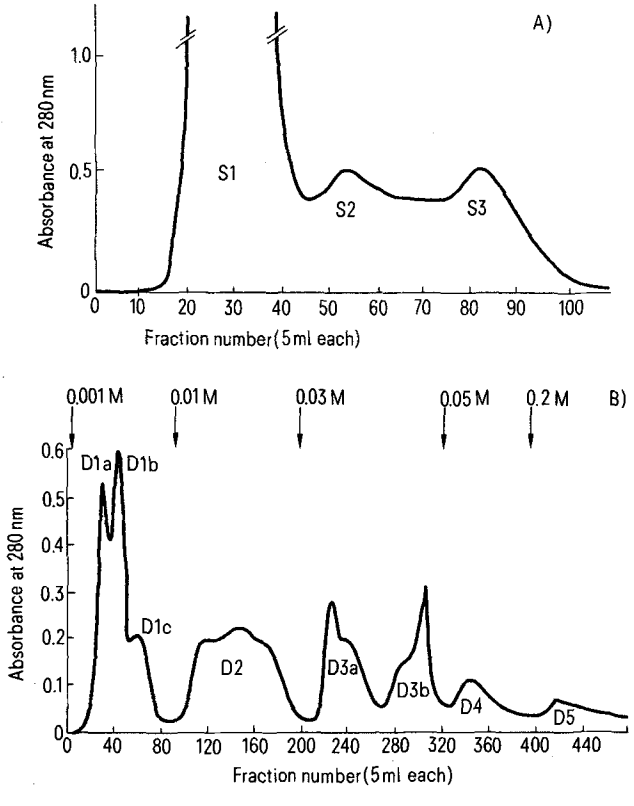

Figure 1. Purification of frog skin lectins by chromatography. *A* Typical gel filtration pattern of the crude extract *(Xenopus laevis)* on Sephadex G-75 (4.0 × 40 cm) in 0.1 M NaCl. Flow rate was adjusted to 80 ml/h. *B* Elution pattern of chromatography of S2 obtained by gel filtration A. Chromatography carried out on DEAE-cellulose (2.0 × 35 cm). The elution was carried out with stepwise increase in the molarity of phosphate buffer (pH 6.8). The molarity was increased as indicated from 0.001 to 0.2 M. Flow rate was 30 ml/h.

Table 1. Separation of reactivity of each fraction after gel filtration of crude *Xenopus laevis* skin extracts on Sephadex G-75

	S1 Fr. No. 15–45	S2 Fr. No. 46–72	S3 Fr. No. 73–110
Tumor cells			
Ehrlich	−	+	−
S-180	−	+	−
AH109A	−	±	+
AS653	−	±	+
Erythrocytes			
Human A	+	±	−
B	+	±	−
O	+	±	−

Tumor cells were harvested, and washed and suspended in physiological saline: concentration adjusted to 10^7/0.1 ml. Sample solution (2.0 mg/ml) was mixed with equal volume of cell suspension and evaluated. +, obvious agglutination of 10^7 tumor cells and 10^8 erythrocytes in a lectin solution with a concentration 200 μg/0.2 ml; ±, a faint agglutination; −, no agglutination under the same conditions as above.

Table 2. Inhibition of Dlc-induced S-180 cell agglutination by hapten sugars (mM) and glycosaminoglycan (μg)

Hapten and glycosaminoglycan	Concentration of hapten or glycosaminoglycan yielding 100% inhibition of the lectin activity
D-Fucose	23.5–47.0
L-Fucose	> 100
D-Arabinose	> 100
L-Arabinose	18.2
D-Galactose	20.5
L-Galactose	> 100
N-Acetyl-D-galactosamine	> 100
D-Glucose	> 100
L-Glucose	> 100
N-Acetyl-D-glucosamine	> 100
Lactose	5.9
Lactulose	3.2
Melibiose	16.5
Heparine	250

Minimum quantity of hapten or glycosaminoglycan per 0.2 ml that inhibited cytoagglutination of the S-180 tumor cell suspension caused by 3 agglutination doses of Dlc.

formosus all failed to agglutinate tumor cells such as Ehrlich ascites cells, S-180 cells, AH109A cells and AS653 cells[11], and human, mouse, rat or chicken erythrocytes, *Xenopus laevis* skin extract agglutinated both tumor cells and human erythrocytes. Table 1 gives the reactivities of each fraction of the extract from *Xenopus laevis* separated by gel filtration on Sephadex G-75. S1 (Fr. No. 15–45) agglutinated human erythrocytes without showing any specificity for A, B and O blood groups[12], but had no effect on erythrocytes of other species and on tumor cells, whereas S2 (Fr. No. 46–72) and S3 (Fr. No. 73–110) preferentially agglutinated xenogenic tumor cells: S2 agglutinated tumor cells grown as ascites in ddY mice, such as Ehrlich and S-180 ascites cells, while S3 agglutinated only tumor cells maintained in Donryu rats, such as AH109A cells and AS653 cells. We have recently found that a lectin, lactose-binding protein, from eggs of *Xenopus laevis* strongly agglutinates various tumor cells as well as sialidase-treated human erythrocytes[13]. Agglutination of S-180 cells by the egg lectin was inhibited by D-fucose, L-arabinose, D-galactose and lactose. Since the agglutinability of Ehrlich ascites cells and S-180 cells by the egg lectin increases after brief treatment with sialidase, and since lectin-induced hemagglutination occurs only after digestion of erythrocytes by sialidase, it seems likely that the egg lectin recognizes terminal D-galactosyl residues. A lectin with similar specificity isolated from embryos and oocytes of *Xenopus laevis* has quite recently been described by Roberson et al.[10]. The sugar specificity of this lectin reported by Roberson et al. is similar to that of the egg lectin, and these lectins may be identical.

After S2 was separated by chromatography on a column of DEAE-cellulose, the lectin activity was found in a peak labelled Dlc (Fr. No. 50–90), which was homogeneous by electrophoresis on cellulose acetate in barbiturate buffer (pH 8.6) (fig. 2). The minimum quantity of Dlc required for agglutination of S-180 cells was 60 μg/ml, while that for intact human erythrocytes was 4000 μg/ml. Agglutination of S-180 cells by the Dlc fractions was inhibited by D-fucose, L-arabinose, D-galactose, lactulose and lactose (table 2), while hemagglutination by the lectin derived from S1 was inhibited by lactulose and lactose but not by D-fucose, L-arabinose and D-galactose[12].

Conclusion. Both Dlc (skin-derived) and the egg lectin agglutinate Ehrlich cells and S-180 cells (mouse tumor cells) but do not agglutinate AH109A or AS653 cells (rat tumor cells). Sialidase-treated human erythrocytes are very strongly agglutinated by the egg lectin and very weakly by Dlc.

Dlc seems to recognize not only the configurations of the 2-, 3- and 4-hydroxyl groups of the terminal sugar but also its β-D-glycosidic linkage, while the lectin derived from S1 seems to recognize only a β-glycosodic linkage containing a D-galactosyl end-group.

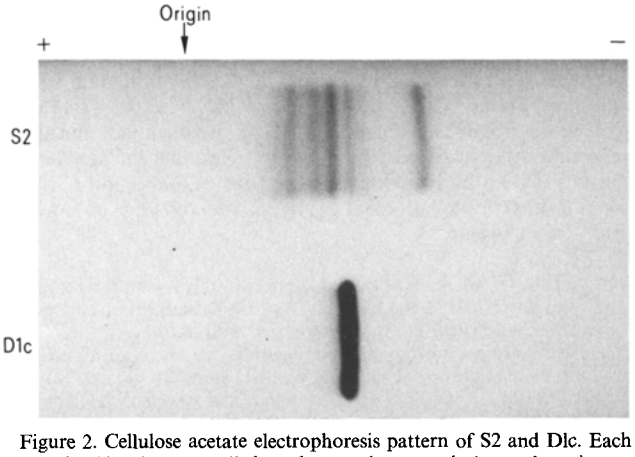

Figure 2. Cellulose acetate electrophoresis pattern of S2 and Dlc. Each sample (10 μg) was applied to the membrane and electrophoresis was carried out at a constant current of 0.8 mA/cm for 45 min.

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Stereochemical course of pheromone biosynthesis in the arctiid moth, *Creatonotos transiens*[1,2]

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Summary. The biosynthetic conversion of a pyrrolizidine alkaloid (heliotrine, **IV**) to a male moth pheromone (hydroxydanaidal, **III**) is found to proceed with inversion of configuration at the remaining asymmetric center (C-7).

Dihydro-5H-pyrrolizines, such as danaidone (**I**), danaidal (**II**) and hydroxydanaidal (**III**), constitute an interesting group of compounds secreted by the males of certain Lepidoptera[3–6]. These substances, which must be biosynthesized from any of several plant alkaloids, such as heliotrine (**IV**) or monocrotaline (**V**)[3–6], have been demonstrated to function as pheromones in some cases[4,7,8]. In recent studies of 2 Asian species of the genus *Creatonotos* (Arctiidae), *C. gangis* L. and *C. tran-*