Purification of Galactose-Binding Phytoagglutinins and Phytotoxin by Affinity Column Chromatography Using Sepharose

Recently we[1] reported that phytoagglutinins having different sugar specificity showed different agglutinability against several kinds of murine tumor cell lines and consequently these phytoagglutinins could be useful as a tool in the investigation of surface structure of the tumor cells. BURGER et al.[2,3] recently communicated that wheat germ agglutinin agglutinates the virally transformed cells more strongly than the normal cells and the phenomenon might closely relate with the malignancy of the cells. In order to have more precise and unambigous knowledge concerning this relation, the use of purified phytoagglutinin having high binding specificity with a definite sugar would be necessary.

This time, phytoagglutinins which specifically bind galactose were purified by use of affinity column chromatography through Sepharose, a galactose-containing polysaccharide. The purification of glucose-binding phytoagglutinin, such as concanavalin A, by use of Sephadex column chromatography has been reported by So and GOLDSTEIN[4].

Materials and methods. 100 g each of meal made from seeds of *Ricinus communis*, *Sophora japonica*, *Momordia charantia* or *Abrus precatorius* were extracted with 500 ml of phosphate-buffered saline (pH 6.8) at 4°C for 2 days. To the crude extract thus obtained was added $(NH_4)_2SO_4$ and the precipitate that occurred between 0.3 to 0.6 saturation of the salt was taken, and it was dissolved in 100 ml of the same saline and dialyzed against water. The dialyzed solution was centrifuged and the supernatant lyophilized (Fraction I). 500 mg of the Fraction I were dissolved in 40 ml of the phosphate-buffered saline. The solution was applied onto a column of Sepharose 4B and the column eluted with the same saline. The absorbance at 280 nm and the agglutinating activity against human O-erythrocytes of the effluents were estimated. After disappearance of the protein in the effluents, the elution media was substituted with 0.1 *M* galactose solution. The peak fraction which had high agglutinin activity was taken, dialyzed against water and the dialysate was lyophilized. The assay of the agglutinin activity against several cell lines was performed according to the previous paper[1], and the toxicity was expressed by concentrations of the phytoagglutinin, which caused 50% growth-inhibition (IC_{50}) of the *in vitro* culture of Yoshida ascites sarcoma cell[5].

Results. Agglutinins obtained from *Ricinus communis*, *Momordia charantia* and *Abrus precatorius* could be purified by affinity chromatography using Sepharose column, while the agglutinin of *Sophora japonica* was not retained on Sepharose (Figures 1 and 2). In the Table, the agglutinability of the purified phytoagglutinins against human O-erythrocytes and Yoshida sarcoma cells are summarized.

The agglutinins from *Ricinus communis* and *Abrus precatorius* showed high agglutinating activities against both kinds of cell lines, while that from *Momordia charantia* did not show the activity against Yoshida sarcoma cells.

This observation indicated that agglutination specificity of each agglutinin which was bound on Sepharose is not the same.

When thus purified phytoagglutinin from *Ricinus communis* was applied to gel-filtration through Biogel P150, it was separated into 2 peak fractions as is shown in Figure 2b. It is noteworthy that the faster elutable protein by the gel-filtration had high agglutinin activity, while the slower elutable 1 showed only slight activity, though it was retained on Sepharose (Figure 2c). On the other hand, the slower elutable protein showed higher toxicity against Yoshida sarcoma cells than the faster elutable protein.

Discussion. Convanavalin A, a glucose-binding phytoagglutinin, obtainable from Jack Bean *(Canavalia ensiformis)* has been shown to have the activity to transform human peripheral lymphocytes[6]; to be useful as a reagent in the research on relation between structures and functions of the cell surfaces. Furthermore, this agglutinin in

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Agglutination titer and cytotoxicity of phytoagglutinins obtained by ammonium sulfate fractionation and subsequent purification by affinity chromatography and gel filtration

Origin	Agglutinating activity						Cytotoxicity
	Human O-erythrocytes			Yoshida sarcoma cells			
	crude	Fract. 1	purified	crude	Fract. 1	purified	IC_{50} (μg/ml)
Abrus precatorius	512	1024	2048[a]	2048	2048	4096[a]	0.004[a]
Momordia charantia	512	1024	16384[a]	8	4	0[a]	>1000[a]
Sophora japonica	32	256	512[a]	1	8	16[a]	300[a]
Ricinus communis	1024	4096	8192[a]	512	1024	2048[a]	0.06[a]
Peak I			16384[b]			4096[b]	0.20[b]
Peak II			1024[b]			256[b]	0.02[b]

The agglutinin solution (16 mg/ml) were diluted serially with phosphate buffered saline, and agglutinating activity represent the highest dilution giving detectable agglutination. Fr. 1: see text. [a]after purification by affinity chromatography. [b]after successive purification by affinity chromatography and gel filtration.

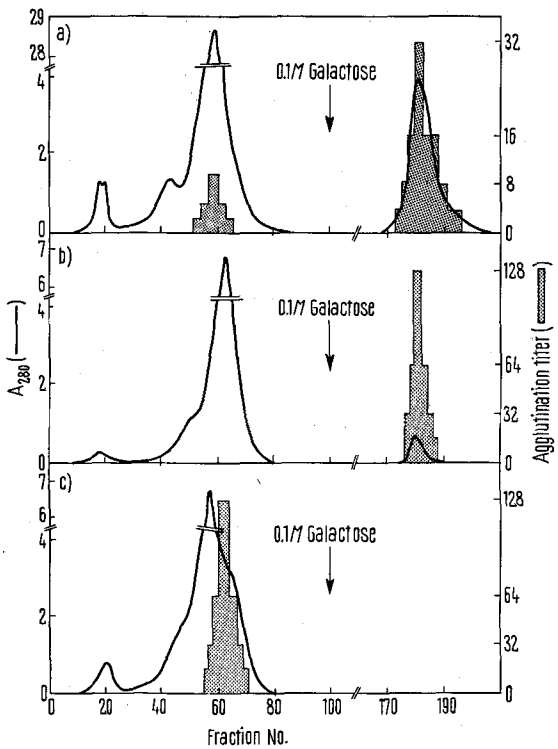

Fig. 1. Purification of galactose-binding agglutinin on Sepharose 4B. a) *Abrus precatorius*; b) *Momordia charantia*, and c) *Sophora japonica*.

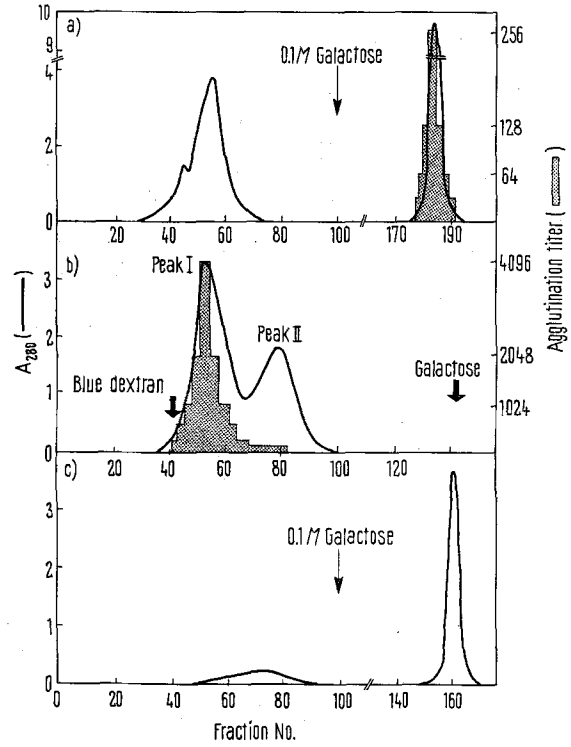

Fig. 2. Purification of *Ricinus communis* agglutinin on Sepharose 4B and Biogel P150. a) Sepharose 4B column chromatography of Fraction I, b) Biogel P150 column chromatography of purified agglutinin separated in a), and c) Sepharose 4B column chromatography of peak II in b).

a form that bound to Sepharose which was previously activated with BrCN, was utilized for the purification of glucose containing glycoproteins[7]. Such wide application of concanavalin A was possible because it had been purified directly by chromatography through Sephadex column which can specifically bind this agglutinin. This time, we could purify galactose-binding phytoagglutinins from several plants such as *Abrus precatorius*, *Ricinus communis* and *Momordia charantia* by use of Sepharose column chromatography. Thus, the purified galactose-binding phytoagglutinins could give more precise information concerning the relation between structure and function of cell surface.

The phytoagglutinin from *Sophora japonica* could not be retained on Sepharose column, though it can bind galactose. This might be explained by the specificity of this agglutinin to α-galactosyl structure involved in type-specific substance of human A and B erythrocytes which are agglutinatable by this agglutinin, as Sepharose has β-galactosyl structure.

It has long been known that phytoagglutinins are toxic to several cells or animals, but the identity between phytotoxin and phytoagglutinin has never yet been confirmed. Recently WALDSCHMIDT-LEITZ et al.[8] separated ricin and agglutinin from *Ricinus communis* and reported that the molecular weight of ricin is approximately one half of that of the agglutinin. The protein contained in Peak II fraction of Figure 2b in our experiment was obtained by gel-filtration from galactose binding agglutinin fraction of *Ricinus communis* and it might be identical with ricin in several aspects. Since this protein binds galactose but does not actively agglutinate erythrocyte and Yoshida sarcoma cells, there is a possibility that this protein is a monovalent agglutinin related to the agglutinin of *Ricinus communis*. Further research on this protein is now in progress.

Zusammenfassung. Phytoagglutinine, welche Galaktose spezifisch binden, wurden mit Chromatographie an Sepharose gereinigt. Zwei Fraktionen aus *Ricinus communis*-Agglutinin wurden durch Gelfiltration mittels Bio-gel getrennt: die früh eluierte Fraktion zeigte starke Agglutinationsaktivität und schwache Toxizität, während die später eluierte Fraktion eine schwache Agglutinationsaktivität bei starker Toxizität zeigt.

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