THE MECHANISM OF THE CYTOTOXICITY OF RICINUS COMMUNIS PHYTOAGGLUTININ TOWARD RAT ASCITES TUMOR CELLS

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Summary: Ricinus communis phytoagglutinin having a high cytotoxicity toward rat ascites tumor cells inhibited the protein and DNA synthesis, but not RNA synthesis, of the cultured cells in vitro. Phytoagglutinin covalently attached to large polymers of Sepharose also showed the inhibition of DNA synthesis and the inhibition was counteracted by addition of galactose. The results indicate that interaction of Ricinus communis agglutinin with surface membrane may cause the cellular metabolic alterations.

It is known that some phytoagglutinins which bind to sugar moieties on the cell surface membrane exhibit cytotoxicity toward tumor cell lines[1-4]. Previously we prepared highly purified phytoagglutinins, which are specific for terminal D-galactose-like residues, by use of a specific affinity column chromatography of Sepharose, and observed that the phytoagglutinins obtained from Ricinus communis and Abrus precatorius possess strong cytotoxicity toward rat ascites cells cultured in vitro[5]. This report deals with the mechanism of the cytotoxicity of the Ricinus communis phytoagglutinin toward the rat ascites tumor cells.

Materials and Methods

Ricinus communis agglutinins were purified as described previously[5]. Thus, the crude extract of Ricinus communis beans was charged on a column of Sepharose 4B and the protein peak obtained by eluting with 0.1 M galactose was named RCS. The RCS fraction was further subdivided into two protein peaks by filtration through a column of BioGel P150. The first peak fraction which had a high agglutinating activity toward human O

erythrocytes and the second fraction which had a weak agglutinating activity were named RCS-I and RCS-II, respectively.

The agglutinin was covalently attached to Sepharose according to the method of Kristiansen et al.[6] Sepharose (40 ml) was activated by CNBr and was reacted with RCS (400 mg). The amount of protein which conjugated with Sepharose was 99.4 % of the protein added. The RCS-Sepharose complex was washed with 2 l of physiological saline (PS) containing 0.1 M lactose and with 10 l of PS, and then stored at 4°. Immediately before use, it was further washed four times with PS.

Thymidine-^{3}H (6.7 Ci/mmole), uridine-^{3}H (14 Ci/mmole) and valine-^{3}H (2.88 Ci/mmole) were purchased from New England Nuclear Corp.

Yoshida ascites sarcoma (YS) cells were harvested from donor rats (adult female Donryu strain) 3-4 days after intraperitoneal inoculation of 10^6 parent cells. The cells were cultured in assay tubes in serum-free 199 medium or serum-free minimal essential medium (MEM) which was devoid of valine. To the harvested YS cells (2×10^5 cells) in 0.9 ml of culture medium was added 0.3 ml of phytoagglutinin diluted with the same medium and incubated at 37°. Each of the radio isotopic compound was diluted to 10 μCi/ml with phosphate-buffered saline (pH 7.4) and 0.1 ml of the solution was added to the culture medium. The reaction was terminated by adding ice-cold PS. Radioactivity of Cl_3CCOOH-insoluble fraction of the reaction mixture was determined by the method of Takeishi et al.[7].

Results and Discussion

The effect of RCS on the DNA, RNA and protein synthesis of YS cells cultured in vitro was studied and the results are given in Table I. When YS cells were incubated with RCS, the amount of DNA synthesized during the incubation was almost the same at 2 hr as the control, but decreased significantly at 3 hr. On the other hand, the RNA synthesis was not affected even at 3 hr. A strong inhibitory effect of RCS was observed

Table I

YS cells were cultured in 199 medium with or without RCS (2.5 μg/ml). Thymidine-^{3}H, uridine-^{3}H or valine-^{3}H was added and the radioactivity incorporated into the acid insoluble fraction of the cells was measured. Each value represents an average of results of three experiments differing from the mean by less than 10 %.

TABLE I

Effects of Ricinus communis phytoagglutinin

on the macromolecular synthesis of Yoshida ascites cells

		Radioactivity incorporated (cpm) at incubation time of			
		0.5 hr	1 hr	2 hr	3 hr
DNA synthesis	− RCS	230	693	1,759	3,277
	+ RCS	251	813	1,532	1,964
RNA synthesis	− RCS	1,524	3,239	7,792	15,111
	+ RCS	2,202	3,801	6,916	15,365
Protein synthesis	− RCS	87	130	265	289
	+ RCS	54	94	143	161

for the protein synthesis: a significant inhibition was noted already from the early stage of the incubation. It thus seemed that RCS exerted inhibitory effect on protein synthesis first, and then inhibited DNA synthesis. These effects may constitute the mechanism of the cytotoxic action of Ricinus communis agglutinins.

Since RCS can agglutinate YS cells[5], it will have the binding sites to galactose moieties on the cell surface membrane and therefore it is possible that an interaction of the agglutinin with surface membrane structure is the trigger of the cytotoxicity. To investigate this possibility we prepared the phytoagglutinin covalently bound to large polymers of Sepharose which cannot enter the YS cells and studied whether the derivative has the cytotoxicity or not.

Table II

YS cells were incubated in 199 medium with the phytoagglutinin-Sepharose complex in the presence and absence of sugar (3 mg/ml) for 3 hr at 37°. The mixture was then pulse-labeled for 1 hr with thymidine-^{3}H. The radioactivity incorporated in the control (– RCS) experiment was 1.25×10^4.

TABLE II

Effect of phytoagglutinin-Sepharose complex

on DNA synthesis of Yoshida ascites cells.

Sugar added	Radioactivity incorporated (% of control)				
	RCS-Sepharose (μl/ml)			Con A-Sepharose (μl/ml)	
	0.015	0.15	1.5	0.15	1.5
None	94	72	25	95	82
galactose	98	99	98	–	–
glucose	93	67	33	–	–

Microscopic examination showed that the YS cells can be adsorbed on the surface of the RCS-Sepharose complex (data not shown). In the presence of galactose this adsorption did not take place, whereas in the presence of glucose no inhibition was observed in the adsorption, indicating that the polymer-bound RCS can still bind to the D-galactose-like residue on the cell surface in a specific manner.

As Table II shows, the amount of DNA synthesized in the YS cells was decreased by the presence of the RCS-Sepharose complex, and it was dependent on the amount of the complex added. Furthermore, the inhibitory action of the complex was counteracted by the presence of galactose, but not by the presence of glucose.

The possibility was considered that the agglutinins may be detached from the polymer during incubation and the resulting free RCS caused the inhibition. This possibility was excluded by the fact that little inhibitory activity was present in the solution obtained by filtering the reaction mixture

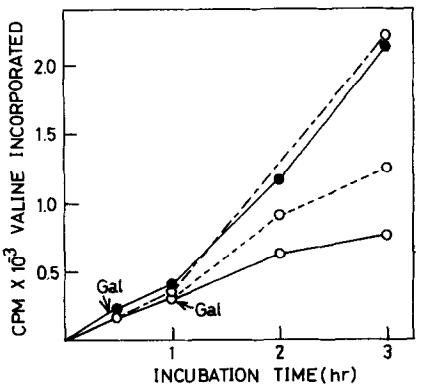

Fig. 1 Effect of galactose on the protein synthesis of YS cells treated with RCS-II.

YS cells were cultured in MEM medium which was devoid of valine with or without RCS-II (2.5 μg/ml). To the medium, galactose was added to 0.1 M concentration 30 min or 1 hr after the start of the incubation.
●—●: control (– RCS-II); O—O : + RCS-II; O- - -O : + RCS-II, + galactose at 30 min; O---O : + RCS-II, + galactose at 1 hr

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through millipore filter. Thus, the mixture containing YS cells and RCS-Sepharose in varying amounts was incubated for 3 hr at 37° and the filtrate collected from each mixture was examined for its ability to inhibit the DNA synthesis: [DNA synthesis, % of control] /[amount of RCS-Sepharose in the mixture before filtration] ; 100 %/0.15 (μl/ml), 90.5 %/1.5 (μl/ml). Further, the filtrate obtained likewise from the medium containing only RCS-Sepharose complex had no inhibitory effect.

Concanavalin A-Sepharose complex was also prepared by the same way as RCS-Sepharose complex ; the phytoagglutinin is known to be specific for D-glucose- and D-mannose-like residues. This polymer-bound agglutinin inhibited the DNA synthesis only to a small extent, indicating that the effect observed with RCS-Sepharose was not due to an artifact produced in the process of preparing the polymer bound agglutinin.

RCS-II has about ten times higher cytotoxicity toward YS cells than RCS-I[5], and we examined if the protein synthesis could be rescued from the inhibitory effect of RCS-II by addition of galactose. As illustrated in Fig. 1,

when galactose was added to the RCS-II treated cells 30 min or 1 hr after the start of the incubation, the inhibitory effect of RCS-II was hindered. When galactose was added to the control YS cells, there was no stimulation of the protein synthesis.

On the basis of these results it is tempting to suggest that Ricinus communis agglutinin exerts its cytotoxicity toward YS cells by attaching to the sugar chains containing D-galactose-like residues on the cell membrane. Although it has recently been shown[8] that ricin, the Ricinus communis toxin without hemagglutinating activity, can directly inhibit cell-free protein synthesis, it does not necessarily relate to its in vivo action. Lin et al.[9] have demonstrated that ricin has a strong cytotoxicity toward murine ascites tumor cells and inhibits the protein and DNA synthesis. However, it is not known whether or not our phytoagglutinin preparation contains the toxin Lin et al. have isolated. It should also be noted that during the preparation of this manuscript Nicolson and Blaustein[10] reported purification of phytoagglutinins from Ricinus communis by the same method as ours[5].

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