EFFECT OF *RICINUS COMMUNIS* LECTINS ON THE MEMBRANE FLUIDITY OF HUMAN PERIPHERAL LYMPHOCYTES

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SUMMARY

The mobility of *Ricinus communis* lectins bound to lymphocyte cell surface was determined by fluorescence polarization of fluorescein-labeled lectins. *R. communis* hemagglutinin and *R. communis* toxin have high mobility. Furthermore, the change of membrane fluidity upon binding of the lectins to lymphocytes was measured by fluorescence polarization of fluorescent hydrocarbon embedded in the membrane. The hemagglutinin, the toxin and its binding subunit apparently increased the membrane fluidity. The hemagglutinin was also found to have mitogenic activity against human peripheral lymphocytes.

Two lectins have been isolated from *Ricinus communis* seeds (1-3). One is a molecular weight 120,000 lectin which has strong hemagglutinating activity but relatively weak cytotoxic activity (*R. communis* hemagglutinin). In contrast, the other, which has a molecular weight of 60,000, shows strong cytotoxic activity but very weak hemagglutinating activity (*R. communis* toxin). The latter lectin has recently been reported to consist of two subunits (4,5), i.e. the non-toxic binding subunit (B-chain) which is capable of binding to galactose-containing carbohydrates, and the toxic subunit (A-chain) which lacks binding sites for the carbohydrates. In this paper, we will describe the effect of *R. communis* hemagglutinin, *R. communis* toxin and its subunits on the membrane fluidity of human peripheral lymphocytes.

MATERIALS AND METHODS

R. communis seeds were obtained from local market. *R. communis* hemagglutinin and *R. communis* toxin were purified according to the method of Tomita *et al.* (1). *R. communis* hemagglutinin used for mitogenic assays against human peripheral lymphocytes was further purified by column chromatography on DE-

52 (Whatman) according to Olsnes *et al.* (6). The subunits of *R. communis* toxin were separated and purified by the method of Olsnes *et al.* (4).

R. communis lectins were conjugated with fluorescein isothiocyanate by the method of Smith and Hollers (7). The binding reaction of the fluorescent lectin thus obtained to lymphocytes was carried out by the method of Inbar *et al.* (8) as follows: Purified lymphocytes (10^7 cells/ml) in 0.01 M phosphate buffered saline, pH 7.2, were incubated with a 1 : 1 mixture of a F-lectin* (0.5 mg/ml) and the same but unlabeled lectin (0.5 mg/ml) for 15 min. at 37°. The cells were then washed with the phosphate buffered saline to remove the free lectin. After dilution with the phosphate buffered saline to give a final concentration of 5×10^6 cells/ml, the fluorescence measurement was immediately performed.

Labeling of the lymphocytes with 1,6-diphenyl-1,3,5-hexatriene (Tokyo Kasei Co.) was performed by the method of Inbar *et al.* (9). To 2 ml of the DPH*-labeled lymphocyte suspension in the phosphate buffered saline (5×10^6/ml) was added 0.2 μg of a lectin and incubation was performed at 37° for 30 min. The temperature was then adjusted to 25° in a water bath, and the suspension was immediately used for fluorescence measurements.

Fluorescence polarization and intensity were measured with a fluorescence spectrophotometer (Hitachi model MPF-2A) with a rotating polarizer. The wavelengths of polarized bands used for exitation were 435 nm for F-lectins and 366 nm for DPH. The emission intensities polarized parallel and perpendicular to the direction of polarization of the exitation beam were measured, and by use of these values and the Perrin equation (10), the rotational relaxation time of a free F-lectin, ρ_0, and the rotational relaxation time of a F-lectin bound to lymphocytes, ρ, were calculated according to Inbar *et al.* (8). The parameter, ρ_0/ρ, was defined as "the degree of mobility" that extends from 0 (immobilized state) to 1 (fully mobile state).

* Abbreviations: F-lectin, fluorescent lectin; DPH, 1,6-diphenyl-1,3,5-hexatriene.

The change of microviscosity of the surface membrane lipid layer upon binding of a lectin was determined by measuring the fluorescence polarization of DPH embedded in the lymphocyte membrane by the methods of Shinitzky *et al.*(11), Cogan *et al.* (12) and Shinitzky and Barenholz (13).

Human peripheral lymphocytes purified by the method of Kawaguchi *et al.* (14) were cultured by the method previously described (15). Radioactivity assay of [5-^{3}H]uridine incorporation was performed by adding [5-^{3}H]uridine (1 μCi, the Radiochemical Centre, England) to each culture tube (3-4 x 10^5 lymphocytes) after 24 hr culture. Twenty-four hours later, the cells were processed as described previously (16), and the counts per minutes of each sample were determined.

RESULTS AND DISCUSSION

Recently, it has been shown that the mitogenic F-lectins bound to lymphocytes have a high degree of mobility (usually 0.70-0.85) close to that of free F-lectin in PBS, while the non-mitogenic F-lectins bound to lymphocytes have a relatively low degree of mobility (usually 0.20-0.35) compared to the corresponding free F-lectin (8,17). As shown in Table I, fluorescein-labeled *R. communis* hemagglutinin bound to lymphocytes show a high degree of mobility which is almost the same degree as the mitogenic lectins. Also in the case of fluorescein-labeled *R. communis* toxin bound to lymphocytes, the degree of

TABLE I

The degree of mobility of *R. communis* lectins bound to human peripheral lymphocytes[a].

Lectins	ρ_0	ρ	ρ_0/ρ
	n sec		
R. communis hemagglutinin	19.7	29.2	0.68
R. communis toxin	10.0	21.9	0.46

[a] Average value of duplicate experiments. Experimental details are given in the text.

TABLE II

Effect of *R. communis* lectins on degree of fluorescence polarization (P) and the apparent microviscosity ($\bar{\eta}$) obtained with human peripheral lymphocytes labeled with DPH at 25°[a].

Lectins	P	$\bar{\eta}$(poise)
R. communis hemagglutinin	0.269	3.2
R. communis toxin	0.270	3.3
B-chain	0.262	3.1
A-chain	0.282	3.7
None	0.278	3.5

[a] Average value of duplicate experiments. Experimental details are given in the text.

mobility is significantly higher than the non-mitogenic lectins. Since the thermal mobility of cell surface receptors might be controlled by the fluid state of the membrane lipid layer, the change of microviscosity of the surface membrane lipid layer upon binding of *R. communis* lectins was determined by

TABLE III

Effect of *R. communis* hemagglutinin on the [5-^{3}H]uridine incorporation by human peripheral lymphocytes[a].

Lectins (μg/ml)		[5-^{3}H]Uridine incorporation (cpm)
R. communis hemagglutinin	(10)	420
	(1)	590
	(0.1)	740
	(0.01)	1,940
	(0.001)	980
	(0.0001)	770
Concanavalin A	(10)	3,050
None		710

[a] Average value of five experiments. Experimental details are given in the text.

measuring the fluorescence polarization of DPH which could be easily introduced into the hydrocarbon region of the membrane lipid layers. The results summarized in Table II indicate that the binding of *R. communis* hemagglutinin, *R. communis* toxin or one of its subunits (B-chain) to the membrane apparently increased the membrane fluidity. The other subunit (A-chain) of the toxin, which is devoid of binding property to the membrane, does not affect the membrane fluidity. These results may suggest that the initial step in the toxicity of *R. communis* toxin is the binding of its binding subunit (B-chain) to cell membrane followed by the increase of the membrane fluidity which in turn enables the toxic subunit (A-chain) to enter the cell to exert its toxic effect. Furthermore, in order to test the mitogenic activity of *R. communis* hemagglutinin, its effect on the incorporation of [5-^{3}H]uridine by human peripheral lymphocytes after 48 hr culture was determined. As shown in Table III, the hemagglutinin enhanced the incorporation at the concentration as low as 0.01 μg/ml. Higher concentration of the hemagglutinin decreased the incorporation to even a lower level than the control value, possibly due to the toxicity which tenaciously remained in the hemagglutinin in spite of its apparent homogeneity by ultracentrifugation and disc gel electrophoresis.

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