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TEMPERATURE-SENSITIVE AGGLUTINABILITY OF HUMAN ERYTHROCYTES BY LECTINS

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SUMMARY

Lectins from *Phaseolus vulgaris*, wheat germ, and soybean, agglutinated trypsinized human A, B and O erythrocytes to a higher extent than nontrypsinized erythrocytes, and concanavalin A only agglutinated trypsinized erythrocytes. The three types of L-fucose-binding molecules from *Lotus tetragonolobus* agglutinated trypsinized A, B and O erythrocytes, whereas nontrypsinized O but not A and B erythrocytes were agglutinated only by types of molecules with four binding sites. The erythrocytes were agglutinated by the lectins from wheat germ and soybean at 24 °C and at 4 °C, and by the lectins from *Phaseolus*, *Lotus* and concanavalin A only at 24 °C. This indicates that agglutination by these three lectins requires a temperature-sensitive activity on the cell surface membrane.

INTRODUCTION

Some proteins and glycoproteins can agglutinate various types of red blood cells. Among such molecules are the lectins[1] from *Phaseolus vulgaris*, *Lotus tetragonolobus*, wheat germ, soybean and concanavalin A[2–5]. It has been shown that concanavalin A[7–10] and the lectins from wheat germ[11] and soybean[12,13] can agglutinate malignant transformed fibroblasts. The malignant cells are agglutinated by the lectins from wheat germ and soybean at 24 °C and at 4 °C, but by concanavalin A only at 24 °C. This indicates that the sites for concanavalin A, but not the sites for the other two lectins, are associated with a specific temperature-sensitive activity on the surface membrane[14]. A temperature-sensitive activity is also associated with the activation of lymphocytes to undergo DNA synthesis and form blast cells (H. Ben-Bassat, M. Imber and L. Sachs, unpublished results). The present experiments were undertaken to compare the agglutinability of trypsinized and nontrypsinized human A, B and O erythrocytes by the lectins from *Phaseolus*, *Lotus*, wheat germ, soybean and concanavalin A; to determine the agglutinating activity of the three types of L-fucose-binding molecules[15] from *Lotus*; and to determine whether there is a difference in the temperature sensitivity of agglutination by these five lectins.

MATERIALS AND METHODS

Human erythrocytes

A, B and O human erythrocytes were freshly collected in 0.38% sodium citrate.

The plasma was then removed and the erythrocytes were washed 3 times with phosphate-buffered saline (pH 7.2). For trypsinization, 10^9 cells in 10 ml buffered saline were incubated with 10 ml 0.25% trypsin solution (Difco laboratories, 1:300) for 30 min. at 37 °C, and the cells washed 3 times with buffered saline.

Agglutination assay

To test for agglutination, 0.5 ml lectin diluted in buffered saline was mixed with 0.5 ml erythrocyte suspension ($5 \cdot 10^6$–$8 \cdot 10^6$ cells per ml) in buffered saline in a 35-mm petri dish[7]. The density and size of aggregates was scored in a scale from − to ++++ after 30 and 90 min of incubation at 24 ± 2 °C or 4 ± 2 °C for trypsinized and nontrypsinized cells, respectively. Trypsinized erythrocytes always agglutinated more rapidly than nontrypsinized cells. The concentrations of lectins in the figures are given as dilutions for the lectin from wheat germ and as μg/ml for the other lectins. The results were reproducible with a variation of one (+). The data in the figures are an average of five experiments.

Lectins

Concanavalin A (Miles–Yeda); soybean[5,6] and *Lotus*[15,17] were purified lectins, and the lectins from *Phaseolus* (Difco laboratories) and wheat germ[11,18] were crude extracts. The lectin from *Lotus tetragonolobus* seeds (Thompson and Morgan Ltd, Ipswich, England) was first purified by affinity chromatography[17] using agarose epsilon amino caproyl fucosamine (Miles-Yeda). The three types of L-fucose-binding molecules, A, B and C, were then separated on a DEAE-cellulose column[15,16] and identified on cellulose acetate electrophoresis with the microzone system (Bechman Model R-101). The purified molecules were stored at −20 °C for not more than 4 weeks.

RESULTS

Agglutination by the lectins from wheat germ, soybean, Phaseolus and concanavalin A

Although glycolipids from group A inhibited agglutination of tumor cells by wheat germ agglutinin more strongly than glycolipids from B and O[19], in the present experiments the lectin from wheat germ agglutinated A, B and O erythrocytes to the same extent, and the lectin from soybean agglutinated group A to a higher extent than B and O[5] (Table I). Trypsinized erythrocytes (Fig. 1 right) were more agglutinable

TABLE I

AGGLUTINABILITY OF NONTRYPSINIZED A, B AND O ERYTHROCYTES BY LECTINS AT 24 °C

The lectin concentrations are given as μg/ml, except for the lectin from wheat germ which is given as dilutions.

Type of lectin	*Lectin concentration*	*Agglutination of blood groups*		
		A	*B*	*O*
Phaseolus vulgaris	250	+++	+++	++
Lotus tetragonolobus A and C	250	−	−	+++
Concanavalin A	250	−	−	−
Wheat germ	1:32	++++	++++	++++
Soybean	250	+++	+	++

than nontrypsinized cells (Fig. 1 left). Trypsinized and nontrypsinized erythrocytes were agglutinated by both lectins at 24 °C and 4 °C (Fig. 1). The addition of 0.1 M *N*-acetyl-D-glucosamine and *N*-acetyl-D-galactosamine to wheat germ and soybean respectively, completely inhibited the agglutination. The lectin from *Phaseolus* agglutinated trypsinized and nontrypsinized A, B and O erythrocytes, whereas concanavalin A did not agglutinate A, B and O cells (Table I) unless they were treated with trypsin. Agglutination by both lectins was temperature sensitive (Fig. 2). Addition of 0.1 M α-methyl-D-mannopyranoside and 0.2 M *N*-acetyl-D- galactosamine to

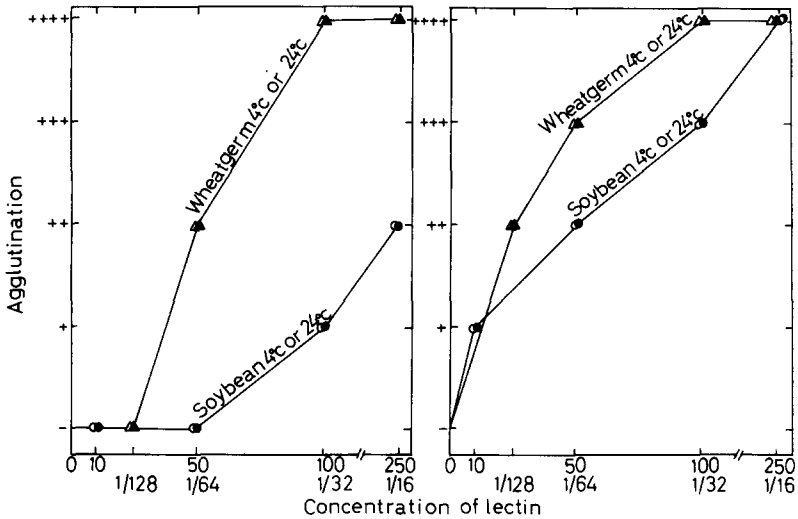

Fig. 1. Non-temperature-sensitive agglutination of O erythrocytes by the lectins from wheat germ and soybean. Left: nontrypsinized erythrocytes after 90 min incubation. Right: trypsinized erythrocytes after 30 min incubation. Open symbols, 4 °C; closed symbols, 24 °C.

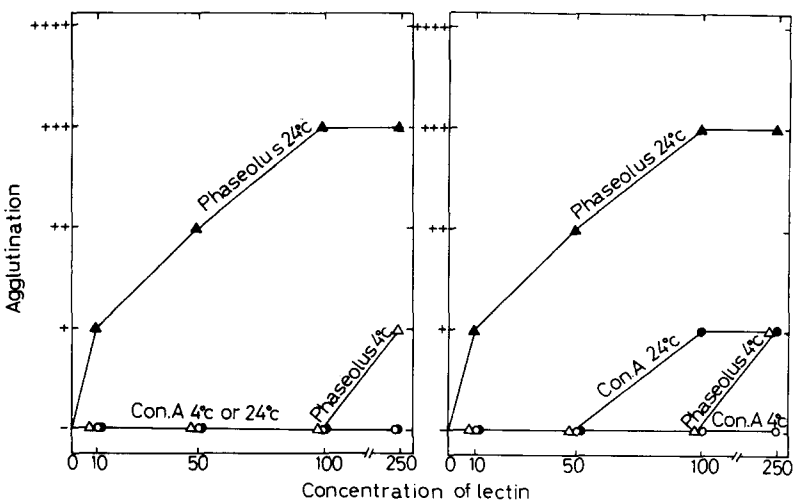

Fig. 2. Temperature-sensitive agglutination of O erythrocytes by the lectins from *Phaseolus* and concanavalin A (Con. A). Left: nontrypsinized O erythrocytes after 90 min incubation. Right: trypsinized O erythrocytes after 30 min incubation. Open symbols, 4 °C; closed symbols, 24 °C.

concanavalin A and to the lectin from *Phaseolus* respectively, completely inhibited the agglutination by concanavalin A and decreased the agglutination by *Phaseolus*.

Agglutination by the 3 types of L-*fucose-binding molecules from Lotus*

Three types of L-fucose-binding molecules, A, B and C[15], were purified by affinity chromatography[17] and separated by a DEAE-cellulose column[15]. Nontrypsinized group O erythrocytes, but not A and B, were agglutinated by molecules of

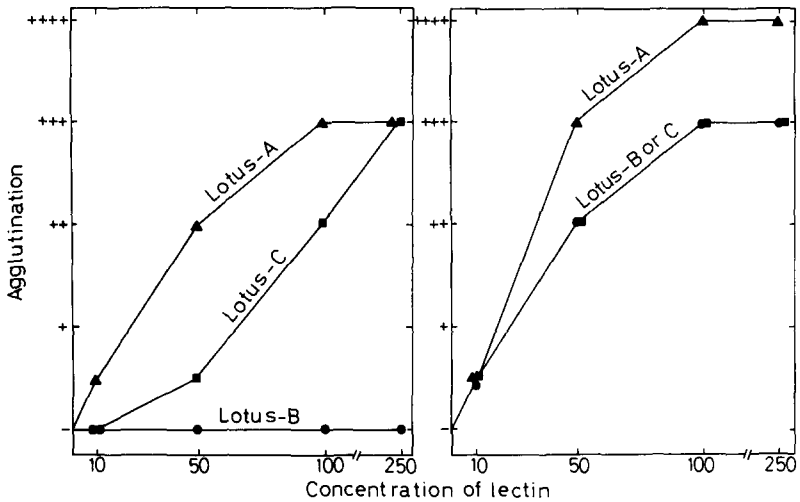

Fig. 3. Agglutination by the three types of L-fucose-binding molecules, A, B and C, from *Lotus* at 24 °C. Left: nontrypsinized O erythrocytes after 90 min incubation. Right: trypsinized O erythrocytes after 30 min incubation. Similar results were obtained with trypsinized A and B erythrocytes.

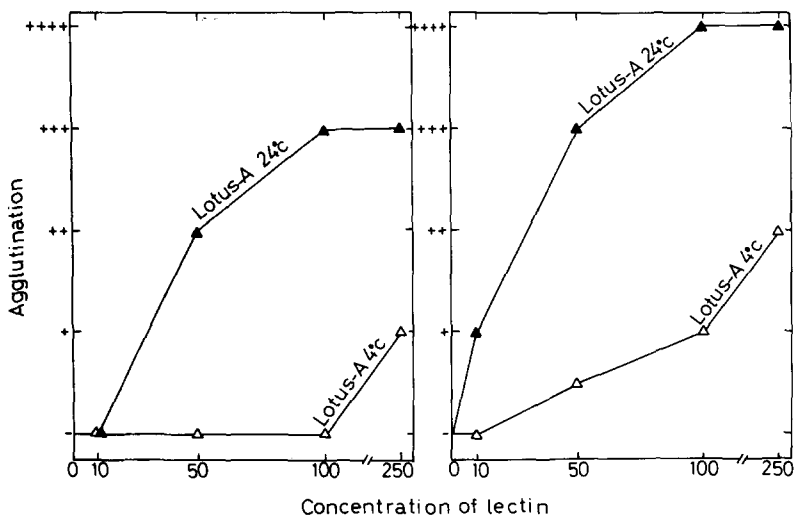

Fig. 4. Temperature-sensitive agglutination by A molecules from *Lotus*. Left: nontrypsinized O erythrocytes after 90 min incubation. Similar results were obtained with C molecules. Right: trypsinized O erythrocytes after 30 min incubation with A, B and O erythrocytes with A, B and C molecules.

types A and C (Table I) but not by molecules of Type B (Fig. 3 left). However, trypsinized A, B and O erythrocytes were agglutinated by all three types of molecules (Fig. 3, right). The agglutination of trypsinized and nontrypsinized erythrocytes was temperature sensitive (Fig. 4) and was completely inhibited by 0.01 M L-fucose. The lectin containing molecules A and B[16] (Miles-Yeda) that had been stored at −20 °C for more than 6 months did not agglutinate nontrypsinized O erythrocytes, and also gave a lower degree of agglutination with trypsinized A, B and O erythrocytes. This indicates, that the agglutinating activity of the L-fucose-binding lectin from *Lotus* decreased after storage at −20 °C. Freshly prepared A and C molecules also do not agglutinate any of the normal or transformed fibroblasts tested[16] with the exception of trypsinized normal hamster fibroblasts, and freshly prepared B molecules did not agglutinate even these cells.

DISCUSSION

The present results have shown that of the five lectins tested, the agglutination of human erythrocytes by the lectins from *Phaseolus*, *Lotus* and concanavalin A, and not by the lectins from wheat germ and soybean, was temperature sensitive. This indicates that erythrocyte agglutination by three of these lectins requires a temperature-sensitive activity on the surface membrane. Requirement for a temperature-sensitive activity on the cell surface associated with concanavalin A but not with wheat germ and soybean lectins have also been found in fibroblasts[14] and in the activation of lymphocytes. This temperature-sensitive activity may be a metabolic activity[14] and/or a nonmetabolic temperature dependent structural rearrangement. The gain or increase of agglutinability after trypsin treatment did not change the temperature sensitivity of the agglutination by any of the lectins. Experiments with the lectin from *Lotus* have shown, that nontrypsinized O erythrocytes can be agglutinated by two types of molecules that have been reported to have four binding sites for L-fucose but not by a type of molecule with two binding sites[15]. It will be of interest to determine the number of binding sites per molecule that are required for agglutination by other lectins.

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