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THE EFFECT OF TEMPERATURE ON CONCANAVALIN A-MEDIATED AGGLUTINATION OF CELLS WITH RIGID RECEPTORS

V.K. JANSONS and J.A. PAKTOR

Department of Microbiology, College of Medicine and Dentistry of New Jersey, New Jersey Medical School, Newark, N.J. 07103 (U.S.A.)

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Summary

The agglutination of a yeast, *Candida albicans*, by concanavalin A has been described. The agglutination was cell-number dependent. Prolonged incubation (60 min) was needed to reach maximum agglutination at 37°C. The rate but not the extent of agglutination was temperature dependent. The dimeric forms of concanavalin A, obtained either at low pH or after succinylation, agglutinated the yeast cells as well as the tetramer. Temperature changes affected the agglutination of yeast cells by dimers and by tetramers to the same extent.

Introduction

The agglutination of mammalian cells by lectins, certain plant and invertebrate proteins, has been investigated intensively during recent years [1,2]. As discussed in detail in several reviews, agglutination of mammalian cells is a complex process, influenced by many factors, including cell surface charge, rigidity and receptor mobility [3,4]. Agglutination is temperature dependent; furthermore, it has been reported that within the same cell line some lectins show temperature-dependent agglutination while some others do not [5]. The lectin receptors in the plasma membrane are carbohydrate moieties of as yet incompletely characterized heterogeneous glycoproteins and, possibly, glycolipids [2—4, 6—8].

Lectins also bind to carbohydrates in cell walls of microorganisms [1,2,9]. A lectin, concanavalin A, which shows temperature-dependent agglutinability of mammalian cells, binds to a yeast, *Candida albicans*. The concanavalin A receptor in this yeast is an α-linked mannan side chain emanating from a backbone which is a structural component of the rigid cell wall [10—12].

In the present communication we describe concanavalin A-mediated agglu-

tination of this yeast. We also show that the effect of temperature on agglutination, which has been explained on the basis of receptor mobility in mammalian cells [5] can also be demonstrated in this system with a rigid, nonmobile receptor.

Concanavalin A was purchased from Pharmacia Fine Chemicals. Succinylation of concanavalin A was carried out as described by Gunther et al. [13]. *C. albicans* (ATCC 10261) was grown overnight at 37°C with gyratory agitation in a medium containing 1% yeast extract (Difco) and 1% glucose. Under these conditions the dimorphic fungus grows entirely in the yeast form [14]. The stationary culture cells were heat killed by autoclaving (15 min, 121°C) or washed rapidly, fixed for 5 h in 2.5% glutaraldehyde, then washed again in phosphate-buffered saline at pH 7.3 or, when indicated in 0.145 M NaCl adjusted to pH 4.0 with 0.05 M sodium acetate buffer. Routinely, cells were handled sterilely, stored at 5°C and washed again in appropriate buffers prior to use. Cell counts were done in a hemocytometer. Agglutination assays were set up in plastic tubes since at acid pH the concanavalin A-yeast cell complexes tended to adhere tightly as a layer to the walls of glass tubes. 2-fold dilution series of concanavalin A were prepared. Reaction mixtures, containing the indicated number of cells and μg of concanavalin A in 0.1 ml of buffer, were incubated in constant temperature reciprocal shaker (80 oscillations/min). Agglutination was scored between 0 and +4 as previously described for mammalian cells [15] except that the whole reaction mixture was gently poured onto the slide for microscopic examination. Scoring was done on coded triplicate samples.

Concanavalin A readily agglutinated the yeast cells (Fig. 1). There was no difference between the agglutination of cells killed by heat or by glutaraldehyde treatment. In contrast to mammalian cells, the cell-lectin aggregates were fragile. The concentration of concanavalin A necessary to agglutinate cells depended on cell number and incubation time (Table I). While very dense cell suspensions were difficult to score, low concentrations agglutinated less well. The optimum cell number was found to be 10^8 cells/ml. The concentration of concanavalin A necessary for half maximal agglutination (+2) with this cell number was 12.5 μg/ml at 37°C. The full extent of agglutination was reached in 60 min. It was surprising to note this relatively long time period needed for maximal agglutination with low concentrations of concanavalin A or at low cell densities even in this rigid receptor system. Similar results have been ob-

TABLE I

EFFECT OF CELL NUMBER AND TIME OF INCUBATION ON THE AGGLUTINATION OF *C. ALBICANS* BY CONCANAVALIN A AT pH 7.3, 37°C

Cells/ml	Time (min)				
	5	15	30	60	180
10^8	100 *	50	25	12.5	12.5
$5 \cdot 10^7$	>500	250	100	50	25
10^7	>500	>500	>500	250	250

* Concentration of concanavalin A (μg/ml) necessary for half maximal agglutination.

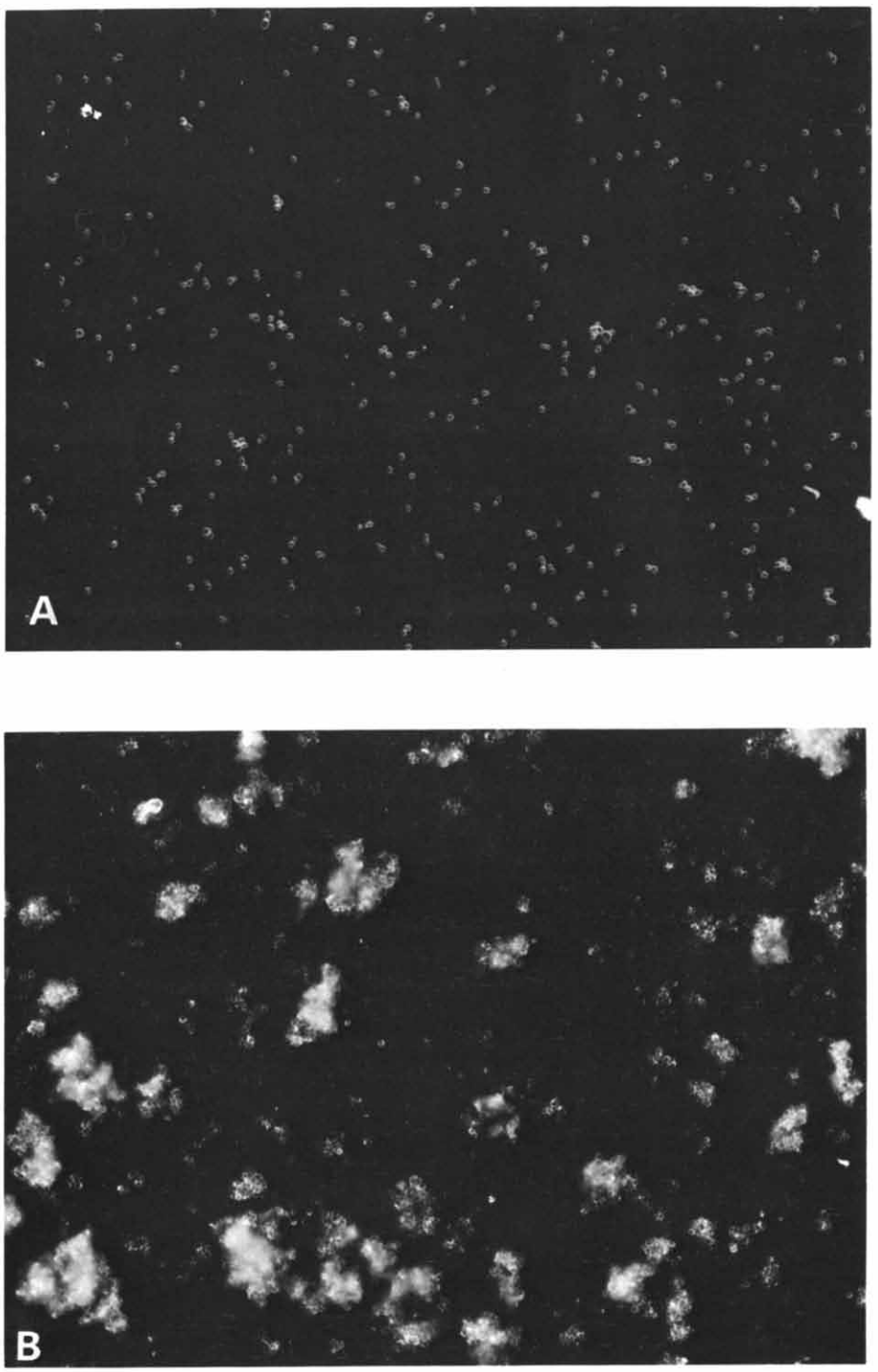

Fig. 1. Concanavalin A-mediated agglutination of *C. albicans*. (A) Control. (B) Yeast cells agglutinated by concanavalin A (50 μg/ml). Dark field microscophy X120.

TABLE II

EFFECT OF TEMPERATURE ON THE AGGLUTINATION OF *CANDIDA ALBICANS* BY CONCANAVALIN A

	Temperature (°C)		
Time	4	15	37
10 min	>500 *	100	25
60 min	500	50	12.5
120 min	250	12.5	12.5
16 h	25	12.5	12.5

* Concentration of concanavalin A (μg/ml) necessary for half maximal agglutination of 10^8 cells/ml.

tained with erythrocytes and the importance of considering cell numbers and time of scoring when comparing results from different laboratories has been emphasized [16]. Agglutination was inhibited in the presence of the specific inhibitor monosaccharides: α-D-methylmannoside and α-D-methylglucoside. Full inhibition of half maximal agglutination was achieved by preincubation of concanavalin A (12.5 μg/ml) with $2 \cdot 10^{-3}$ M α-D-methylmannoside or $4 \cdot 10^{-3}$ M α-D-methylglucoside.

Lowering the temperature of incubation increased the time needed for maximal agglutination at a given concanavalin A concentration. Thus, the rate rather than the extent of agglutination was affected. Upon prolonged incubation cells agglutinated almost equally well at 4, 15 and 37°C (Table II). Noonan and Burger [17] have reported that mouse fibroblasts bind more concanavalin A at 22°C than at 0°C. However, the amount of concanavalin A bound at 0°C was sufficient to agglutinate cells at 22°C [17]. We obtained similar results although direct binding studies were not carried out. When yeast cells were preincubated at 4°C with varying concentrations of concanavalin A, then washed three times in phosphate-buffered saline at 4°C and reincubated at 37°C, the agglutination titers were similar to those obtained with cells incubated directly at 37°C without washing.

As already mentioned, the concanavalin A-mediated, temperature-dependent agglutination of viable mammalian cells has been attributed to receptor mobility or some other temperature-sensitive membrane function [5]. These factors, or course, cannot contribute to the temperature effect observed in the present system which consists of rigid and metabolically inert particles. However, it has also been suggested that the effect of temperature in concanavalin A-mediated agglutination might be due to dissociation of the lectin molecule [18,19]. While at 37°C concanavalin A exists predominantly as a tetramer at neutral pH, with the lowering of the temperature there is an increase of dissociation of tetramers into dimers which would agglutinate cells less well than the tetramers [18,19].

This suggestion was further explored in our system by setting up agglutination assays under two additional conditions which also favor the dimer form: at pH lower than 5.6 and after succinylation [13]. The specificity of agglutination in these systems was ascertained by hapten inhibition.

The rigidity of the yeast cell wall permitted to study the agglutination reac-

TABLE III

EFFECT OF TEMPERATURE ON THE AGGLUTINATION OF *C. ALBICANS* BY MODIFIED FORMS OF CONCANAVALIN A

Temperature (°C)	Concanavalin A, control		Succinylated concanavalin A
	pH 7.3	pH 4.0	pH 7.3
4	>500 *	500	500
37	25	25	25

* Concentration of concanavalin A (μg/ml) necessary for half maximal agglutination of 10^8 cells/ml in 30 min.

tion at pH 4.0. There was no difference in agglutinability at this pH as compared to pH 7.3 at 37°C (Table III). Furthermore, a decrease of temperature affected the agglutination at pH 4 to the same extent as at pH 7.3.

Concanavalin A treatment with succinic anhydride was carried out twice to maximize the extent of succinylation [13]. The agglutinating ability of the succinylated concanavalin A preparation was compared to that of non-treated concanavalin A using guinea pig erythrocytes. A 20-fold increase in the concentration of succinylated concanavalin A was needed to achieve similar agglutination. However, there was no difference in the agglutination of yeast cells by the two lectin preparations at pH 7.3 or pH 4.0 at 37°C. At 4°C the succinylated concanavalin A again showed the temperature-dependent agglutination observed with the non-treated concanavalin A at pH 7.3 and at pH 4.0 (Table III). These experiments indicate that the temperature effect in this system cannot be explained on the basis of dimer-tetramer transition.

One of the lectins which has been reported not to show temperature-dependent agglutination of mammalian cells is wheat germ agglutinin [5]. It has also been demonstrated that wheat germ agglutinin in contrast to concanavalin A exhibits structural stability when analyzed by ultracentrifugation under varying conditions of pH and temperature [19]. It will be interesting to study the wheat germ agglutinin-mediated agglutination of chemically defined, rigid receptor systems available in other microorganisms.

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