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SUCCINYLATED AND ACETYLATED CONCANAVALIN A ACTIVATE THE CLASSICAL COMPLEMENT PATHWAY

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SUMMARY: Succinylated and acetylated concanavalin A, but not the native lectin, lyse sheep erythrocytes in the presence of guinea pig complement. The effect appears to be specific since succinylated wheat germ agglutinin is inactive and hemolysis is inhibited selectively by α-D-methylglucopyranoside. Hemolytic activity is enhanced by preincubation of succinylated lectin-sensitized cells with complement at 4° and correlates with the number of molecules of the first component of complement bound to cells that were treated with succinylated concanavalin A and then washed.

INTRODUCTION

Concanavalin A is a lectin isolated from the Jack bean that shows binding specificity for glucose and mannose residues in glycoconjugates (1-3). Because of this specificity, concanavalin A has been used in affinity chromatography (4,5), to assess the biological significance of cell surface glycoproteins in normal and neoplastic cells (3,6,7), and to activate lymphoid cells (3,7).

At pH 7.2 concanavalin A is tetravalent while succinylated and acetylated concanavalin A are divalent (8). Although these derivatives are 500-fold less effective than the parent lectin in agglutinating erythrocytes, they retain the same sugar specificity and binding activity (8). In this communication we report that both acylated lectins, but not native concanavalin A or succinylated wheat germ agglutinin, are able to bind to sheep erythrocytes and activate guinea pig complement leading to cell lysis in the absence of antibody. The degree of lysis depends on the dose of succinylated or acetylated derivative and correlates with the fixation and activation of complement[1] C1 to lectin-treated cells.

[1]Abbreviation: Complement C1, the first component of guinea pig complement.

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MATERIALS AND METHODS

Cells. Sheep erythrocytes were collected and washed as described in (9).

Reagents. Concanavalin A, α-D-methylglucopyranoside (the sugar specific for concanavalin A binding sites), glucose, galactose, N-acetylglucosamine, and N-acetylgalactosamine were purchased from Sigma Chemical Co., St. Louis, MO. Succinylated concanavalin A and succinylated wheat germ agglutinin were obtained from Vector Laboratories, Burlingame, CA. Acetylated concanavalin A was prepared by the method of Gunther et al. (8) and obtained as a white solid after dialysis and lyophillization. Both acylated lectins had agglutination titers approximately 1/500 that of native concanavalin A against sheep erythrocytes as described in (8).

Buffer. Reagents were prepared in veronal buffered 0.15 M saline containing 0.00015 M Ca^{+2}, 0.001 M Mg^{+2} and 0.1% gelatin, pH 7.2.

Complement. Fresh frozen guinea pig serum as a source of complement was obtained from JEM Laboratories, Bethesda, MD, and stored at -30°. Complement was absorbed 3 times with sheep erythrocytes (10^9/ml serum) at 4° for 30 min to remove natural antibodies and stored in 1 ml aliquots at -30°.

Hemolysis. Percent lysis was determined by comparing the optical density at 412 nm of the supernatant fluid of test samples to the value obtained for cells lysed 100% by addition of water. In each experiment, controls included cells incubated in buffer alone, in complement alone, or with test reagent alone.

Quantification of complement C1. The number of cell-bound activated complement C1 molecules was determined on a molecular basis by the C1 fixation and transfer test (10).

RESULTS AND DISCUSSION

Hemolytic activity of lectin derivatives plus complement. Erythrocytes (0.1 ml; 10^9 cells/ml) were incubated with buffer or with increments of lectin derivative (0.05 ml) at concentrations up to 1 mg/ml reaction mixture at 37° for 90 min. Buffer (0.5 ml) was added to bring the cells to 1.5 x 10^8/ml, and 0.1 ml was added to guinea pig complement (1.1 ml guinea pig serum diluted 1/85 and shown in preliminary experiments to be excess) and incubated at 37°. After 90 min, the degree of lysis was determined. In another experiment the initial cell concentration was 1.5 x 10^8/ml, so no dilution was necessary before incubation with complement. Acetylated concanavalin A, native concanavalin A, or succinylated wheat germ agglutinin [with specificity for N-acetylglucosamine (2)] were tested in the same way at the same concentrations. The results are shown

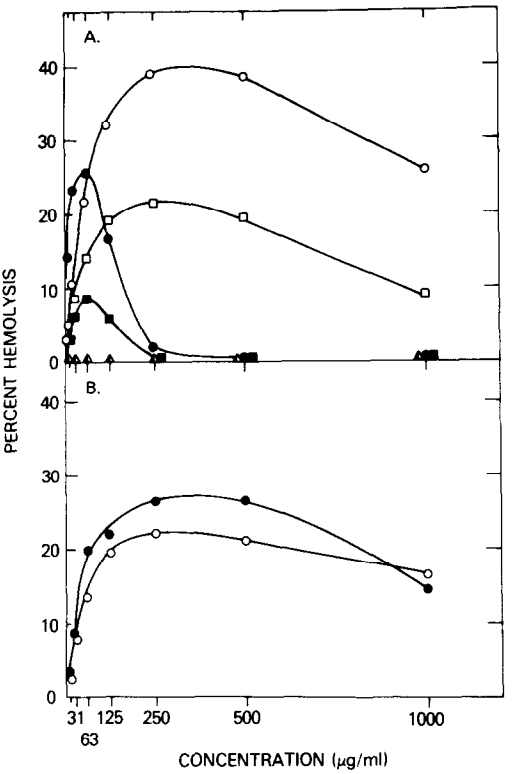

Figure 1A: Hemolytic activity of lectins plus complement against sheep erythrocytes. Cells (0.1 ml; 10^9/ml) were incubated with increasing amounts of succinylated concanavalin A (○), acetylated concanavalin A (□), native concanavalin A (△), or succinylated wheat germ agglutinin (▲), diluted to 1.5 x 10^8 cells/ml, and 0.1 ml suspension incubated with excess complement. Similarly, a starting cell concentration of 1.5 x 10^8/ml (0.1 ml) was incubated with succinylated (●) or acetylated (■) concanavalin A. Other details are given in the text. B. Effect of washing on the hemolytic activity of succinylated concanavalin A. Cells at either 10^9/ml (●) or 1.5 x 10^8/ml (○) were incubated with increasing amounts of succinylated concanavalin A, washed with buffer, resuspended at 1.5 x 10^8/ml, and 0.1 ml incubated with excess complement.

in Fig. 1A. Both concanavalin A (open triangles) and succinylated wheat germ agglutinin (filled triangles) were inactive. However, succinylated (circles) and acetylated (squares) concanavalin A gave significant hemolysis in the presence of complement. For each dose-response curve there was an optimal amount of lectin derivative that gave maximum lysis depending on the number of cells used. Succinylated concanavalin A at 450 μg/ml gave approximately 40% lysis with 10^9 cells/ml while 63 μg/ml gave 25% lysis when 1.5 x 10^8 cells/ml were used. Similar results were obtained with

acetylated concanavalin A except maximum lysis observed at the high or low cell concentration (22% or 9%) was less than the lysis observed with the succinylated derivative. Inhibition at the highest levels of succinylated concanavalin A was minimal when treated cells were washed twice with 4.5 ml portions of buffer before incubation with complement (Fig. 1B). Thus cell-bound lectin derivative is hemolytically active and reduced lysis at high doses with no washing (Fig. 1A) may be due to inhibition of C1 activity by free fluid phase acylated lectin. We showed earlier (11) that fluid phase concanavalin A inhibits the functional activity of C1 in antibody-complement mediated hemolysis. Also, acetylation and succinylation both acylate amino groups. However, the acetyl group is neutral and gives no ionic character to the lectin, while succinylation introduces free carboxylate groups. Since both derivatives give lysis with complement (Fig. 1A), the activity of succinylated concanavalin A is not due solely to behavior as a polyanion, some of which are known to activate complement C1.

Effect of sugars and temperature. The inactivity of succinylated wheat germ agglutinin suggests that lysis induced by the acylated concanavalin A derivatives may be specific. Specificity also was demonstrated by comparing the ability of α-D-methylglucopyranoside and other sugars to inhibit the activity of succinylated concanavalin A. Titration curves like those shown in Fig. 1A were obtained over a range of succinylated concanavalin A concentrations in buffer or in the presence of 50 µl 0.01 M sugar. Based on a preliminary experiment, this was the minimum amount of α-D-methylglucopyranoside required to give 100% inhibition of lytic activity. In contrast, the curves shown in Fig. 2A demonstrate that galactose and N-acetylgalactosamine did not inhibit, while sucrose and N-acetylglucosamine reduced lysis only 17 and 25%, respectively. Thus the sugar specific for concanavalin A was the most effective inhibitor.

The effect of preincubation with complement at 4° before incubation at 37° also was tested with cells (10^9/ml) that were treated with increments

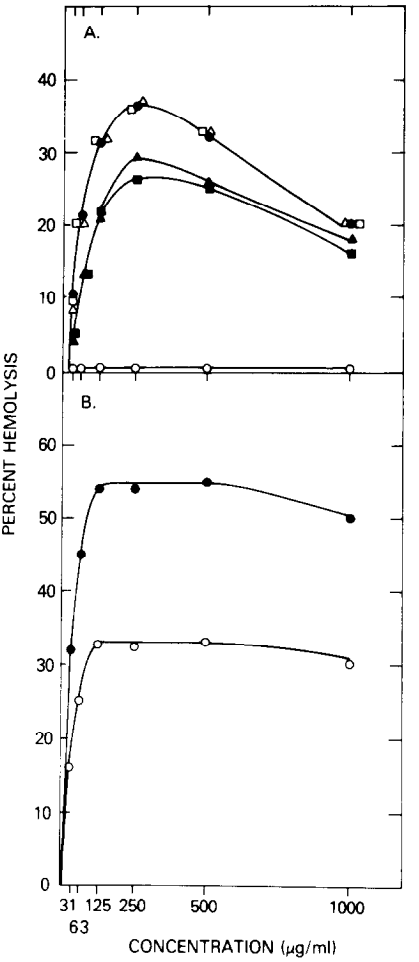

Figure 2A. Effect of sugars on the hemolytic activity of succinylated concanavalin A. Cells (0.1 ml; 10^9/ml) were incubated with increasing amounts of succinylated concanavalin A in the presence of 0.05 ml buffer (●) or 0.01 M solutions of α-D-methylglucopyranoside (○), sucrose (▲), galactose (△), N-acetylglucosamine (■), or N-acetylgalactosamine (□). After dilution to 1.5 x 10^8 cells/ml, 0.1 ml aliquots were incubated with excess complement. B. Effect of preincubating succinylated concanavalin A-treated cells with complement in the cold. Cells (0.1 ml; 10^9/ml) were treated with increasing amounts of succinylated concanavalin A (0.05 ml), washed, and resuspended at 1.5 x 10^8/ml. Aliquots (0.1 ml) were incubated with complement at 4° for 90 min, then at 37° for 90 min (●), or directly at 37° for 90 min (○).

of succinylated concanavalin A, washed with buffer, then resuspended at 1.5 x 10^8 cells/ml. Aliquots (0.1 ml) were added to one set of tubes containing complement at 4° and incubated for 90 min before a second similar incubation was carried out at 37°. A second set of tubes contained cells incubated

Fixation and Transfer of Complement C1 by Cells Treated with Lectins[a]

Lectin	Dose (μg/ml)	Complement C1 Molecules Transferred per Cell
None	---	< 1
Concanavalin A	1,000	< 1
Succinylated Concanavalin A	250	5
	500	9
	1,000	21

[a] Sheep erythrocytes (0.1 ml; 10^9/ml) and lectin or buffer (0.05 ml) were incubated at 37° for 90 min, washed twice with 4.5 ml buffer, and the number of complement C1 molecules bound and activated determined by the C1 fixation and transfer test (10).

directly at 37° for 90 min. The results in Fig. 2B show that preincubation at 4° caused a significant enhancement in lysis at each point in the titration curve. This result is similar to the increased lysis observed when erythrocytes sensitized with immunoglobulin G antibodies to Forssman antigen are carried through a cold preincubation step (12). With immunoglobulin G the enhancement is due to increased binding of C1 (12). In contrast to succinylated concanavalin A, the native lectin gave no lysis with complement even after preincubation in the cold (Fig. 2B).

Quantification of complement C1 fixation. Based on these results we tested if binding and activation of complement C1 played a role in the hemolytic activity of succinylated concanavalin A. The number of complement C1 molecules bound to cells treated with the acylated lectin and then washed was determined quantitatively by the C1 fixation and transfer test (10).

The results in the Table show that the number of C1 molecules bound to the succinylated lectin-treated erythrocytes was approximately proportional to the dose of lectin used to treat the cells. There was no binding of complement C1 to cells alone or cells treated with native concanavalin A. Thus the ability of the bivalent lectins to bind and activate the first component of complement leading to activation of the remaining components

and ultimately to cell lysis appears to be a plausible mechanism of action. Either succinylation or acetylation may expose active groups which are dormant or hidden in the native tetravalent molecule.

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