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Inactivation of Herpes Simplex Virus by Concanavalin A

MICHIO ITO AND ALMEN L. BARRON

Department of Microbiology, School of Medicine, State University of New York at Buffalo, Buffalo, New York 14214

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The infectivity of herpes simplex virus type 1 (HSV-1) was inactivated after treatment with either concanavalin A (ConA) or periodate. Phytohemagglutinin, wheat germ agglutinin, pokeweed mitogen, and neuraminidase failed to inactivate the virus. The effect of ConA could be specifically inhibited or reversed by the addition of α-methyl-D-glucoside or α-methyl-D-mannoside. Evidence was obtained that HSV-1 inactivated by ConA could adsorb to host cells. Viral aggregation was not a major mechanism in the inactivation of HSV-1 by ConA. Under the experimental conditions employed, inactivation of HSV-1 was faster by ConA than by antiserum and less temperature dependent. A ConA-resistant fraction was detected which appeared to adsorb less quickly than untreated virus, and penetration of ConA-resistant fraction was strikingly slow. The presence of aggregates in the virus preparation did not appear to account for the ConA-resistant fraction. Inactivation of viral infectivity by ConA was obtained only with enveloped viruses, since HSV-1, HSV-2, pseudorabies, and vesicular stomatitis virus were inactivated and vaccinia and echovirus type 6 were not.

It has been demonstrated that virus-specific glycoproteins appear on the plasma membranes of cells infected with herpes simplex virus (HSV) as well as on the envelope of HSV virions (15, 21). The biological significance of virus-specific glycoproteins on the plasma membranes, as well as their importance as viral-specific antigens, remains to be elucidated. In our laboratory we have been interested in studying the nature of an antigen(s) which appears on the surface of cells infected with HSV and other herpesviruses (17, 19; M. Ito and A. L. Barron, Proc. Soc. Biol. Med., in press).

Increase in agglutininability of cells by concanavalin A (ConA) has been shown after infection with both oncogenic (5, 6, 8, 9, 16, 20) and nononcogenic viruses (4, 7, 28, 31, 33). ConA may have a direct effect on a wide variety of viruses including myxoviruses (4), arboviruses (7, 24), oncornaviruses (10, 30), paramyxoviruses (23), and herpesviruses (23, 26). In a number of these studies, ConA was found to form visible and sedimentable aggregates of virions. Inactivation of infectivity after ConA treatment has been reported for myxoviruses (4) and herpesviruses (23, 26).

Virions which contain glycoproteins in their envelopes would be expected to react with ConA since lectins have a specific affinity for carbohydrates. Although a number of publications have appeared on the inactivation of viruses by ConA, little is known of the mechanism involved. Inactivation of HSV by ConA was first described by Okada and Kim (23), and our study is an outgrowth of their investigation. In this communication we describe studies on the interaction of HSV with ConA which provide a basis for explaining the mechanism of inactivation.

The sensitivity of other enveloped as well as nonenveloped viruses to ConA was investigated, as well as the effect of other lectins and substances which react with polysaccharides. In addition, the presence of a small fraction in the virus population resistant to ConA was detected in the course of this investigation, and experiments on the nature of this ConA-resistant fraction are reported as well.

MATERIALS AND METHODS

Cells. A cell line from rabbit kidney (BIRK) was employed to prepare virus stocks (17). Cells were grown in Eagle basal medium in Hanks balanced salt solution containing 10% fetal calf serum, penicillin (200 U/ml), and streptomycin (200 μg/ml). HSV plaque assays were performed in BGM cells of African green monkey kidney origin (3) grown in 30-ml plastic flasks (Falcon Plastics, Los Angeles, Calif.). BGM cells were cultivated in Eagle minimum essential medium (MEM) in Earle balanced salt solution (bicarbonate, 1.8 mg/ml) supplemented with 10% fetal calf serum and antibiotics. After virus inoculation, cells of both lines were maintained in MEM supplemented with 3% serum.

Viruses. HSV-1, McIntyre VR 3 strain, and

HSV-2, MS strain, were employed as described previously (17). HSV strains were plaque-purified twice in BGM cells prior to preparation of virus stocks. Pseudorabies virus, Aujeszky strain, was also passed in BIRK cells (17). Vaccinia virus, NY strain, was derived from commercial calf lymph vaccine and passed in BIRK cells (18). Simian adenovirus (SV15) was kindly supplied by H. D. Mayor, Baylor College of Medicine, Houston, Tex., and passed in BGM cells. Vesicular stomatitis virus (VSV), Indiana strain, grown in chicken embryos was obtained from T. D. Flanagan of our department. Echovirus type 6, D'Amori strain, from the virus collection of this laboratory was also propagated in BGM cells.

Infectivity assay. Plaque titration for measuring infectivity was performed in BGM cells (17). Phosphate-buffered saline (PBS) containing 0.3 mM calcium chloride and 1.0 mM magnesium chloride was employed as diluent. Daniel modified Eagle medium (Grand Island Biological Co. [GIBCO], Grand Island, N.Y.) containing 3% fetal calf serum and 0.7% agarose (Seakem, Bausch and Lomb, Rochester, N.Y.) was used as an overlay medium for plaque assay of HSV and pseudorabies viruses. The agarose was replaced by 1.2% Noble agar (Difco, Detroit, Mich.) for assay of all other viruses. After incubation at 36 C, cells were stained with a second overlay medium consisting of the modified Eagle medium, 3% newborn-calf serum, 0.007% neutral red, and 1.2% Noble agar.

Chemicals. The preparation of ConA used was purchased from Nutritional Biochemicals Corp. (NBC), Cleveland, Ohio (lot no. 5594, crystallized two times); 1 ml of solution contained 41.5 mg of protein and was homogeneous by polyacrylamide gel electrophoresis at pH 4.5 according to the supplier. The ConA was used without further purification in our laboratory. Phytohemagglutinin-P (Difco) and pokeweed mitogen (GIBCO) were prepared according to the supplier and were considered as undiluted in our experiments. Crude wheat germ agglutinin was prepared from wheat germ lipase (Schwarz Mann, Orangeburg, N.Y.) (2). Potassium metaperiodate was obtained from Matheson, Coleman and Bell Co., Norwood, Ohio. Neuraminidase (Behringwerke Ag., Marburg-Lahn, Germany) was a gift from H. Fuji of our department. α-Methyl-D-glucoside was obtained from Sigma Chemical Co., St. Louis, Mo.; α-methyl-D-mannoside was from Calbiochem, San Diego, Calif., and *n*-acetyl-D-glucosamine was from NBC.

Filtration. In filtration experiments of untreated and ConA-treated virus, membrane filters (Millipore Corp., Bedford, Mass.; 13-mm diameter, 0.45 and 0.22 μm) were pretreated with 10% fetal calf serum essentially as described by Wallis and Melnick (32). A 5-ml volume of 0.05 M Tris buffer (pH 7.2) containing 10% fetal calf serum was passed through the filters by using a Swinney-type adaptor and syringe. Coated filters were washed with 10 ml of Tris buffer without serum and then used for filtration. A 2-ml volume of the virus preparation was filtered, and the first 1 ml of filtrate was discarded before the remaining filtrate was collected for titration.

RESULTS

Inactivation of HSV. Among the lectins and mitogens tested, only ConA was found to decrease the infectivity of HSV-1 (Table 1). Phytohemagglutinin, wheat germ agglutinin, and pokeweed mitogen had no effect. The modest increase in plaque numbers obtained with wheat germ agglutinin was not considered significant since tests performed with other lots failed to show this slight increase. It was also found that periodate was a strong reagent for inactivation of HSV, but negative results were obtained with neuraminidase.

Inhibition and reversal of ConA activity. Inactivation of HSV-1 by ConA was blocked specifically by the simultaneous addition of α-methyl-D-glucoside or α-methyl-D-mannoside but not by *n*-acetyl-D-glucosamine (Table 2). Even at a concentration of 0.3 M α-methyl-D-glucoside, complete inhibition of ConA was not

TABLE 1. *Inactivation of HSV-1 by concanavalin A and periodate*

Treatment of HSV-1				Infectivity of HSV-1 (PFU)		
Substance	Amount	Temp (C)	Time	Untreated	Treated	Reduction (%)
Concanavalin A	0.5 mg/ml	25	1 h	1,410	15	98.9
Phytohemagglutinin-P	Undiluted	25	1 h	1,410	1,740	0
Wheat germ agglutinin	10 mg/ml	25	1 h	1,410	2,520	0
Pokeweed mitogen	Undiluted	25	1 h	1,410	1,480	0
Periodate	0.01 M	25	15 min	600,000	0	100.0
Neuraminidase	100 U/ml	37	1 h	210	194	7.6

[a] Equal volumes of appropriate concentrations of ConA or other lectins and mitogens in PBS were mixed with HSV-1 and incubated for 1 h at 25 C. Thereafter, the mixtures were diluted at least 100 times to avoid any direct effect on the host cells, and the residual infectivity was assayed. For treatment with periodate, 3 volumes of freshly prepared 0.01 M solution of periodate in PBS were mixed with 1 volume of HSV. After 15 min at 25 C, the periodate was neutralized by addition of 4 volumes of 1% glycerol in PBS, and residual infectivity was assayed. In the case of neuraminidase, equal volumes of the enzyme solution (100 U/ml) in PBS were mixed with HSV and incubated for 1 h at 37 C.

TABLE 2. *Inhibition of concanavalin A inactivation of HSV-1*

Sugar[a]	Infectivity (PFU) of HSV-1 mixed with		Reduction (%)
	PBS	Con A	
None	2,090	40	98.1
α-Methyl-D-glucoside:			
0.3 M	2,370	1,600	32.6
0.1 M	1,860	1,200	35.6
α-Methyl-D-mannoside:			
0.1 M	1,830	1,340	26.8
n-Acetyl-D-glucosamine:			
0.1 M	1,900	79	95.8

[a] HSV-1, ConA (0.1 mg/ml), and sugar (final concentration 0.1 or 0.3 M) were mixed simultaneously and incubated for 1 h at 25 C prior to the infectivity assay.

obtained. To learn whether the inactivation could be reversed after treatment with ConA, the experiment summarized in Table 3 was performed. ConA inactivation was reversed by the addition of either α-methyl-D-glucoside or α-methyl-D-mannoside.

Adsorption of HSV inactivated by ConA to cell cultures. Since reversal of inhibition had been demonstrated, it was possible to determine whether HSV-1 inactivated by ConA could adsorb to BGM cells. HSV-ConA mixtures were inoculated and, after incubation, the cells were washed extensively. Recovery of infectious virus from the cells was attempted by the addition of α-methyl-D-glucoside or α-methyl-D-mannoside. Infectious virus was recovered under these conditions, and the results indicated that HSV inactivated by ConA could adsorb to cells (Table 3).

Possible role of aggregation in inactivation of HSV-1 by ConA. An attempt was made to determine whether viral aggregation was a major mechanism in the inactivation of HSV-1 by ConA. Appreciable recovery of infectious virus after inactivation by ConA and filtration was obtained with α-methyl-D-mannoside (Table 4). This would not have occurred if aggregation was the major mechanism, since the aggregates would not have passed through the filter. The effect of aggregation was also studied in experiments in which HSV-1 treated with ConA was exposed to sonication (Table 4). There was no change in infectivity as a result of the sonication of ConA-treated virus, which also supported the idea that aggregation was not important in inactivation of the virus.

Kinetics of inactivation of HSV by ConA or antiserum. Inactivation of HSV-1 by ConA was compared to neutralization by specific antiserum at 5, 25, and 36 C (Fig. 1). At the concentration used (serum, 1:40; ConA, 0.1 mg/ml), inactivation by ConA was quite rapid,

TABLE 3. *Reversal[a] of concanavalin A inactivation of HSV-1 and recovery[b] of HSV-1 inactivated by concanavalin A from cell cultures*

Treatment of HSV-1	Control (PFU)[c]	α-Methyl-D-glucoside (PFU)		Control (PFU)	α-Methyl-D-mannoside (PFU)	
		Reversal	Recovery		Reversal	Recovery
PBS	115,000	92,000	112,000	29,000	20,000	21,000
ConA	140	27,800	33,100	50	4,000	21,600

[a] Appropriate dilutions of HSV-1 were mixed with 0.3 mg/ml of ConA or PBS, and the mixtures were incubated for 1 h at 25 C. After incubation, HSV-ConA mixtures were treated with α-methyl-D-glucoside or α-methyl-D-mannoside, final concentration 0.1 M, and directly assayed for infectivity with PBS as diluent.

[b] HSV-ConA and HSV-PBS mixtures were diluted at least 100 times after incubation for 1 h at 25 C, and directly inoculated into BGM cells which were incubated for 2 h at 25 C to permit adsorption of virus. Inoculum was discarded and the cells were washed three times with PBS. Either α-methyl-D-glucoside or α-methyl-D-mannoside (0.5 ml of 0.1 M solution) was added to appropriate flasks which were incubated for 30 min at 25 C. Flasks were then overlaid to assay for infectivity.

TABLE 4. *Role of aggregation in inactivation of HSV-1 by concanavalin A*

First treatment[a]	Filtration[b]	Second treatment[c]	PFU[d]	Infectivity %
PBS	+	PBS	29,000	100
		PBS + α-MM[e]	26,500	88.6 (100)
		ConA	220	0.74
		ConA + α-MM	11,600	38.8 (43.6)
ConA	+	PBS	144	0.48
		PBS + α-MM	5,700	19.1 (21.5)
PBS	—	—	4,230	100
		Sonically treated[f]	4,430	108.8
ConA	—	—	38	0.89
		Sonically treated	23	0.52

[a] Equal volumes of appropriate dilution of HSV-1 were mixed with either PBS or ConA (0.1 mg/ml) and incubated for 1 h at 25 C.

[b] Preparations were filtered through a 0.45-μm membrane filter (Materials and Methods).

[c] HSV-PBS filtrate was mixed with either PBS or ConA as described in footnote a. HSV-ConA filtrate was mixed with PBS and incubated for 1 h at 25 C. The mixtures were diluted at least 100 times, inoculated into BGM cells, and incubated for 2 h at 25 C, and the inoculum was removed.

[d] Flasks overlaid to assay for infectivity.

[e] α-Methyl-D-mannoside (α-MM) (0.5 ml of 0.1 M solution) was added, and flasks were incubated for 30 min at 25 C before overlay.

[f] After treatment of HSV-1 with either PBS or ConA, mixtures were sonically treated for 45 s with a Branson model W-185C sonifier at a setting of 75 W, diluted at least 100 times in PBS to avoid reaggregation, and assayed for infectivity.

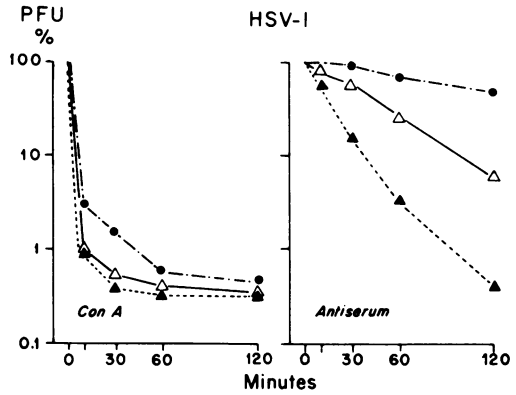

FIG. 1. *Kinetics of inactivation of HSV by ConA or antiserum. Equal volumes of HSV-1 (approximately 10^6 PFU) were mixed with 0.1 mg/ml of ConA or PBS. After incubation at 5, 25, or 36 C for varying periods of time, the mixtures were immediately diluted at least 100 times in PBS to prevent any further effect of ConA and then assayed for infectious virus. A hyperimmune serum against HSV-1 (no. 284) was produced in a rabbit as described previously (17). (This serum had a neutralization titer (80% plaque reduction) of 2,560.) An equal volume of HSV-1 (approximately 10^6 PFU/ml) was mixed with 1:40 dilution of heat-inactivated antiserum. After incubation at 5, 25, and 36 C for varying periods of time, the mixtures were immediately diluted 10 to 1,000 times in PBS and assayed for infectious virus. Residual infectivity was expressed as a percentage of infectivity of HSV-1 incubated either with PBS or preimmune rabbit serum at the corresponding temperature. Symbols: ●, 5 C: △, 25 C; ▲, 36C.*

with the major reduction of infectivity occurring in 10 min (not temperature dependent). Maximal reduction of infectivity was obtained between 30 and 60 min. Neutralization by antiserum was slower and obviously temperature dependent, and maximal reduction of infectivity even at 36 C was not complete after 60 min.

Influence of aggregates on ConA-resistant fraction. Approximately 0.2 to 1% of the infectivity could still be recovered after treatment with ConA at concentrations of 0.1 to 0.5 mg/ml. The nature of this ConA-resistant fraction was explored further. Experiments were designed to investigate whether large aggregates present in the virus preparation were responsible for the ConA-resistant fraction. Stock virus was diluted and filtered as described above. The infectivity of untreated filtrate was compared to filtrate reacted with ConA. As seen in Table 5, 58.8% of infectivity was recovered after filtration through a 0.45-μm filter and 2.8% was recovered after filtration through a combination of a 0.45- and a 0.22-μm filter. When the combination of filters was used, it was considered highly unlikely that aggregates could be present in the filtrate. Treatment of the filtrates with ConA still resulted in the detection of a small ConA-resistant fraction, and the percentage of ConA-resistant fraction detected was essentially the same as that of unfiltered virus.

Adsorption and penetration of the ConA-resistant fraction. Adsorption and penetration of untreated HSV-1 and the ConA-resistant fraction were compared (Fig. 2). Adsorption of untreated virus occured rapidly, and maximal adsorption was obtained within 1 h at either 25 or 36 C, whereas penetration was temperature dependent and more rapid at 36 than at 25 C. Adsorption of the ConA-resistant fraction proceeded somewhat more slowly than untreated virus, and penetration appeared to be markedly delayed. After 2 h of incubation, more than 95% of the adsorbed virus was still neutralized by antiserum.

Effect of ConA on a variety of viruses. ConA selectively inactivated enveloped viruses (Table 6). More than 95% of infectivity was lost for HSV-1, HSV-2, pseudorabies, and VSV. No loss of infectivity was obtained in the case of the nonenveloped viruses, SV15, vaccinia, and echovirus type 6.

DISCUSSION

The inactivation of HSV by ConA was first reported by Okada and Kim (23). In the present study, other lectins in addition to ConA and other substances which react with polysaccharides were investigated for their effect on HSV infectivity. Marked inactivation of HSV was obtained with ConA and periodate, but phytohemagglutinin, wheat germ agglutinin, pokeweed mitogen, and neuraminidase had no effect. Periodate inactivation of viruses is not a generalized phenomenon in that infectivity of

TABLE 5. *Influence of aggregates on the concanavalin A-resistant fraction*

Filtration[a]	Treatment[b]	PFU	Recovery (%)	ConA-resistant fraction (%)
None	PBS	350,000	100	
	ConA	1,240		0.35
0.45 μm	PBS	206,000	58.8	
	ConA	2,480		1.21
0.45 + 0.22 μm	PBS	9,770	2.8	
	ConA	56		0.57

[a] A 2-ml volume of a 1:100 dilution of stock HSV-1 was filtered as described in Materials and Methods.

[b] An equal volume of filtered or nonfiltered HSV-1 was mixed with either PBS or 0.1 mg of ConA per ml and incubated for 1 h at 25 C prior to the plaque assay.

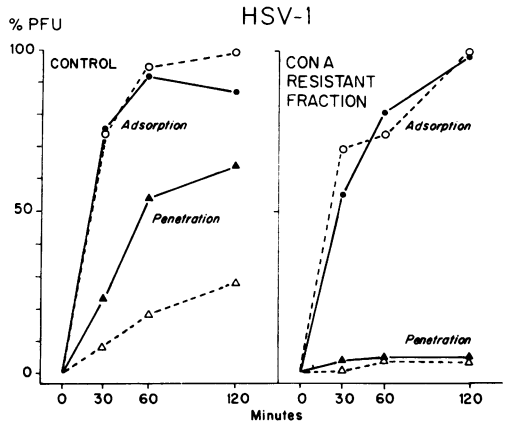

FIG. 2. *Adsorption and penetration of untreated HSV-1 and the ConA-resistant fraction. Equal volumes of HSV-1 were mixed with PBS or ConA (0.5 mg/ml) and incubated for 1 h at 25 C. Thereafter, the mixtures were diluted 10,000 times for PBS-treated virus (control) or 100 times for ConA-treated virus in PBS. The remaining infectious virus was designated ConA-resistant fraction. Flasks were inoculated with 0.2 ml of either the ConA-resistant fraction or control and incubated at 25 C, or 36 C for 30, 60, or 120 min. Four flasks of each set were removed, and the monolayers were washed three times with PBS in order to remove any unadsorbed virus. Two flasks were overlaid for assay of adsorbed virus, and the other two flasks were each treated with 0.2 ml of a 1:20 dilution of anti-HSV-1 serum (no. 284) for 1 h at 36 C to neutralize virus which had adsorbed but not penetrated the cells. These flasks were then overlaid without further washing. Symbols:* O, *adsorption 25 C;* ●, *adsorption 36 C;* Δ, *penetration 25 C;* ▲, *penetration 36 C.*

human adenovirus type 1 was not affected by the same treatment as used for HSV (M. Ito, unpublished data).

In considering the mechanism of action of ConA on HSV, we investigated the possibility of aggregation as being of primary importance in view of the known aggregation activity of ConA reported previously (4, 23, 24, 30). Our data obtained from filtration experiments failed to substantiate that aggregation was the major mechanism involved. Loss of infectivity may be due to interaction between ConA and HSV virions similar to virus neutralization by antiserum. Kinetic studies revealed that inactivation of HSV-1 by ConA was more rapid and less temperature dependent than neutralization. However, the mechanism of inactivation could still be similar, since ConA-binding sites present on the virion envelope are essential for the infectious process, and by blocking these sites the virions are rendered noninfectious. In our experiments, adsorption to cells was found to occur by ConA-treated HSV. Thus, the mechanism of inactivation could not be ascribed to failure at the adsorption step. Apparently, inactivated virus adsorbs to cells, and blockage may occur at the penetration step or later in the infectious cycle.

Our experiments revealed the presence of a small fraction of HSV-1 that was resistant to ConA. This fraction represented approximately 0.2 to 1% of the stock virus population. In spite of repeated treatment of HSV-1 with ConA or exposure to concentrations of ConA as high as 25 mg/ml, the level of resistant fraction was unchanged (M. Ito, unpublished data). The detection of such a fraction was not reported by Okada and Kim (23), who observed 100% loss of infectivity of HSV-1. The differences in the results might be explained by the assay systems used in the two studies, since Okada and Kim used a liquid overlay containing HSV antibodies, whereas in our studies the plaque procedure involved an overlay of agarose without any addition of antiserum. The ConA-resistant fraction could be accounted for by the presence of aggregates in the stock virus suspension, but filtration experiments demonstrated that the presence of aggregates in the virus suspension did not appreciably contribute to the ConA-resistant fraction. Analysis of the ConA-resistant fraction in comparison to untreated HSV-1 revealed that penetration of the ConA-resistant fraction was much slower as measured by sensitivity to neutralizing antibody. However, the resistant fraction did eventually penetrate and produce infection as evidenced by plaque for-

TABLE 6. *Inactivation of various viruses by concanavalin A*[a]

Viruses	PBS (PFU)	ConA (PFU)	Reduction (%)
Herpesviruses			
HSV-1	776	9	98.8
HSV-2	832	5	99.4
Pseudorabies	413	12	97.1
Rhabdoviruses			
VSV	386	19	95.1
Adenoviruses			
SV15	138	159	0
Proxviruses			
Vaccinia	267	288	0
Picornaviruses			
Echovirus type 6	63	64	0

[a] Equal volumes of appropriate dilution of viruses were mixed either with PBS or ConA (0.1 mg/ml) and incubated for 1 h at 25 C. Thereafter the mixtures were diluted at least 100 times in PBS, and residual infectivity was assayed in BGM cells.

mation. One could speculate that the virions which compose the ConA-resistant fraction possess receptors for ConA and that interaction with ConA does occur. These virions adsorb to the cells, but infectivity is reactivated by the release of ConA inhibitors at the cell surface, since the surface of animal cells has been shown to contain abundant ConA-receptor substances which act as ConA inhibitors (1, 11). The difference between the ConA-resistant fraction and the susceptible population might be due to the number of ConA receptors on the virions or to the strength of the binding, and reactivation may not take place in the case of the susceptible population.

In addition to the inactivation of herpesviruses, reduction in infectivity was also obtained with a rhabdovirus in our studies, but no effect was obtained with the poxvirus, adenovirus, or picornavirus tested. Thus it would appear as if inactivation of viruses by ConA is limited to enveloped viruses. In experiments on inactivation of viruses by ConA, attention should be paid to the reagents selected. In our early experiments on HSV, we found that lactalbumin hydrolysate and serum proteins could markedly inhibit ConA activity and had to be excluded from the assay system (M. Ito, unpublished data).

Envelopment of HSV virions occurs at the nuclear membrane and membranes of cytoplasmic vacuoles (12, 22). Since ConA reacts directly with virions, it can be assumed that these membranes also contain ConA-binding sites as well as plasma membranes (31). Uninfected cells may code for ConA-binding sites, and it is not known at present if some or all of the ConA-binding sites found in HSV infection are coded for by the virus.

Glycoproteins play a significant role in the structure of the envelope of HSV virions, and virus-coded glycoproteins appear on the surface of infected cells (15, 21). These glycoproteins are probably associated with the HSV antigens detected on the cell surface by various serological procedures (13, 14, 17, 25, 27, 29). Recently, antigens induced by HSV have been separated using ConA affinity chromatography (26). The use of lectins with an affinity for specific carbohydrates provides a new approach for the investigation of cell membranes altered by viral infection.

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