NOTES

Binding Effects of Concanavalin A on a Coronavirus

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ABSTRACT

Concanavalin A, a phytagglutinin, binds to the envelope of hemagglutinating encephalomyelitis virus, a Coronavirus. Concanavalin A treated virus suspensions lose their hemagglutination properties and there is a transient interference with infectivity. Electron micrographs show the Concanavalin A as a granular deposit adhering to the viral envelope and there is aggregation of the virus. Concanavalin A does not bind to virions stripped of their envelopes.

RÉSUMÉ

La concanavaline A, une phyto-agglutinine, s'attache à l'enveloppe des virions du virus hémagglutinant de l'encéphalomyélite porcine, lequel est un coronavirus. Les suspensions virales ainsi traitées perdent leur propriété hémagglutinante et la concanavaline A supprime temporairement l'infectiosité du virus. Après une imprégnation négative, la concanavaline A forme un dépôt granuleux qui adhère à l'enveloppe virale; on constate aussi des agrégats de virions. La concanavaline A ne s'attache pas aux virions dépouillés de leur enveloppe.

Concanavalin A (Con A) is a phytagglutinin isolated from the jack bean *Conavalin ensiformis* by Sumner and Howell in 1936 (15). It has the property of combining with polysaccharides and glycoproteins to form precipitates. The binding action is inhibited and reversed by α-glucopyranosides and α-mannopyranosides. Con A will bind to the glycoprotein of enveloped viruses (3, 12, 13) and tissue cultures infected with such viruses (2, 9, 12). The effect is to cause agglutination or clumping of the virus or infected cells and an interference in some virus activities such as hemagglutination (HA) and infectivity.

Coronaviruses are enveloped viruses whose petal shaped surface structures contain ether sensitive substances (1). Reported here are the effects of Con A on the infectivity, HA and electron microscopic appearance of hemagglutinating encephalomyelitis virus (HEV), a Coronavirus isolated from pigs (7, 8).

HEV strain 2 (HEV-2) was propagated in primary pig kidney (PK) cell cultures by methods previously reported (6). In some experiments a pig kidney cell line, IB-RS-2, was used (5). This cell line was grown in Eagles Minimal Essential Medium (MEM), Hank's based, supplemented with 10% fetal calf serum. Eagles Basal Medium (BME), Earle's based, without serum was used as maintenance medium in both cell systems.

In some experiments virus was concentrated by centrifuging supernatant fluids from infected cultures at 2000 rpm for ten minutes to remove cell debris, then at 50,000 xg for one hr. The pellets were resuspended in distilled water or 0.05 M tris.HCL buffer (pH7.4) at 50 to 100 x the original virus concentration.

Freeze-dried Con A[1] was dissolved in 0.15 N sodium chloride solution (N-saline) or distilled water.

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[1]Pharmacia Fine Chemicals, Uppsala, Sweden.

Infectivity of virus suspensions was determined by titration in PK tube cultures. The end points were determined by the presence or absence of HA in the medium overlay.

Preliminary trials on the effect of Con A on primary PK cells and IB-RS-2 cell line showed that concentrations above 10 ug/ml caused rounding and detachment of the cells within a few hours. The effect was gradient, with only a few cells affected by 20 ug/ml and complete destruction of all cells exposed to 100 ug/ml. Similarly, chicken erythrocyte suspensions were agglutinated by 20 ug/ml or higher concentration of Con A. The agglutination was prevented or reversed by 0.2 Mα-methyl-D-glucoside.

Because of these effects, tests were designed to avoid exposing cell cultures to more than 10 ug/ml where possible. In one series of tests, virus suspensions containing between 10^5 and 10^8 infectious doses were mixed with concentrations of Con A up to 1000 ug/ml. The mixtures were incubated for one hr at 37°C, then diluted in tenfold steps to beyond their expected end points. Control mixtures of virus and N-saline were similarly treated. Each set was inoculated into PK tube cultures and incubated for four days at 37°C. At 1000 ug/ml the end points of infectivity of both Con A treated and control virus suspensions were the same but there was an apparent repression of virus multiplication in the dilutions containing a residual 10 ug/ml or more Con A. This result is shown in the example in Table I. In this case, where 1000 ug/ml Con A were mixed with the virus, the first dilution, 10^{-1}, contained still 100 ug/ml Con A. This is enough, as anticipated, to destroy the PK cells. In the next dilution, 10^{-2}, 10 ug/ml Con A remained and was apparently enough to prevent infection of the cells. Dilutions beyond this point were equally infectious in each set.

Based on these results, a second series of tests was undertaken in which HEV was diluted in BME containing 10 ug/ml Con A in parallel with control titrations without Con A. In all trials 10 ug/ml Con A reduced the end point by 2 log 10 dilutions or more. In some experiments, as an additional step, a part of each virus/Con A dilution was further diluted 1:10 in BME prior to inoculation in order to dilute the Con A to 1 ug/ml, reducing the expected titre of virus 1 log 10 dilution in consequence. In fact, this step resulted in a partial recovery of infectivity. The results of one such trial are shown in Table II.

TABLE I. Comparison of Titres Between HEV in N-Saline (Control) and HEV Exposed to 1000 ug/ml Con A for one hour

Dilution	HEV Control	HEV in Con A	Conc. Con A ug/ml	Con A Control
10^{-1}	5[a]	5[b]	100	5[b]
10^{-2}	5	0	10	0
10^{-3}	5	5	1	0
10^{-4}	5	5	0.1	0
10^{-5}	3	3		
10^{-6}	0	0		

[a]HA positive tubes/5
[b]Agglutination of chicken RBC due to excess Con A

In a third series of tests, cell cultures in roller tubes were exposed to 10 ug/ml Con A for one hr at 37°C then rinsed twice with BME. HEV titrations were then carried out in these Con A treated cells and in untreated control cells. No difference in end points could be detected.

In tests on the effects of Con A on hemagglutination, concentrated virus antigen was diluted in twofold steps in N-saline starting with a 1:10 dilution, in 0.2 ml amounts. Con A in N-saline in different concentrations was added in 0.2 ml amounts to each dilution except the controls, where N-saline substituted for Con A. After 15 minutes at room temperature 0.4 ml of 1:200 chicken red cells were added to each tube. The results of repeated tests showed that amounts of Con A above 0.5 ug/ml markedly reduced the hemagglutination of chicken erythrocyte suspension by HEV (Table III). The inhibitory action of Con A was reversed by the presence of 0.2 Mα methyl-D-glucoside.

Concentrated virus suspensions, untreated or treated for 15 min or longer with 200 to 1000 ug/ml Con A, were examined under a Hitachi HU12A electron microscope. The suspensions were stained by the negative contrast method using 2% phosphotungstate adjusted to pH 7.2 with N KOH. Carbon stabilized formvar films on 400 mesh copper grids were used. In Con A treated specimens the normal fringe characteristic of the Coronavirus (Fig. 1) was obscured by a heavy deposit of particulate material to the point where individual peplomers could not be distinguished (Fig. 2). Furthermore, most virions were aggregated into large, electron dense masses. In smaller clumps or on the edges of larger ones, individual virus particles

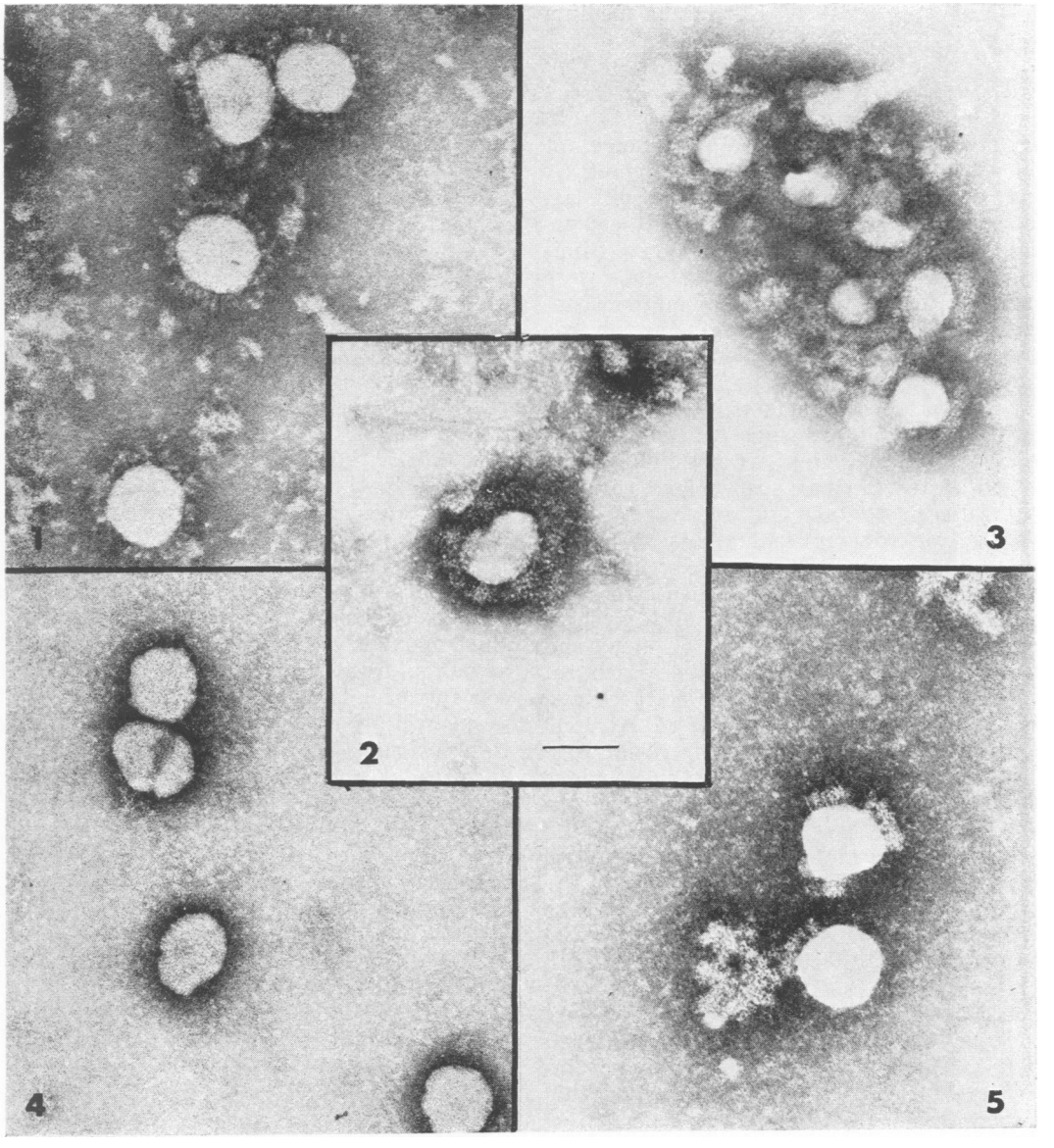

Fig. 1. Normal HEV-2 stained by the negative contrast method.

Fig. 2. Single particle of HEV treated with 1000 ug/ml Con A in H_2O. The Con A completely obscures the viral envelope.

Fig. 3. Small aggregation of HEV particles treated with 1000 ug/ml Con A in H_2O.

Fig. 4. HEV particles treated with cobra venom (Naja naja) — the phospholipase enzyme has removed all but fine traces of the envelope.

Fig. 5. HEV treated with bromelain. This enzyme has partly removed the envelope. Con A has bound to the remaining envelope, but not to the stripped areas of the viron. Bar (Fig. 2) = 100 nm.

TABLE II. Comparison of Titration of HEV in BME, in BME + 10 ug/ml Con A and in BME + 10 ug/ml Con A Diluted 1:10 after One hour Incubation

Dilution	BME Control	BME + Con A 10 ug/ml	BME + Con A Dil to 1 ug/ml
10^{-1}	5[a]	5	
10^{-2}	5	3	5
10^{-3}	5	4	5
10^{-4}	5	0	3
10^{-5}	3	0	0
10^{-6}	0	0	0
10^{-7}	0	0	0

[a]HA positive tubes/5

TABLE III. HA Titres of HEV in N-Saline (Control), N-Saline + Con A and in N-Saline + Con A + 0.2M α-methyl-D-glucoside

Con A ug/ml	N Saline	α-methyl-D-glucoside
0.....	128[a]	128
0.5.....	8	64
1.....	2	128
2.....	0	64
5.....	0	16

[a]Reciprocal of highest dilution showing HA

were seen bound together by the particulate matter (Fig. 3).

To investigate the effects of Con A on naked virions, suspensions of virus were treated with hooded cobra venom *(Naja naja)*[2] which contains phospholipase A and with bromelain,[3] a protease. Hooded cobra venom, obtained as a dehydrated powder, was dissolved in 0.05 M tris. HCL buffer (pH 7.4) at a concentration of 10 mg/ml. Equal amounts of HEV and venom were mixed and incubated at 37°C for 30 minutes. The virions were completely stripped of their peplomers by this treatment and subsequently failed to bind Con A (Fig. 4).

Bromelain was prepared by the method of Compans (4) and used in the same manner as cobra venom. Bromelain was less effective than cobra venom in removing the fringe and in instances where virions retained some peplomers, Con A bound selectively to those areas (Fig. 5).

Treatment of HEV with cobra venom completely inactivated the virus. Bromelain treatment reduced viability but did not inactivate the virus completely.

[2]Sigma Chemical Company, St. Louis, Missouri.

[3]Sigma Chemical Company, St. Louis, Missouri.

It is apparent from these results that Con A binds with components in the envelope of HEV. Since binding of Con A is to long chain carbohydrate hydroxyl groups, it is probable that the binding sites in HEV, as with other viruses, are glycoprotein groupings in the viral peplomers (4), which along with the ether sensitive glyolipids form the main components of the peplomers. The removal of one or more of these components renders the virus inactive.

The results of the HA trials indicate a strong interference by Con A in the interaction of virus and erythrocyte. It would appear the interference is simply mechanical since the viral envelope is completely obscured by a thick layer of Con A, as seen in the electron micrographs. It indicates too that the HA reaction of HEV is a property specifically of the glycoprotein in the envelope. The result with HEV differs from that with Sendai virus where HA activity was diminished but not lost on exposure to Con A (12).

Con A interferes with the infectivity of HEV only in a transient way. The presence of a minimum of 10 ug/ml during the attachment and growth phases prevented multiplication of HEV. However simply diluting the virus/Con A mixture below this concentration restored infectivity. This differs markedly from the more permanent effects shown by Con A on Sendai virus and Herpes simplex (12) and on Semliki forest virus (13). In each case infectivity was greatly reduced by Con A and could only be restored by treatment with α-methyl glycosides. Cell cultures treated with Con A, then washed free were no less susceptible to HEV than normal cell cultures.

Snake venoms are mixtures of enzymes including phospholipase A (14) which is responsible for the removal of viral membranes (11). Although their use with viruses is complicated by the frequent presence of snake herpes-like virus (10) and much organic material, *Naja naja* venom served to strip the membrane completely from HEV. No herpes virus was seen in the preparation used. The virus after treatment with venom was inactive. The enzyme splits phospholipids and in HEV must have acted on the lipoprotein portion of the envelope causing detachment from the underlying protein core. The protease of bromelain digested the glycoprotein part of the viral membrane.

With each enzyme, virus particles

stripped of the membrane did not bind Con A, indicating the absence of exposed carbohydrate groups on the virion core.

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