Agglutination of Japanese Encephalitis Virus with Concana valin A

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Received for publication 9 September 1974

Results of experiments have indicated that reduction in biological activities at high concentrations of Japanese encephalitis virus is caused by aggregates of the virus by concanavalin A. The possibility exists that the concanavalin A binding site is different from hemagglutination and antireceptor sites of Japanese encephalitis virus.

Many enveloped viruses contain components of glycoproteins and are agglutinated by such substances as concanavalin A (Con A) or Ricinius communis agglutinin (1–3, 5, 6).

This report describes the agglutination of Japanese encephalitis virus (JEV), a group B arbovirus, which is enveloped and contains the component of glycoprotein (7). JEV grown in BHK-21 cells was purified as described before (8). The virus in the infected tissue culture fluid (usually 5 to 10 liters) was precipitated by zinc acetate (0.05 M) and resuspended in saturated EDTA solution (pH 7.8). After the suspension was centrifuged at 100,000 × g for 60 min at 4 C, the pellet was resuspended in STE buffer (0.13 M NaCl, 0.01 M Tris-hydrochloride, and 10⁻³ M EDTA), which was then subjected to sucrose density gradient (10 to 40% [wt/vol]) centrifugation at 100,000 × g for 60 min at 4 C. The visible virus band was collected, diluted with STE, and centrifuged at 100,000 × g for 60 min at 4 C. The pellet was again centrifuged on the same sucrose gradient. A constant PFU-to-hemagglutination unit (HAU) ratio (5 × 10⁴) was obtained across a gradient (Fig. 1), and a homogeneous virus population was observed under an electron microscope (Fig. 2). The virus was collected by centrifugation, and final suspensions were made in phosphate-buffered saline (pH 7.4; PBS of Dulbecco and Vogt [4] but lacking Ca and Mg) containing 0.1% bovine serum albumin. To 0.2 ml of the virus suspension was added 0.2 ml of various concentrations of Con A in PBS; after 60 min at room temperature, the mixture was centrifuged at 1,000 × g for 15 min at 4 C, and the supernatant was assayed for HAU and PFU (8). The precipitate was washed once and then dissociated by 0.4 ml of 0.1 M α-methyl-d-mannoside (α-MM) for 60 min at room temperature. After centrifugation to remove any remaining aggregates, the solubilized precipitates were assayed for HAU and PFU.

When 100 μg of Con A per ml was added to the virus suspension, the visible aggregates (Fig. 3B) were formed following a decline of HAU and PFU after 60 min at room temperature. The reduction of the biological activities of JEV by agglutination with Con A was different between PFU and HAU; the remaining HAU in the supernatant was 0.1% and PFU was 1% (Table 1). The aggregate reversed about 10 to 20% of biological activities by 0.1 M α-MM, and complete inhibition by α-MM against binding of Con A to virus was seen when 0.1 M α-MM

FIG. 1. Sucrose-gradient centrifugation of JEV. The virus preparation was subjected to sucrose-gradient centrifugation and assayed for PFU (○) and HAU (●).
was added to the mixture before the agglutination of virus with Con A occurred (Table 1). To examine more exactly the effect of Con A on the biological activities of JEV, two experiments were done. In the first experiment, various concentrations of Con A and equal volumes of a constant concentration of JEV were mixed, and the resulting biological activities were determined. The titers of infectivity and hemagglutination decreased on increasing the concentration of Con A, reached a plateau at 10 to 200 μg of Con A per ml, and slightly recovered at a high concentration (200 μg/ml) of Con A (Fig. 4). The degrees of reduction of HAU and PFU differed, and the hemagglutination activity was much reduced by Con A. Essentially the same results were obtained in the second experiment; when a constant concentration (100 μg/ml) of Con A
was added to various concentrations of virus, the HAU was decreased more than the PFU at a high concentration of virus (Fig. 5A, B). Although the reason why reduction of HAU and PFU were different was not clear, the possibility that such a difference might be caused by breakdown of virions was eliminated as follows. (i) In experiments in which the virus suspension was incubated at room temperature for 60 min without Con A, the PFU-HAU ratios showed no difference before and after the incubation; (ii) similarly, no difference was noted in the sucrose density gradient data before and after incubation. From the response in biological activities of JEV to Con A, hemagglutination and an-

**Table 1. Reduction of biological activities with aggregates appearing in a mixture of JEV and Con A and its recovery by α-MM**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>HAU/ml (×10³)</th>
<th>PFU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS only</td>
<td>51,200</td>
<td>2,700</td>
</tr>
<tr>
<td>Con A(100 μg/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supernatant</td>
<td>75</td>
<td>30</td>
</tr>
<tr>
<td>Precipitate</td>
<td>50</td>
<td>7.5</td>
</tr>
<tr>
<td>Con A + α-MM(0.1 M)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>After precipitation</td>
<td>4,000</td>
<td>510</td>
</tr>
<tr>
<td>Before precipitation</td>
<td>51,200</td>
<td>3,000</td>
</tr>
</tbody>
</table>

a An equal volume of PBS was added to the virus suspension.

b The precipitate and the supernatant of the JEV-Con A mixture were obtained by centrifugation at 1,000 × g for 15 min.

c The precipitate was resuspended in the original volume of PBS.

d The precipitate obtained from (b) was resuspended in the original volume of PBS containing α-MM(0.1 M).

e An equal volume of Con A (200 μg/ml) was added to the virus suspension containing α-MM(0.2 M).

**Fig. 4. Retention of hemagglutination activity and infectivity of JEV after treatment with Con A.** Equal volumes of a constant concentration of JEV and various concentrations of Con A were mixed, and the resulting hemagglutination titer and infectivity were determined after 60 min at room temperature.

**Fig. 5. Dependency of aggregate formation with Con A on concentration of JEV.** Serially twofold diluted JEV was mixed with an equal volume of 200 μg of Con A per ml. After 60 min at room temperature, the resulting HAU (A) and PFU (B) were determined.
tireceptor sites on the virion are possibly different.

Furthermore, the rates of reduction of HAU and PFU were decreased by decreasing the JEV concentration (i.e., increasing the relative concentration of Con A per virus particle) (Fig. 5A, B). These results indicated that the reduction of biological activities at a high concentration of virus was caused by aggregates of the virus by Con A, and that the same viral activities were not reduced when a lower concentration of virus was mixed with the same amounts of Con A (i.e., the relative concentration of Con A per virus particle increased). These observations indicated the possibility that the Con A binding site was different from hemagglutination and antireceptor sites of JEV.

We wish to express our gratitude to S. Hotta, Department of Microbiology, Kobe University, for his kind advice and suggestions.

LITERATURE CITED