Immunological Activity Associated with the Nucleocapsid and Envelope Components of an Arbovirus

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Complement-fixing antigens were detected in the nucleocapsid and envelope of Sindbis virus. Neutralizing antibodies are directed against antigens in the viral envelope.

Arboviruses are small lipid-containing ribonucleic acid (RNA) viruses composed of an inner nucleocapsid core (ribonucleoprotein) and an outer lipoprotein envelope (1, 3). The phospholipids present in the envelope of Sindbis, a group A arbovirus, appear to be derived from cellular phospholipids (5), whereas the protein is specified by the viral genome (4). The virion contains two major structural proteins: one associated with the viral envelope and the second associated with the RNA in the nucleocapsid (6, 7). The immunological activity of these components is reported here.

Sindbis virus labeled with ^3H -tryosine and -methionine, or with ^3H -uridine, or with ^{32}P was purified by differential centrifugation and zonal sedimentation through a linear sucrose gradient (6). The purified virus was then treated with a final concentration of 0.2% desoxycholate (DOC) for 20 min at 4 C and subjected to an additional centrifugation in a 15 to 30% sucrose gradient (Fig. 1). The separated nucleocapsid and envelope fractions were used to determine which component is involved in the complement fixation and viral neutralization reactions. Antiserum was prepared in rabbits against purified Sindbis virus, and the serum was adsorbed with chicken embryo cells until it no longer reacted with cell antigens in a complement fixation test.

Complement-fixing antigens were detected in both the nucleocapsid and envelope components (Fig. 2). The complement-fixing activity associated with the envelope preparation was not thought to result from contamination of the envelope fraction with nucleocapsid protein, because the viral RNA level at the top of the gradient after DOC treatment of uridine-labeled virus indicated that only 10% of the virus preparation

was degraded. In addition (Fig. 3), the level of complement-fixing activity associated with the intact nucleocapsid did not increase after disruption by osmotic shock.

To define the role of each of these structural components in viral neutralization, antiserum prepared against Sindbis virus was adsorbed with either envelope or nucleocapsid preparations. Envelope and nucleocapsid preparations were obtained by treating 10^{10} PFU of purified Sindbis virus per ml with DOC and separating them by

FIG. 1. Sedimentation pattern of ^3H -uridine-, ^3H -methionine- and ^3H -tyrosine-, and ^{32}P -labeled Sindbis virus preparations after treatment with 0.2% desoxycholate. The results presented in this figure represent the combined results obtained from several gradients. Sedimentation was from right to left. The detergent-treated virus preparations were centrifuged through a 15 to 30% linear sucrose gradient at $63,000 \times g$ for 3 hr.

FIG. 2. Complement-fixing activity associated with the nucleocapsid and envelope components of the Sindbis virion. Microcomplement fixation tests were conducted with 5 units of guinea pig complement per well. The results are expressed as the reciprocal of the highest antigen dilution showing 50% hemolysis.

sucrose gradient centrifugation. The envelope or nucleocapsid preparation was mixed with an equal volume of anti-Sindbis serum (1:50 dilution), and the mixture was incubated overnight at 4 C. Adsorbed antiserum preparations were then tested for their ability to neutralize purified Sindbis virus. As indicated in Fig. 4, the titer of purified Sindbis virus was reduced approximately 100-fold with unadsorbed serum. Adsorption with nucleocapsid protein did not alter the ability of the immune serum to neutralize Sindbis virus. However, adsorption of the serum with envelope preparations reduced the ability of the serum to neutralize intact virus by 90%. An additional adsorption of the immune serum with nucleocapsid or envelope preparations failed to alter these neutralization curves. The level of DOC employed did not reduce the neutralizing ability of immune serum. These data indicate that neutralizing antibodies are directed against antigens in the viral envelope and that the antigenic determinants associated with the envelope and membrane proteins are not identical.

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FIG. 3. Complement-fixing activity associated with the Sindbis nucleocapsid before (O) and after (●) osmotic shock. The sucrose gradient-purified nucleocapsid peak was ruptured by a 3:1 dilution in tris-(hydroxymethyl)aminomethane-NaCl buffer and subjected to an additional sucrose gradient centrifugation (15 to 30% sucrose at 63,000 $\times$ g for 3 hr). The radioactivity and optical density presented were determined after osmotic shock of the nucleocapsid. Sedimentation was from right to left.

FIG. 4. Neutralization studies conducted with anti-serum adsorbed with envelope or nucleocapsid preparations of Sindbis virus. Plaque reduction neutralization tests (2) were performed in primary CE cell culture. A constant dilution of each serum preparation was mixed with an equal quantity of purified virus. The serum-virus mixtures were incubated at 4 C for 1 hr. Monolayer cultures were inoculated with appropriate dilutions, and the inoculum was adsorbed for 1 hr at 37 C. The cultures were washed with Hanks balanced salt solution and overlaid with agar as in the standard plaque test.

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