EFFECTS ON α-MANNOSIDASE ON THE ACTIVE SITE OF JAPANESE ENCEPHALITIS VIRAL RECEPTOR

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D-Mannose, D-ribose and L-rhamnose have a significant inhibitory activity against the virus-binding capacity of pigeon red cell and the cellular receptor (CR) in the case of JaGAr-01 strain of Japanese encephalitis (JE) virus. Moreover, polysaccharides containing terminal D-mannose inhibit viral haemagglutination (HA) and infectivity of the strain mentioned (*1*, *2*). These facts suggested that D-mannose or a D-mannose-like substance may be involved in the active site of CR for JE virus. To confirm this suggestion, an active fragment which included the active site of the CR was separated from complete CR and examined for its properties.

The active fragment was extracted from JE viral receptor purified from bovine brain tissues (*3*) by shaking in hot phenol. Cold ethanol was added to the water phase freed from phenol and the ethanol supernatant treated with 10% $HClO_4$. The supernatant obtained after centrifuging in the cold was passed through CM-cellulose and Sephadex G-15 column. The final preparation was dialysable and consisted of some carbohydrates. It could bind with JaGAr-01 strain at 20° C instead of at 0° C, and inhibited markedly viral HA under the assay condition reported previously (*1*).

Inactivation of active fragment with α-mannosidase. The active fragment was treated with purified α-mannosidase extracted from livers of marine gastropods (*4*) in 0.01 M acetate buffer (pH 4) at 37° C for 1 hr and assayed for HA inhibitory activity. After enzymatic treatment the activity of active fragment was completely destroyed. No decline of activity was found in the control in which active fragment was treated with buffer containing the same quantity of bovine serum albumin as the enzyme protein.

Inhibition of JE virus plaque formation by treatment of virus-sensitive cells with α-mannosidase. The result described above led us to ascertain the inactivation of CR for JE virus with α-mannosidase. The viability of chick embryo cells (CEC) was not detectably damaged at pH 5, though CR separated from intact cells was soon inactivated at acidic pH as shown previously. Monolayers of CEC were exposed to α-mannosidase in Hanks' balanced salt solution adjusted to pH 5 with KH_2PO_4. Control cells were exposed to the pH 5 buffer alone. After 1 hr at 37° C, both enzyme-treated and control cells were infected with a given quantity of JE virus. The plaque numbers in 2 experiments were as follows:

Treatment	Plaque numbers	
α-Mannosidase	259	27
pH 5 buffer alone	399	45
No treatment	387	48

The results indicate that plaque formation in the enzyme-treated cells was reduced as compared with control cells. The reduction of plaque numbers may be explained by a decease in the number of viral receptors on CEC owing to the enzymatic digestion. Moreover, a remarkable reduction of plaque size was concomitantly observed in enzyme-treated CEC.

The present results support our previous suggestion that D-mannose or D-mannose-like substance may be involved in a part of the active site of CR for JaGAr-01 strain of JE virus.

References

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