Temperature-Sensitive Behavior of the Hemagglutinin in a Temperature-Sensitive Mutant Virion of Sindbis¹

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The hemagglutinating ability of a temperature-sensitive mutant virion was reversibly lost when the temperature of hemagglutination was increased from 27 to 37 C.

Temperature-sensitive mutant phages grow adequately at a permissive temperature, but fail to grow at a higher temperature because of the complete loss of a function (6). This loss of a function is the result of a single mutational event involving a nucleotide change which leads to an amino acid replacement in a viral protein. This substituted amino acid may so weaken the internal bonding of the viral protein that the structure of the protein is radically changed by a rise in temperature and the protein becomes biologically inactive (9). Although many temperature-sensitive (ts) mutants of animal virus have been obtained, there have been few reports on the thermostability of the mutant gene products (5). This paper describes the functional ability of the hemagglutinin of one ts mutant of Sindbis virus at two different temperatures.

The ts mutant (ts 23) and a heat-resistant wild-type (HR) strain of Sindbis virus were kindly supplied by E. Pfefferkorn, Hanover, N.H. The mutant is unable to grow in cells incubated at 40 C, whereas that temperature is permissive for the growth of wild-type Sindbis virus. Stock virus preparations were produced in primary cultures of chick embryo cells at 27 C. Genetic characteristics of the mutants have been reported previously (1, 2). Biochemical studies indicated that ts 23 is defective in the viral membrane protein (3, 10). The hemagglutination test of the ts 23 virions was performed by the method of Clarke and Casals (4). Growth medium containing viruses was cleared of cell debris by low-speed centrifugation and filtration through a membrane filter (0.22 μ m pore size). A 0.2% suspension of goose erythrocytes was mixed with an equal volume of the virus diluents to give a

final pH of 5.8, and the mixture was incubated at 25 or 37 C for 1 hr. Glass tubes were used throughout. The hemagglutinin tiers were reciprocals of the highest dilution of virus in which a positive pattern was present. Corresponding virus titers were determined by plaque assay on monolayers of chick embryo cells which were incubated at 27 C.

Hemagglutination reactions by ts 23 and HR were performed at different temperatures. As shown in Table 1, virus diluents containing the same number of plaque-forming units of ts 23 and HR had the same hemagglutinin titers at 25 C. However, the ability of ts 23 to hemagglutinate goose erythrocytes was lost at 37 C, whereas the hemagglutinin titer of HR virion was temperature-insensitive and remained constant at both temperatures. To determine whether incubation at 37 C would affect the hemagglutinin titer reversibly, medium containing ts 23 virion was incubated at 37 C for various lengths of time and then cooled to 25 C; the hemagglutination test was performed at 25 C. It was found that the hemagglutinin titer of ts 23 at 25 C remained unchanged after incubation at 37 C for 12 hr if the hemagglutination reaction was carried out at 25 C; HR behaved similarly.

For the ability of hemagglutination to change drastically and reversibly with temperature, the hemagglutinin must consist of a temperature-sensitive moiety, presumably protein. Since the hemagglutinating activity of Sindbis is associated with some structure of the viral envelope (7), and the viral envelope of the Sindbis virus contains one single protein, the membrane protein (8, 10), the hemagglutinin probably involves the membrane protein moiety. Biochemical studies indicated that ts 23 is defective in the viral membrane protein (3, 10). It is reasonable to speculate that

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TABLE 1. *Hemagglutinin titers at 25 and 37 C*

Virus	Plaque-forming units at 27 C	Temp of hemagglutination	Hemagglutinin titer
		C	
ts 23	2×10^9	25	1,024
		37	<2
HR	2×10^9	25	1,024
		37	1,024
ts 23 heated at 37 C for 12 hr		25	1,024
HR heated at 37 C for 12 hr		25	1,024

the ts 23 virion probably contains a substituted amino acid in its membrane protein. This substituted amino acid may cause the hemagglutinin to lose its functional configuration for the hemagglutination reaction at 37 C but not at 25 C. This change of the functional configuration at 37 C is reversible.

At the nonpermissive temperature, Sindbis ts 23 can adsorb to and penetrate the host cell, but viral maturation is defective (3, 10). If the temperature sensitive defect in the membrane protein is assumed to be the only defect in ts 23, it is reasonable to conclude that the active site in the membrane protein responsible for hemagglutination is the same site that is involved in viral maturation. On the other hand, viral adsorption

and penetration to the host cell either do not involve this site or do not involve the viral membrane protein at all.

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