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Preliminary Report

Hemagglutinins from L Cells Treated with Trypsin

Recently, Youngner and Rubinstein (1962) have isolated two hemagglutinins from chorioallantoic membrane suspensions (supernatant at 2,600 rpm) of embryonated eggs infected with vaccinia virus; one was heat stable and virus specific and the other was heat labile and corresponded with the normal tissue agglutinin present in uninfected membrane suspensions (supernatant at 2,600 rpm).

The present paper deals with two hemagglutinins obtained from mouse fibroblast L cells treated with trypsin and not infected by virus; a heat labile one in the supernatant at 3,000 rpm (SU fraction) and a heat stable one in the sediment at 3,000 rpm (SE fraction). Studies were made on the relation between the two hemagglutinins from uninfected cells treated with trypsin and from cells infected by HA^+ virus.

Two types of the IHD strain of vaccinia virus were used. One produced hemagglutinin and the other did not (Fujio, 1962). Viral samples were obtained by freezing and thawing infected L cells and centrifuging the resulting mixture at 3,000 rpm for 10 minutes to remove cellular debris. Supernatants were prepared containing 5.0×10^7 $TCID_{50}$ virus per ml, containing 80 HA units of hemagglutinin in HA^+ virus-infected samples and which were free of demonstratable hemagglutinin in HA^- virus-infected samples. Supernatants were obtained from L cells infected with either HA^+ or HA^- virus. These which incubated with 0.5 per cent trypsin at 37°C for one hour and then soybean trypsin inhibitor was added to stop the trypsin activity. These supernatants were then found to have 1280 HA units. Thus, the hemagglutinating activity had increased. It is interesting that hemagglutinin was produced from not only HA^+ virus-infected cells but also from HA^- virus-infected cells after these cells had been treated with trypsin. The activity of the hemagglutinin produced by treatment with trypsin was reduced when the solution was heated at 56°C for 60 minutes but the activity of the hemagglutinin produced by HA^+ virus infection was unaffected by heat treatment. The hemagglutinin produced by trypsin treatment was identical in its behavior with the 30 cream fraction described by Youngner and Rubinstein (1962).

Immediately after centrifugation no hemagglutinating activity was detected in the supernatant at 3,000 rpm (SU fraction) of uninfected L cells which had not been treated with trypsin. When this fraction was incubated with 0.5 per cent trypsin solution at 37°C for one hour, hemagglutinating activity was detectable. When the sediment at 3,000 rpm (SE fraction) from uninfected L cells was incubated with trypsin, its hemagglutinating activity increased to a higher level than that of the SU fraction. This suggests that hemagglutinins present in the original L cells in an inactive form might be activated by trypsin. The activated hemagglu-

tinin of the SU fraction was inactivated by heating at 56°C, whereas that of the SE fraction was heat stable. These results are shown in Table 1.

Table 1. Hemagglutinating Activity of Various Fractions from L Cells Infected and not Infected by Vaccinia Virus

Hemagglutinin from:	Infectivity titer/ml*	HA units			
		Before trypsin treatment	After trypsin treatment**	Heat treatment after trypsin treatment*** 30 min.	60 min.
SU fraction from HA+ Virus infected cells	5.0×10^7	80	—	80	80
SU fraction from HA+ virus infected cells	5.0×10^7	80	1280	80	80
SU fraction from HA− virus infected cells	5.0×10^7	<10	1280	80	40
SU fraction from uninfected cells	—	<10	1280	160	40
SE fraction from uninfected cells	—	<10	2560	1280	1280
Phosphate buffered saline (pH 7.2)	—	<10	<10	<10	<10

* Titration of infectiyity was carried out in L cells by the 50 per cent end point method ($TCID_{50}$).

** Mixtures of 0.5 ml of each ftaction and 0.5 ml aliquots of trypsin solution were incubated at 37°C for one hour before HA titration.

*** Heated at 56°C

The activity of these hemaggltuinins was detectable with only certain fowl red blood cells, which were susceptible to hemagglutinin from L cells infected by HA+ virus. These hemagglutinins agglutinated mouse red blood cells, but not rabbit and human red blood cells. The correspondence in susceptibility not only between different species but also between different individuals within a species is probably due to the presence of a simillar molecular structure which responsible for the union of the agglutinins with the red cell surface, irrespective of whether the hemagglutinins are obtained by trypsin or by HA+ virus infection.

Inhibition tests were carried out with antiserum to study the relation between the hemagglutinins obtained from uninfected cells treated with trypsin and from cells infected by HA+ virus. The activity of the two kinds of hemagglutinins obtained from uninfected cells treated with trypsin was strongly inhibited by normal rabbit serum and HA+ and HA− virus immune rabbit sera. Probably this inhibition of hemagglutination was non-specific since the agglutination by the two kinds of hemagglutinins from uninfected cells treated with trypsin was completely inhibited by normal mammalian sera (bovine or mouse) and egg albumin. In contrast, the activity of hemagglutinin obtained from cells infected by HA+ virus was inhibited by HA+ virus immune rabbit serum but not by HA− virus immune rabbit serum or normal rabbit serum. However, the hemagglutinating

activity of the HA+ virus infected supernatant after heating at 56°C for 30 minutes was strongly inhibited by HA+ and HA− virus immune sera and normal rabbit serum (Table 2). These facts indicate that the two kinds of hemagglutinins obtained from uninfected cells treated with trypsin are non-specific and the hemagglutainin obtained from cells infected with HA+ virus is virus-specific.

Table 2. HI Titers of HA^+ and HA^- Vaccinia Virus Immune Rabbit Sera and Normal Rabbit Serum against Various of Uninfected and Infected L Cells

Cells	Fraction	HI titer against 10 HA units of various fractions (HI units/ml)		
		HA^+ virus immune rabbit serum	HA^- virus immune rabit serum	Normal rabbit serum
Uninfected cells	SU fraction*	2048	2048	2048
	SE fraction*	2048	2048	2048
HA^+ virus infe-	SU fraction	256	8	4
cted cells	SU fraction**	2048	2048	2048

* Treated with trypsin.

** Heated at 56°C for 30 minutes.

The two kinds of hemagglutinin from uninfected cells treated with trypsin and from cells infected by HA+ virus were unaffected by twenty-four hours dialysis against phosphate buffered saline (pH 7.2). They were soluble in ether and chloroform, inactivated at below pH 5.5 and precipitated in 50 per cent ammonium sulfate solution. These facts suggest that these hemagglutinins are "lipoproteins". The serological specificity of the hemagglutinins obtained from cells infected by HA+ virus, their inactivation of the hemagglutinins by heat and at below pH 5.5, and their precipitation by half saturated ammonium sulfate all suggest that the protein component is rather labile.

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