INVESTIGATION OF ELECTROPHORETIC MOBILITY AND IMMUNOSPECIFICITY OF THE NEURAMINIDASES OF INFLUENZA VIRUS AND HOST CELLS

Kh. Zh. Zhumatov, E. S. Isaeva,
Z. K. Chuvakova, and O. G. Stetsenko

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Preparations of purified neuraminidases of influenza virus and of cells of the chorioallantoic membrane of chick embryos were investigated by zonal electrophoresis. The enzyme of strains A2 (Singapore/57) and A2 (Alma-Ata 397/67) of influenza virus was shown to be electrophoretically less heterogeneous than that of strains A2 (Alma-Ata 454/65) and A2 (USSR 0467/69). Components similar to cellular neuraminidase in electrophoretic mobility are constantly present in the virus neuraminidase. Virus-specific neuraminidase can by differentiated serologically in the zones of the paper after electrophoresis.

According to one report the neuraminidase of influenza virus (strain V-LI), isolated by disintegration of the viruses with sodium dodecylsulfate, has low electrophoretic mobility and possesses a certain immunological affinity for the neuraminidase of the host cell [3]; the latter can be separated by ultracentrifugation in a sucrose density gradient [4].

This paper describes the results of a comparative investigation of the electrophoretic mobility of neuraminidases obtained from different strains of influenza virus and chorioallantoic cells of chick embryos (CACE) as a widely used medium for the maintenance of this virus. The specificity of the various zones after electrophoresis of the neuraminidase from the virus and CACE cells was determined serologically.

EXPERIMENTAL METHOD

Experiments were carried out with local strains A2 (Alma-Ata 397/57), A2 (Alma-Ata 454/65), and A2 (Alma-Ata 770/64) and reference strains A2 (Singapore 1/57) and A2 (USSR 0467/69) of influenza virus. The reference strains were obtained from the museum of the D. I. Ivanovskii Institute of Virology, Academy of Medical Sciences of the USSR. Before extraction of the neuraminidase, all the strains were subcultured more than 20 times in CACE and their neuraminidase activity on ovomucoid, pH 6.3, was 24 ± 1.9, 8.7 ± 1.3, 16.8 ± 2.5, 18 ± 2.1, and 10.0 ± 0.8 units $\times 10^{-3}$/AE respectively. The CACE were taken from normally developing 12-day chick embryos.

Soluble neuraminidase was isolated from CACE cells and from influenza virus purified and concentrated in the usual way by the methods described previously.

Electrophoresis on paper and subsequent treatment of the paper were carried out as follows. The enzyme was applied in a quantity of 20×10^{-3} units to a strip of electrophoresis paper (mark M), 4.5 cm wide, soaked in Tris-boric acid-versene buffer, pH 8.9 [3], near the cathode, and the paper was placed in an electric field (in the cell of a type ÉFA-1 apparatus). Electrophoresis continued for 3 h with a constant voltage gradient of 10 V/cm. In a medium of alkaline reaction created by the buffer solution the proteins have a negative charge, and in the electric field they migrate along the filter paper toward the anode.

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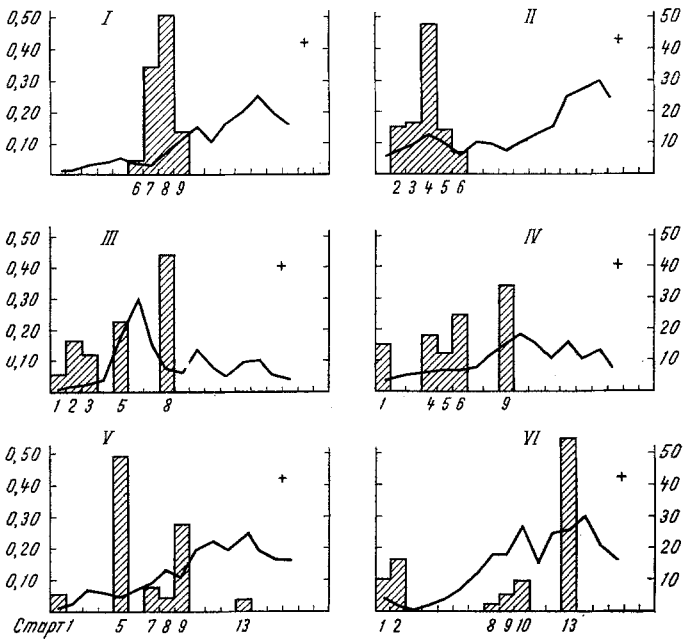

Fig. 1. Localization of neuraminidase activity on paper strip after electrophoresis of neuraminidase preparations from influenza virus A2 [Singapore (I), A2-397 (II), A2-454 (III), A2-0467 (IV), AO-770 (V), and also from normal CACE cells (VI)]. Origin at the cathode. Ordinate on the left, optical density at 280 nm, on the right, neuraminidase activity as a percentage of total activity (columns); abscissa, Nos. of zones of paper after electrophoresis.

After the end of electrophoresis the paper was cut into 16 equal transverse strips (zones). Each zone of the paper was cut up with scissors, covered with 5 ml 0.15 M NaCl, and kept for 30 min at 37°C. The eluates were then tested for the presence of protein (from their absorption at 280 nm) and for neuraminidase activity. To 0.1 ml of the original solution of the enzyme or 1 ml of the eluates from the zones of the paper, 1 ml (5 mg) of ovomucoid dissolved in 0.1 M phosphate buffer, pH 5.5, was added, and the quantity of sialic acids liberated was determined after 1 h (37°C). The quantity of enzyme which, under standard conditions, splits 1 μmole neuraminic acid from the substrate per minute was taken as the unit of enzyme.

Antineuraminidase sera were obtained by immunizing rabbits (2.0-2.5 kg) with purified preparations of neuraminidase. From one to three units of enzyme was introduced beneath the conjunctiva of the upper lid of the left and right eyes alternately [2] for 3 days. The cycle of immunization was repeated three times at intervals of 2 days. Seven days after the last injection the cycle of immunization was again repeated with the same intervals. Nine days after the last injection the animal was exsanguinated and the titer of antineuraminidase antibodies determined by the inhibition of enzyme activity test. For this purpose 0.1 ml of the immune serum (control-normal serum), treated with periodate (to remove nonspecific inhibitors), was added to an equal volume of a solution of neuraminidase containing 13-20 $\times 10^{-3}$ enzyme unit, the mixture was incubated for 30 min at 37°C, after which 0.2 ml (1 mg) of ovomucoid was added, and 1 h later (37°C) a test for sialic acids was carried out by the usual method. The titer of antineuraminidase sera was 1:160-1:320.

EXPERIMENTAL RESULTS

The localization of neuraminidase activity in the enzyme preparations tested by zonal electrophoresis is shown in Fig. 1. The character of electrophoretic mobility of the neuraminidase differed considerably among the five strains of influenza virus. Strain differences were seen near the cathode and in the neutral zone (zones from Nos. 1 to 9). The zones of activity for two enzyme preparations (Fig. 1: I and II) were confluent, suggesting homogeneity of the protein whereas the zones of localization of enzyme activity of the other three strains formed three or four (Fig. 1: III-V) fractions.

TABLE 1. Neutralization of Activity of Neuraminidases from Different Zones after Electrophoresis

Soruce of neuraminidase	No. of zone (antigen)	Dose of antigen (units × 10^{-3})	Titer of antineuraminidase serum	Neuraminidase activity in the presence of serum			
				normal	immune sera against enzyme		
					cellular	A2-454	AO-770
Strain A2-454	1—2	3,61	10^{-1}	3,48	0,28	0	1,36
	3	2,82	10^{-1}	2,72	1,79	0	2,80
	5	4,00	10^{-2}	4,00	4,52	0	3,68
	8	9,10	10^{-1}	9,07	7,86	0	8,98
Strain AO-770	1	1,07	10^{-1}	1,00	0,06	0,67	0
	5	9,62	10^{-2}	9,71	9,70	9,48	0
	7—9	8,25	10^{-1}	8,18	6,49	7,81	0
	13	0,60	10^{-1}	0,58	0,005	0,57	0
CACE cells	1—2	5,02	10^{-1}	5,07	0	1,35	1,70
	8—10	3,00	10^{-1}	3,12	0	2,97	2,81
	13	12,02	10^{-1}	11,99	0	11,97	10,24

Neuraminidase of the CACE cells also was heterogeneous but, unlike the influenza virus enzyme, most of its activity was located in the anodic part after electrophoresis (zone No. 13; Fig. 1: VI).

The preparations of neuraminidase both from influenza virus and from CACE cells contained components with identical electrophoretic mobilities. In order to differentiate them, eluates from the zones of paper after electrophoresis of the enzymes from influenza viruses AO-770 and A2-454 were investigated in the neutralization test with virus and cellular antineuraminidase sera. These sera were treated in the usual way with potassium periodate, dialysed against 0.15 M NaCl (4°C), and heated at 56°C (30 min). In the control test, normal rabbit serum treated in the same way was used. The experiments were repeated three times. The results of one of them (Table 1) show that the virus-specific enzyme of strains A2-454 and AO-770 is located in zone No. 5 after electroresis, and its activity is completely suppressed in the presence of homologous serum. The enzyme located in zones Nos. 1 and 2 after electrophoresis is immunologically identical with cellular neuraminidase. For example, compared with 3.6×10^{-3} enzyme unit of strain A2-454, after treatment with antiserum against cellular neuraminidase only 0.28×10^{-3} unit of activity remained.

The eluate of neuraminidase from zone No. 13 (strain AO-770) likewise was neutralized by cellular antineuraminidase serum. It must be emphasized that eluates from zones Nos. 1 and 2 of the paper after electrophoresis of cellular neuraminidase were partially inactivated by the virus antineuraminidase sera. In all probability the primary antigen of virus neuraminidase, with which the rabbits were immunized, was not completely purified from neuraminidase of the CACE cells.

Heterogeneity of the enzyme from three strains of influenza virus and the host cell was thus demonstrated by zonal electrophoresis. The electrophoretic mobility of the neuraminidase from strains A2 (Singapore 1/57) and A2 (Alma-Ata 397/57) were shown to differ from that of the neuraminidase of strains A2 (Alma-Ata 454/65) and A2 (USSR 0467/69). The enzymes of the last two strains were similar in their electrophoretic mobilities. These results probably reflect variation of the antigenic properties of A2 influenza virus neuraminidase during the years of its circulation in population groups [5].

Neuraminidase of the CACE cells, by contrast to the enzyme of the strains of influenza virus tested, is bound with a component migrating rapidly in an electric field. However, components with equal electrophoretic mobility are found in preparations of both cellular and virus neuraminidase. What is not clear is whether cellular neuraminidase participates in the activation of virus infection. According to the writers' preliminary findings, antiserum against cellular neuraminidase facilitates the viropexis of, for example, influenza virus AO (Alma-Ata 770/64). The neuraminidase of this strain has an electrophoretic mobility identical with that of strain A2 (Alma-Ata 454/65), but their enzymes in zone No. 5 after electrophoresis (Table 1; Fig. 1: III and V) clearly exhibit serological specificity.

In a previous investigation [1], using histoautoradiographic methods the writers demonstrated viropexis of the above AO virus (Alma-Ata 770/64) in the respiratory tract of albino mice. The pathogentic role of the neuraminidases of influenza virus and of the host cell must evidently be represented rather more widely than has hitherto been considered.

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