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SOME PROPERTIES OF MUMPS VIRUS NEURAMINIDASE

H. KLAMM

Institute for Applied Virology, DDR-119 Berlin, German Democratic Republic

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Summary. — Mumps virus strains were characterized by the neuraminidase activity of infected allantoic and amniotic fluids of chick embryos. Ovomucoid was used as substrate for determination of neuraminidase activity. Storage of virus samples at 4 °C led to great losses of enzyme activity. Alkali chlorides, divalent cations, EDTA and N-ethylmaleimide exerted a low to medium inhibitory activity. Surfactants, depending on their type, caused inhibition, activation or no effect on the enzyme — substrate reaction. The pH optimum was 5.6 and 5.3 with mumps virus strains Enders and Jeryl Lynn, respectively. The Michaelis constants of 9.43×10^{-3} and 7.57×10^{-3} mol/l allowed a differentiation of the two mumps virus strains based on their neuraminidase.

Key words: *mumps virus*; *neuraminidase*; *strain differentiation*

Introduction

Mumps virus (genus *Paramyxovirus*) consists of a helical nucleocapsid and an envelope with spikes possessing haemagglutinating, neuraminidase and haemolytic activities. The haemagglutinating and neuraminidase activities are both carried by a single glycoprotein HN (East *et al.*, 1971; Jensik *et al.*, 1976; Örvell, 1978). Neuraminidase (N-acetylneuraminylglycohydrolase E.C. 3.2. 1.18) splits off hydrolytically the α-ketosidically bound N-acetyl neuraminic acid (sialic acid) from appropriate substrates like oligo- and polysaccharides, gangliosides and glycoproteins.

The aim of the present study was to characterize different mumps virus strains based on the properties of their neuraminidase.

Materials and Methods

Mumps virus strains and sources of neuraminidase. Strain Enders in the 55th egg passage was used in the form of untreated allantoic fluid. The vaccine strain Jeryl Lynn was derived from the "Mumpsvax" vaccine (Behringwerke, FRG) and had undergone 3 egg passages in our laboratory. The virus-containing materials were stored at about −65 °C.

Determination of neuraminidase activity. Ovomucoid, containing about 0.9% bound N-acetyl neuraminic acid (NANA) was used as substrate. After precipitation of undesired proteins with a 2 :1 mixture of ethanol and 0.5 M trichloroacetic acid, ovomucoid was prepared by mixing egg white with the double volume of ethanol at pH 6.0. The crude ovomucoid was dissolved in 0.025 M acetate buffer, pH 4.5, and re-precipitated with ethanol. Incubation of the enzyme — substrate mixture and determination of the liberated NANA were carried out by the method of Aymard-Henry *et al.* (1973) as recommended for influenza virus neuraminidase. The substrate concentration was, as a rule, 100 mg/ml.

The Michaelis constant was calculated in the usual way. Reciprocal values of the reaction velocity as measured by optical density and the amount of bound NANA were plotted according to Lineweaver and Burk (1934). The Michaelis constant was calculated from the intersection point of this straight line with the abscissa (Gray, 1976).

Surfactants. The following were used: sodium dodecyl sulphate (SDS) (FERAK, West Berlin); Triton X-100, Hyamin 1622 and Sarkosyl NL-35 (Serva, Heidelberg, FRG); Tween 20 and Tween 80 (Atlas Chemie, Essen, FRG); and Won 100 (VEB Chemische Werke Buna, Schkopau, GDR).

Results and Discussion

Mumps virus-containing materials have to be stored at temperatures below −60 °C, because at higher temperatures their infectivity is rapidly lost. Changes in neuraminidase activity of preparations stored at 4 °C were examined. After thawing the deep-frozen sample, the neuraminidase activity dropped rapidly within the first 24 hr; subsequently, the decrease slowed down. After 169 days the enzyme activity decreased to about 25% of the original value. At this interval, electron microscopy of such preparations revealed no intact, but only broken virus particles.

Alkali chlorides exerted only little effect on neuraminidase activity (Fig. 1). Inhibition was observed in all cases; it was the most marked with potassium chloride, reaching almost 50% with 0.2 M KCl.

The effects of ionic and nonionic surfactants on the activity of mumps virus neuraminidase are illustrated in Fig. 2. Anionic surfactants (SDS, Sarkosyl) blocked the enzyme activity already at low concentrations. The

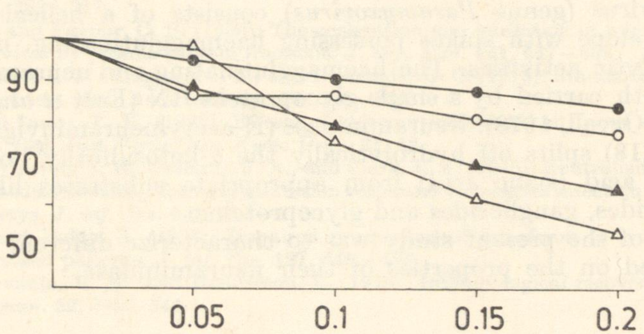

Fig. 1.

Effect of alkali chlorides on mumps virus (strain Enders) neuraminidase
Abscissa: salt concentration (M); ordinate: neuraminidase activity (E_{549})
● CsCl, ○ NaCl, ▲ LiCl, △ KCl

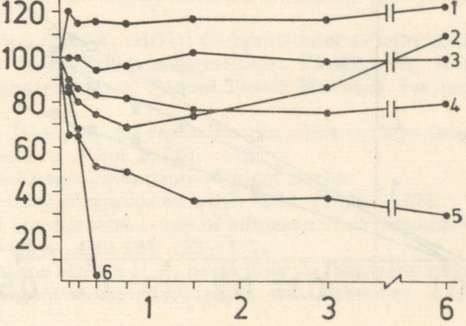

Fig. 2.

Effect of surfactants on mumps virus (strain Enders) neraminidase
Abscissa: surfactant concentration in % (w/v); ordinate: neuraminidase activity in % of control without surfactant
Curves: 1 — Tween 80, 2 — Tween 20, 3 — Won 100, 4 — Triton X-100, 5 — Hyamin 1622, 6 — SDS

cationic Hyamin 1622 caused inhibition (about 70%) but no blockade. Nonionic surfactants enhanced the activity (Tween 80) or had no or little effect (Triton X-100, Won 100). Tween 20 at low concentrations caused inhibition and at high concentrations an enhancement of neuraminidase activity. The enhanced activity could have been due to the splitting off of HN glycoprotein from the virion without affecting the active centre of the enzyme. The relative separation of the glycoprotein could facilitate the cleavage of sialic acid.

The neuraminidases of different ortho- and paramyxovirus strains differ in their pH optima. The lowest pH optimum of 3.8 was reported for Bangor virus with fetuin as substrate (Alexander, 1974) and the highest of 7.0 for influenza A/PR 8 virus with (α, 2 → 3) sialyllactose as substrate (Rafelson *et al.*, 1963). The position of the optimum depends on the kind and concentration of substrate. For the neuraminidase of mumps virus strain Enders, a pH optimum of 5.5 with fetuin as substrate was reported (Brostrom *et al.*, 1971). In the present experiments the following pH optima were determined with ovomucoid used as substrate in a high concentration (250 mg/ml): pH 5.6 for the Enders strain and pH 5.3 for the Jeryl Lynn strain. In spite of a different substrate used, these values are in good agreement with that reported by Brostrom *et al.* (1971). The maxima of neuraminidase activity are rather broad and the peak values are thus difficult to determine accurately. Therefore a biochemical differentiation of mumps virus strains based on pH optima of neuraminidase activity cannot be regarded as reliable.

Bacterial and viral neuraminidases have frequently been activated by the addition of divalent cations, mainly Ca^{2+} and Mg^{2+} (Drzeniek, 1972). I examined the effect on mumps virus neuraminidase of several cations and other substances in a concentration of 0.01 M. An activation was never observed. Slight inhibition was shown by Fe^{2+} (11%), Fe^{3+} (17%), Mg^{2+} (17%), Ca^{2+} (14%), and EDTA (9%). Zn^{2+} (47%), Mn^{2+} (44%) and the mercury-containing Merthiolate (43%) proved to be more potent inhibitors. An increase in EDTA concentration to 0.1 M caused 42% inhibition. N-ethylmaleimide (0.01 M) caused no inhibition, suggesting that no sulfhydryl groups

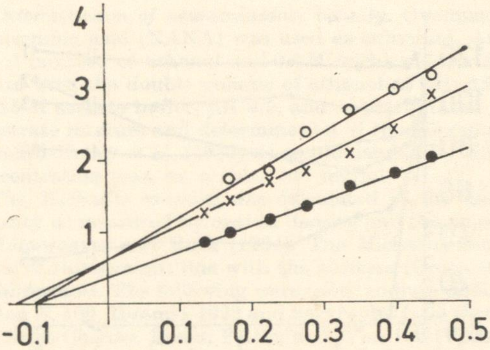

Fig. 3.

Lineweaver-Burk plots of mumps virus neuraminidase with ovomucoid at pH 5.8 Abscissa: $^1/s$ (s = mmol bound NANA per l); ordinate: $^1/v$ (v = velocity of the enzymatic reaction expressed in E_{549}) ○ and ● : Enders strain 1 : 30 and 1 : 15, respectively × : Jeryl Lynn strain

in the active centre of the enzyme are involved in the reaction with the substrate.

The hydrolysis of NANA in appropriate substrates by mumps virus neuraminidase followed the laws of classical reaction kinetics. From the plots shown in Fig. 3 the following Michaelis constants K_M were calculated:

Enders strain	$K_M = 9.43 \times 10^{-3}$ mol/l
Jeryl Lynn strain	$K_M = 7.57 \times 10^{-3}$ mol/l.

The difference was statistically significant. From the common intersection point with the abscissa of the two straight lines for two concentrations of the Enders strain shown in Fig. 3 it may be concluded that the K_M value is independent of the enzyme concentration and thus of virus concentration. This means that highly purified virus preparations are not necessarily required for the determination of Michaelis constants, provided that the virus suspensions used contain no activators or inhibitors.

As distinct from the present results, Leprat (1978), using 2-(3-methoxyphenyl)-N-acetyl neuraminic acid as substrate, found the same Michaelis constant of 0.33×10^{-3} mol/l for neuraminidase of two strains derived from wild mumps viruses. But further studies on wild and vaccine strains of mumps virus (Klamm, unpublished) revealed clear-cut differences in the K_M values. Kendal and Madeley (1969) described different kinetics of influenza virus neuraminidases and stressed that the antigenic site and the active centre of neuraminidase are not identical. According to Thomas *et al.* (1978), influenza virus neuraminidases of the serological subtypes N1 and N2 can, in some cases, be differentiated also by the K_M values. But it remains obscure what is the importance of the K_M value in the biological role of neuraminidase in virus infection.

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