DIFFERENCES IN SUBSTRATE SPECIFICITY OF MYXOVIRUS NEURAMINIDASES(*)

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Received December 8, 1969

SUMMARY

Neuraminidase of NDV splits the (α, 2→8) linkage between two adjacent NANA molecules of disialyllactose almost as easily as the (α, 2→3) linkage between NANA and D-galactose present in 3'-sialyllactose. FPV neuraminidase, however, is barely capable of liberating NANA from disialyllactose within 15 minutes at pH 7.0, although it splits 3'-sialyllactose. These differences in substrate specificity furnish additional proof that neuraminidases of myxoviruses are coded by the viral genome. The enzymes may be used to determine the kind of linkage between NANA and the joint carbohydrate.

Three types of α-ketosidic linkages have been demonstrated between acylated neuraminic acids and different carbohydrates, i.e. 2→3, 2→6 and 2→8 linkages. The enzyme neuraminidase (E.C. 3.2.1.18) from Vibrio cholerae is able to split all three types of linkages between NANA and other carbohydrates (1,2).

On the other hand it was shown that neuraminidases of some myxoviruses (influenza A2 virus, FPV and NDV) hydrolize 2→6 linked NANA much more slowly than 2→3 linked molecules

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*Abbreviations: NDV - Newcastle disease Virus
FPV - Fowl plague virus
NANA - N-acetylneuraminic acid

(3,4). The former compounds even acted as competitive inhibitors on NDV neuraminidase at pH 7.0 ($K_i = 1 \times 10^{-3}$ M), when 3'-sialyllactose was used as substrate (4,5).

METHODS AND RESULTS

The following substrates were used: 3'-sialyllactose (N-acetylneuraminosyl (α, 2→3)galactopyranosyl(β, 1→4)glucopyranose) isolated by the method of Schneir and Rafelson (3) and the disodium salt of disialyllactose (N-acetylneuraminosyl (α, 2→8) N-acetylneuraminosyl (α, 2→3)galactopyranosyl (β, 1→4)glucopyranose) prepared according to Kuhn and Gauhe (6).

V. cholerae neuraminidase was obtained from Behringwerke AG, Marburg/Germany. Intact virions were used as viral neuraminidase. Fowl plague virus, strain "Rostock" (FPV) and Newcastle disease virus strain "Italien" (NDV) were purified by high and low speed centrifugation as described previously (7). The test procedure and the definition of one enzyme unit were given in detail elsewhere (4).

As indicated in figures 1 and 2 the total amount of NANA present can be released within 24 hours from 3'-sialyllactose and disialyllactose by V. cholerae and NDV neuraminidases. A detailed analysis shows a linear correlation between release of NANA and the time of incubation within the first 30 minutes for both enzymes and substrates. The initial velocity rate (v)* for the hydrolysis of disialyl-

* $$v = \frac{\text{initial velocity of disialyllactose hydrolysis}}{\text{initial velocity of 3'-sialyllactose hydrolysis}}$$

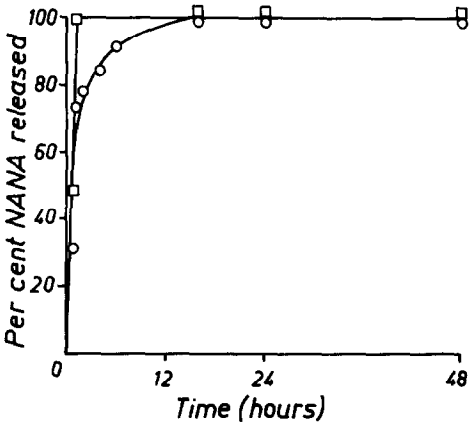

Fig. 1: Release of NANA from 3'-sialyllactose and disialyllactose by V. cholerae neuraminidase. 3 milliunits of enzyme were incubated at 37° C with 3'-sialyllactose (□—□) or disialyllactose (o—o) in 0.5 ml of 0.05 M acetate buffer, pH 5.5 supplemented with 0.9% NaCl and 0.1% $CaCl_2 \cdot 2H_2O$. A total amount of 45 ± 5 µg NANA was used in each test. The liberated NANA was estimated according to Aminoff (9) and expressed as the percentage of total NANA in the sample.

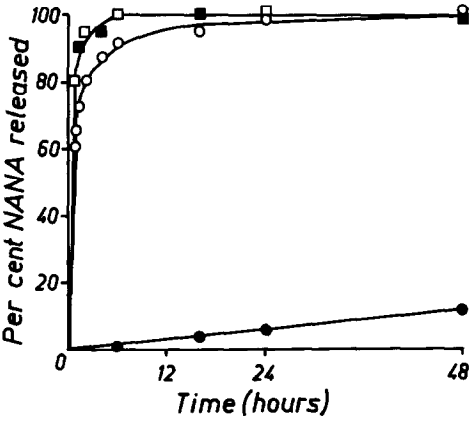

Fig. 2: Release of NANA from 3'-sialyllactose and disialyllactose by NDV and FPV neuraminidase. 25 milliunits of enzyme were used in 0.5 ml of 0.1 M phosphate buffer at pH 7.0 and 37° C. All other data are as given in Fig. 1.

□—□ NDV + 3'-sialyllactose
■—■ FPV + 3'-sialyllactose
o—o NDV + disialyllactose
●—● FPV + disialyllactose

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lactose at pH 5.5 by V. cholerae neuraminidase is v = 0.7 and bei NDV neuraminidase at pH 7.0 v = 0.3. In contrast,

FPV neuraminidase hardly attacks disialyllactose: 25 milliunits of this enzyme preparation released no more than 12% of NANA even during an incubation period of 48 hours.

During short time incubations (15 min.) V. cholerae as well as NDV neuraminidase liberates between 80 - 100% of NANA from 3'-sialyllactose and disialyllactose, when the enzyme activities are sufficiently high. FPV enzyme, however, hydrolyzes only minute amounts of NANA from these substrates (Fig. 3). In several experiments up to 250 enzyme units of FPV never released more than 5% of NANA. Even higher substrate concentrations (200 µg of total NANA per sample) did not alter these results.

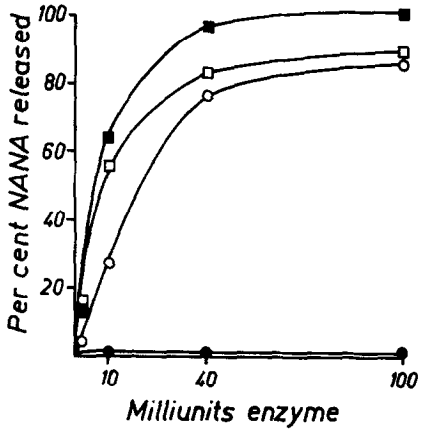

Fig. 3: Action of increasing amounts of FPV and NDV neuraminidase on 3'-sialyllactose and disialyllactose. Incubation time: 15 minutes. Further conditions as in Fig. 2.

A higher amount of NANA was released only if high concentrations of FPV were incubated for 24 hours (Fig. 4). Under such experimental conditions up to 250 milliunits of enzyme liberated 78 $\pm$ 8% of NANA. The velocity ratio between disialyllactose and 3'-sialyllactose for the fowl

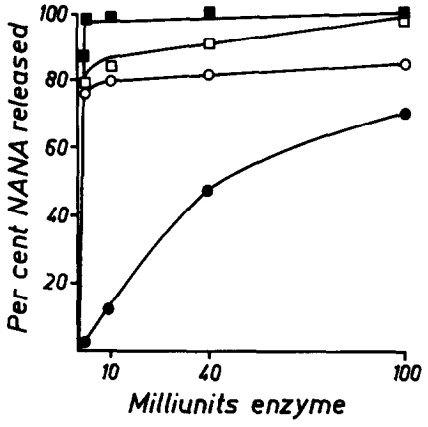

Fig. 4: 24 hours incubation of FPV and NDV neuraminidase with 3'-sialyllactose and disialyllactose. Further conditions as in Fig. 2.

plague virus enzyme was calculated to be $v = 1 \times 10^{-4}$. Neither a thermal inactivation of the neuraminidase in the virus preparations nor a detectable NANA destruction could be observed during the incubation period.

DISCUSSION

Whereas NDV neuraminidase acts on disialyllactose almost as fast as the enzyme of V. cholerae, the reaction rate of the FPV preparation was 10 000 times slower. It is interesting to note that during short incubation periods neither the 2→8 linkage nor the 2→3 linkage of both NANA molecules joined one after another in disialyllactose are cleaved by FPV neuraminidase. Since this enzyme hydrolyzes the 2→3 linkage in 3'-sialyllactose very easily it should be assumed that the resistance of this linkage in disialyllactose is due to the inability of FPV neuraminidase to split the 2→8 linkage of this substrate in an appropriate time. The resistance of the 2→8 linkage against FPV neuraminidase is comparable

to the resistance of the 2→6 linkage against viral neuraminidases (4).

The reported substrate specificity reveals differences in the activity of two viral neuraminidases investigated. These highly differenciated active sites find their counterpart in the antigenic specificities of viral neuraminidases described earlier (7). These observations in combination with inhibition and inactivation studies (8) show that the synthesis of viral neuraminidases is directed by the viral genome.

ACKNOWLEDGEMENTS

The cooperation of Miss M. Orlich and Prof. Rott's encouraging discussions are gratefully acknowledged. The work was supported by the Deutsche Forschungsgemeinschaft.

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