Neuraminidase: the specific enzyme of influenza virus and *Vibrio cholerae*

Over the last years it has become evident that N-acetyl neuraminic acid and diacetyl neuraminic acid (= sialic acid) respectively are present in varying amounts in most mucoproteins and in many glycolipids of animal origin[1]. The biological interest in the acetylated neuraminic acids arises from the facts that (a) absorption of the influenza virus particle to potential host cells, (b) agglutination of erythrocytes by influenza virus and (c) the competitive inhibition of virus haemagglutination by mucoproteins are intimately associated with the neuraminic acid content of the cellular receptors and their chemical analogues, the soluble inhibitory mucoproteins. The influenza virus particle has embedded in its surface an enzyme splitting off from soluble inhibitory mucoproteins the terminal neuraminic acid units; the viral enzyme is imitated in detail by an exo-enzyme produced by *Vibrio cholerae* (RDE)[1]. The molecular structure assigned previously[2] to neuraminic acid has been strongly supported by BLIX *et al.*[3] and by KUHN *et al.*[4]. In natural products, neuraminic acid is invariably found together with other sugars, galactose and/or galactosamine being the most regular associates. Concerning the linkage of neuraminic acid in mucoproteins it was recently established that in bovine submaxillary gland mucoprotein (BSM) the potential keto group of neuraminic acid is linked in glycosidic fashion to its partner, almost certainly a D-galactosamine residue[5]. Whether this glycosidic linkage in BSM is mediated by an O or N atom has still to be ascertained.

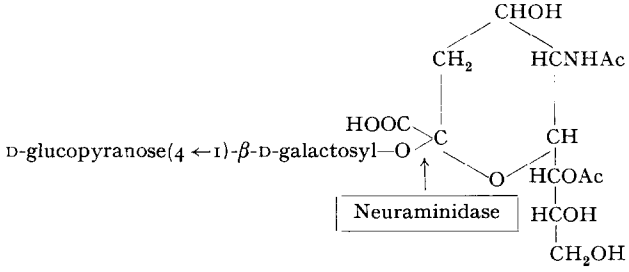

Fig. 1

The uncertainty about the true nature of the glycosidic atom, O or N, joining neuraminic acid in BSM or other mucoproteins to its partner made it desirable to test the activity of the virus glycosidase and of RDE on a neuraminic acid containing compound with well defined glycosidic structure. Neuramin-lactose (N-L) conforms to this requirement. This compound, isolated from mammary glands of rats by TRUCCO AND CAPUTTO[6], consists of diacetyl neuraminic acid and lactose. With the Somogyi reagent, N-L has a reducing value corresponding to 83% of that of an equivalent amount of lactose, whereas the reduction equivalent of free sialic acid is only 15%. N-L is almost completely hydrolysed into its components in 3 min by 0.5 *N* sulfuric acid at 100° C[6]. From these data we may conclude that the reducing group of neuraminic acid is engaged in an O-glycosidic linkage with an OH-group of galactosyl, the reducing group of lactose forming the reducing end of the molecule (Fig. 1). Only a molecular arrangement characterizing N-L as a 2-deoxyglycoside will account for the great ease of acid hydrolysis; a furanoside structure can be excluded. The high reducing power of N-L and the resistance of its lactose moiety towards *Saccharomyces fragilis* β-galactosidase indicates that neuraminic acid is linked to the galactose rather than to the glucose component of lactose (Fig. 1).

Material. (1) Highly purified influenza A virus (WSM) in buffered saline (pH 6.5) of haemagglutinin titre $2 \cdot 10^6$/ml (against 1% fowl erythrocytes) and of enzymic titre $2.2 \cdot 10^5$/ml. (2) Purified RDE in 0.02% $CaCl_2$ solution ($2 \cdot 10^5$ units/ml). (3) Neuramin-lactose containing 48.2% lactose (anthrone method) indicating 95% purity.

Experimental. Assay I: 1 mg N-L in 25 µl virus solution (pH 6.5). Assay II: 1 mg N-L in 25 µl RDE solution (pH 6.0). Controls: (a) 2% lactose (in water); (b) 4% N-L (in water); (c) 2% N-acetyl neuraminic acid (in water); (d) virus solution and (e) RDE solution. Volume of individual control: 50 µl. Assays and controls were kept at 35° C and samples withdrawn at intervals. 3.5 µl of each sample was applied to Whatman No. 1 filter paper and chromatographed by the descending method (20° C).

TABLE I

EFFECT OF VIRAL AND BACTERIAL NEURAMINIDASE ON NEURAMIN-LACTOSE

R_F values of controls and assays after various times of digestion. Chromatograms of set A were run in *n*-butanol/pyridine/water (6:4:3 v/v), of set B first in *n*-butanol/glacial acetic/water (4:1:5 v/v), then in *n*-butanol/pyridine/water in the same direction. Lactose was located with aniline hydrogen phthalate, N-acetyl neuraminic acid with orcinol-trichloroacetic acid or with Ehrlich reagent, N-L with any of the three sprays.

Reactant	*Set A*			*Set B*
	0 h	*2 h*	*16 h*	*2 h*
Neuramin-lactose (N-L)	0.03	0.03	0.03	0.06
Lactose	0.12	0.12	0.12	0.14
N-acetyl neuraminic acid	0.15	0.15	0.15	0.20
N-L + virus	0.02	0.02, 0.12, 0.15	0.12, 0.15	0.06, 0.14, 0.20
N-L + RDE	0.02	—	0.12, 0.15	—
Virus and RDE respectively	0.0	0.0	0.0	0.0

As may be seen from Table I both the influenza virus enzyme and RDE split N-L into its components, completely in 16 h, incompletely (about 20%) in 2 h; the O-acetyl group is easily lost[3] non-enzymically. In a preliminary note, KUHN AND BROSSMER[7] reported the isolation from cow colostrum of crystalline O-acetyl lactaminic acid-lactose, hydrolyzable to 1 mole each of lactose, acetic acid and lactaminic by *N*/100 HCl in the cold. The analysis and properties of lactaminic acid closely agree with those of N-acetyl neuraminic acid. The compound was found to be cleaved into lactose and lactaminic acid by influenza virus B and RDE. However, since the authors are still engaged in the elucidation of the structure of the compound, they did not discuss the type of linkage joining the components and split by the enzyme.

We may conclude from the present data and previous results that the viral enzyme and the vibrio enzyme split off the terminal neuraminic acid unit (in its mono- or diacetyl form) from neuraminyl-lactose, from inhibitory mucoproteins and probably from inhibitory glycolipids. The linkage cleaved in neuraminyl-lactose is of the O-glycosidic type. We propose the name "neuraminidase" for the enzyme and define its action as the hydrolytic cleavage of the glycosidic bond joining the keto group of neuraminic acid to D-galactose or D-galactosamine and possibly to other sugars.

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Note added in proof (31.12.56): The correctness of the proposed structure[2] of N-acetyl neuraminic acid was recently conclusively proved by its synthesis from N-acetyl D-glucosamine and oxaloacetic acid (W. J. CORNFORTH, M. E. DAINES AND A. GOTTSCHALK, *J. Chem. Soc.*, 1957, in press).