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Short communication

Monovalent and polymeric 5*N*-thioacetamido sialosides as tightly-bound receptor analogs of influenza viruses

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Abstract

A possible approach to the development of synthetic inhibitors of influenza virus attachment to host cells is based on the anchoring of the minimum receptor determinant of influenza virus, sialic acid, to a polymeric carrier. In this study, the effect of substitution of oxygen by sulphur in the 5*N*-acetyl moiety of sialic acid on the binding of monovalent and polymeric sialosides by A and B influenza virus strains was investigated. The polymeric inhibitor with pendant 5*N*-thioacetylneuraminic acid residues was found to be more broadly active against different virus strains than the one prepared from the Neu5Ac ligand. Copyright © 1997 Elsevier Science B.V.

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Influenza virus infection is initiated by the attachment of the virus to target cells mediated by specific interactions of the viral hemagglutinin (HA) with sialo-sugar chains of cell-surface glycoproteins and/or glycolipids (reviewed by Paulson, 1985; Wiley and Skehel, 1987). The X-ray structure of HA in complex with sialic acid analogs (Weis et al., 1988; Sauter et al., 1992; Watowich et al., 1994) revealed that the sialic acid binding site is formed by amino acid residues which are mainly conserved during antigenic variation of the virus. It was suggested, therefore, that this site could be a potential target for the design of anti-influenza drugs covering a wide range of virus strains.

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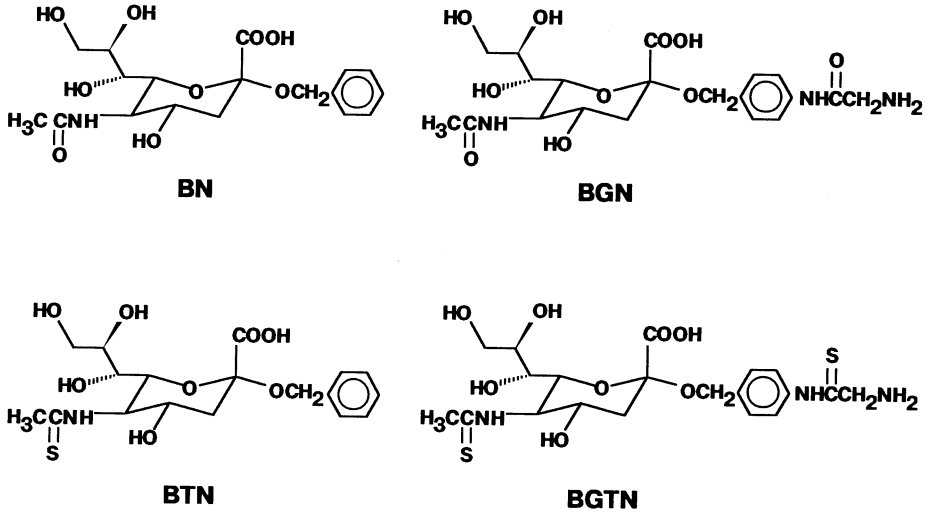

Fig. 1. Structural formulas of sialosides.

There are two main interconnected approaches to the design of tightly bound inhibitors of influenza virus attachment, namely, (i) development of monovalent compounds with high affinity for the receptor-binding site, starting from *N*-acetylneuraminic acid (Neu5Ac) as a scaffolding device (Sauter et al., 1992; Watowich et al., 1994, and references therein), and (ii) design of bi- or multisialylated structures which provide for enhanced binding affinity due to cooperative interactions with the virus particle (Lees et al., 1994; Mochalova et al., 1994, and references therein).

In the first approach, sialic acid analogs with 10–100-fold higher affinity for X31 (H3N2) influenza virus strain have been already obtained by introduction of unique substituents at glycosidic oxygen and/or at 4-*O*-hydroxyl group of sialic acid (Toogood et al., 1991; Weinhold and Knowles, 1992). The explanations of observed affinities in molecular terms were suggested in a study on the crystal structures of the HA complexed with these analogs (Watowich et al., 1994). Substitution of carbonyl oxygen by sulphur in 5*N*-acetamido residue of Neu5Ac (Brossmer and Gross, 1994) also significantly enhanced the binding of monovalent and of polymeric sialosides by the X31 strain (Machytka et al., 1993; Itoh et al., 1995).

In this study, we prepared benzyl α-glycosides of 5*N*-thioacetylneuraminic acid, coupled one of them to a polymeric carrier, and measured binding of the monovalent and polyvalent 5*N*-thioacetyl-Neu analogs to various human A and B influenza viruses. The polymeric inhibitor with pendant 5*N*-thioacetylneuraminic acid residues thus obtained was found to be more broadly active towards different virus strains compared with the one prepared from the Neu5Ac ligand.

Benzyl- and glycylamidobenzyl α-glycosides of 5*N*-acetylneuraminic acid (Fig. 1, BN and BGN, respectively) were synthesized as described earlier (Byramova et al., 1991). The benzyl glycoside of 5*N*-thioacetylneuraminic acid (BTN) and the thioglycylamidobenzyl glycoside of 5*N*-thioacetylneuraminic acid (BGTN) were synthesized by treatment of protected BN and BGN with P_2S_5 and Lawesson's reagent (2,4-bis(*p*-methoxyphenyl)-1,3-dithiadiphosphetane-2,4-disulfide), respectively, followed by deprotection of purified thionated products (Bovin et al., 1992). To prepare polyvalent sialosides, BGN and BGTN were coupled to poly(4-nitrophenyl)acrylate with subsequent hydrolysis of the remaining 4-nitrophenylester side chain groups of the polymer as previously described (Matrosovich et al., 1990; Byramova et al., 1991). Copolymers of acryloylglycylamidobenzylsialoside-co-acrylic acid, thus

Table 1
Binding by influenza viruses of monovalent and polymeric α-benzyl glycosides of 5*N*-acetylneuraminic acid (BN and PBGN, respectively), and of corresponding 5*N*-thioacetyl compounds (BTN and PBGTN)

Virus strain	Binding affinity, K_d (μM Sia)[a]				Amino acid in position 155 of the HA
	BN	BTN	PBGN	PBGTN	
Type A, subtype H3					
X31 (Aichi/2/68)	400	130	>20	3	Thr
X31/HS [226Q][b]	1300	130			Thr
X31/63-E [193N, 226P][b]	630	200			Thr
X31/68X [193R][b]	400	200			Thr
X31/63-D [224–230 deleted][b]	400	160			Thr
X31/63-3 [218E][b]	320	160	8	2	Thr
X31/V9A [218R][b]	400	130	7	0.8	Thr
Port Chalmers/1/73	200	500			Tyr
Victoria/3/75	80	400	6	10	Tyr
Texas/1/77	8	100			Tyr
England/321/77	30	250	0.1	0.05	Tyr
Philippines/2/82	5	80	0.1	0.15	Tyr
USSR/2/85	200	630			Tyr
USSR/3/85	4	50	0.3	0.4	Tyr
Type A, subtype H1					
Fort Warren/1/50			4	0.4	Thr
USSR/90/77	1300	500			Thr
Chile/1/83	500	250	5	1	Thr
Taiwan/1/86	1300	500			Thr
Type B					
Singapore/222/79	500	250	3	0.6	Val
USSR/100/83	400	250	2	0.2	Val
Ann Arbor/1/86			10	3	Val

[a] The binding affinity constants were determined by measuring the ability of the ligand to compete with the binding of the standard peroxidase-labeled fetuin preparation to the solid-phase immobilized virus (Gambaryan and Matrosovich, 1992).
[b] Mutants of X31 were kindly donated by Dr J.J. Skehel. Amino acid substitutions in respect to X31 are specified in square brackets in a single-letter code.

prepared (PBGN and PBGTN, respectively), bore 12 mol% of pendant Neu5Ac or 5*N*-thioacetylneuraminic acid moieties. Both PBGN and PBGTN were prepared using the same batch of poly(4-nitrophenyl)acrylate, to avoid possible differences in their ratio of polymerization and polydispersity.

The binding of monomeric and polymeric α-benzylsialosides and of corresponding 5*N*-thioanalogs by influenza viruses was measured in a competitive solid phase assay as described in our previous papers (Gambaryan and Matrosovich, 1992; Mochalova et al., 1994). The binding affinity constants, formally equivalent to the dissociation constants of the virus–sialoside complexes, were calculated with respect to the concentration of *N*-acetylneuraminic acid to facilitate comparison of monovalent and polymeric analogs. Representatives of currently epidemiologically active influenza virus types and subtypes were included in the study to assess possible variability in the virus recognition of 5*N*-thioacetamido substituent.

The results of the comparative testing of BN and BTN (Table 1) revealed that substitution of the acetyl group at 5*N* of Neu5Ac by the thioacetyl group increased the affinity of the ligand towards type B viruses, H1 subtype type A strains, X31 strain and its variants (H3 subtype HA). The effect was even more pronounced in the

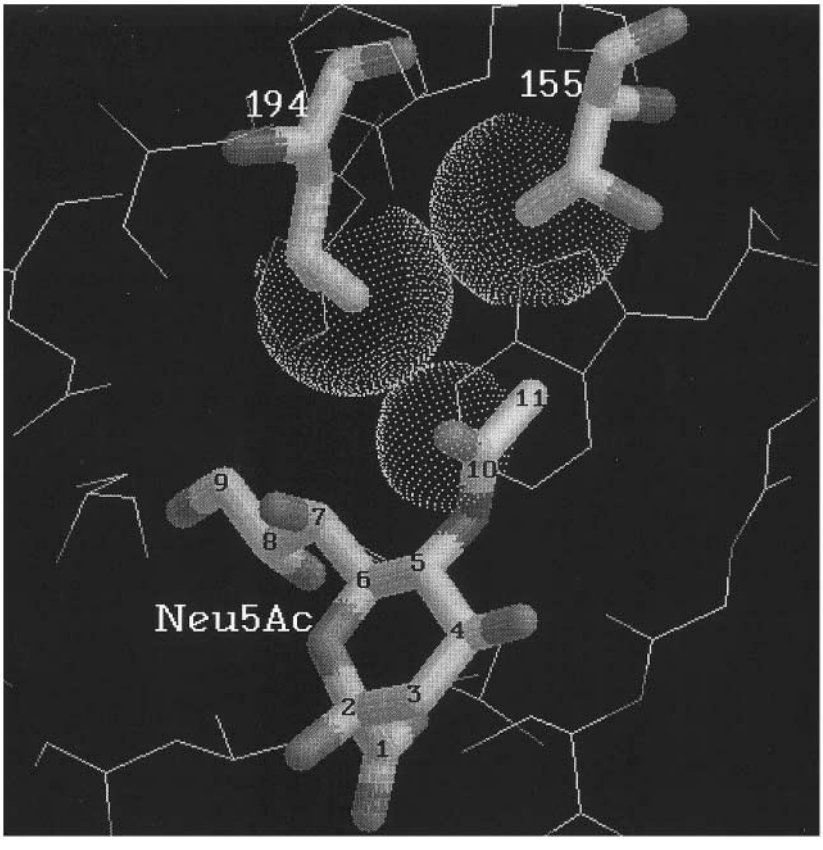

Fig. 2. Closest counterparts of the carbonyl oxygen of *N*-acetamido group of Neu5Ac in the receptor-binding site of X31 influenza virus HA according to X-ray data of Weis et al. (1988) (5HMG entry in the Brookhaven Protein Data Bank). Neu5Ac with its carbon atoms numbered, Leu194, and Thr155 are represented as stick bonds, other amino acids in the site—as wireframe bonds. Van der Waals dot surfaces are shown for the carbonyl oxygen of 5*N*-acetyl substituent, CD2 of Leu194, and CG2 of Thr155. Figure is generated using RasMol 2.5 Molecular Visualisation Program (Roger Sayle, Glaxo Research and Development, Greenford, Middlesex, UK).

case of polymeric analogs; polymer with pendant thioacetylated sialic acid residues (PBGTN) bound to the viruses 4–10 times more avidly as compared to Neu5Ac-based PBGN.

According to the structural data on a disposition of Neu5Ac moiety in the receptor-binding site of X31 (Weis et al., 1988), the carbonyl oxygen of the Neu5Ac 5*N*-acetamido group is in Van der Waals contact with the hydrophobic methyl group of the side chain of Leu194 and in a close proximity to the side chain methyl residue of Thr155, partially shielding off both methyls from the solvent (Fig. 2). To rationalize the higher affinity of thio-analog towards X31 virus, it may be suggested that substitution of 5*N*-acetamido oxygen by less polar and more bulky sulphur atom enhances hydrophobic and Van der Waals interactions of the ligand with this subsite of the receptor-binding pocket. This explanation may be valid also for the H1 and type B strains tested (Ile194/Thr155 and Leu194/Val155, respectively). In HAs of other subtypes of influenza A virus and in type B virus HAs sequenced to date, Leu is in position 194, while mutations in position 155 are mainly conservative (Thr/Val/Leu/Ile), leaving the possibility that the 5*N*-thioacetyl analog could be superior for these strains as well.

Remarkably, although X31 and its variants bound BTN better than BN, in agreement with the earlier data of Machytka et al. (1993) and Itoh et al. (1995), the opposite effect was observed for the other H3 strains tested herein. X31 is a recombinant virus bearing hemagglutinin and sialidase from the A/Aichi/2/68 field strain (H3N2). The latter is one of the first human isolates from the so-called Hong Kong influenza virus era started after introduction of the avian H3 HA into the human population in about 1965 (Bean et al., 1992). During the first years of circulation of the H3 subtype virus in humans, substitution of Thr155 by Tyr155 occurred, and subsequent drift variants of the H3 HA retained Tyr at this position. This substitution seems to be the reason for the inverse mode of recognition of 5*N*-acetyl and 5*N*-thioacetyl analogs by X31 and by the later H3 viruses. Despite a negative effect of the thio-substituent on the affinity of monovalent sialosides for later H3 strains, these strains, with an exclusion of Victoria/3/75, still reveal a higher affinity for the polymeric analog compared with H1, type B viruses and X31.

In summary, the substitution of 5N-acetyl group of sialic acid by the thioacetyl one, leads to an increased affinity of the ligand for H1 subtype A and type B influenza virus strains. Coupling of this ligand to a polyacrylic acid carrier results in preparation with a more uniform inhibitory activity towards different strains of influenza viruses compared to the Neu5Ac-based polymer.

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References

Bean, W.J., Schell, M., Katz, J., Kawaoka, Y., Naeve, C., Gorman, O. and Webster, R.G. (1992) Evolution of the H3 influenza virus hemagglutinin from human and nonhuman hosts. J. Virology 66, 1129–1138.

Bovin, N.V., Byramova, N.E., Tuzikov, A.B., Matrosovich, M.N., Mochalova, L.V. and Gambaryan, A.S. (1992) Viral attachment inhibitors. Patent PCT No. PCT/US92/09745.

Brossmer, R. and Gross, H.J. (1994) Sialic acid analogs and application for preparation of neoglycoconjugates. Methods Enzymol. 247, 153–176.

Byramova, N.E., Mochalova, L.V., Belyanchikov, I.M., Matrosovich, M.N. and Bovin, N.V. (1991) Synthesis of sialic acid pseudopolysaccharides by coupling of spacer-connected Neu5Ac with activated polymer. J. Carbohydr. Chem. 10, 691–700.

Gambaryan, A.S. and Matrosovich, M.N. (1992) A solid-phase enzyme-linked assay for influenza virus receptor-binding activity. J. Virol. Methods 39, 111–123.

Itoh, M., Hetterich, P., Isecke, R., Brossmer, R. and Klenk, H.-D. (1995). Suppression of influenza virus infection by an *N*-thioacetylneuraminic acid copolymer resistant to neuraminidase. Virology 212, 340–347.

Lees, W.J., Spaltenstein, A., Kingery-Wood, J.E. and Whitesides, G.M. (1994) Polyacrylamides bearing pendant α-sialoside groups strongly inhibit agglutination of erythrocytes by influenza A virus: multivalency and steric stabilization of particulate biological systems. J. Med. Chem. 37, 3419–3433.

Machytka, D., Kharitonenkov, I., Isecke, R., Hetterich, P., Brossmer, R., Klein, R.A., Klenk, H.-D. and Egge, H. (1993) Methyl α-glycoside of *N*-thioacetyl-D-neuraminic acid: a potential inhibitor of influenza A virus. A H-NMR study. FEBS Lett. 334, 117-120.

Matrosovich, M.N., Mochalova, L.V., Marinina, V.P, Byramova, N.E. and Bovin, N.V. (1990) Synthetic polymeric sialoside inhibitors of influenza virus receptor-binding activity. FEBS Lett. 272, 209–212.

Mochalova, L.V., Tuzikov, A.B., Marinina, V.P., Gambaryan, A.S., Byramova, N.E., Bovin, N.V. and Matrosovich, M.N. (1994) Synthetic polymeric inhibitors of influenza virus receptor-binding activity suppress virus replication. Antiviral Res. 23, 179–190.

Paulson, J.C. (1985) Interactions of animal viruses with cell surface receptors. In: M. Conn (Ed), The Receptors, Vol. 2, pp. 131–219. Academic Press, Orlando, FL.

Sauter, N.K., Hanson, J.E., Glick, G.D., Brown, J.H., Crowther, R.L., Park, S.-J., Skehel, J.J. and Wiley, D.C. (1992) Binding of influenza virus hemagglutinin to analogs of its cell-surface receptor, sialic acid: analysis by proton nuclear magnetic resonance spectroscopy and X-ray crystallography. Biochemistry 31, 9609–9621.

Toogood, P.L., Galliker, P.K., Glick, G.D. and Knowles, J.R. (1991) Monovalent sialosides that bind tightly to influenza A virus. J. Med. Chem. 34, 3138–3140.

Watowich, S.J., Skehel, J.J. and Wiley, D.C. (1994) Crystal

structures of influenza virus hemagglutinin in complex with high-affinity receptor analogs. Structure 2, 719–731.

Weinhold, E.G. and Knowles, J.R. (1992) Design and evaluation of a tightly binding fluorescent ligand for influenza A hemagglutinin. J. Am. Chem. Soc. 114, 9270–9275.

Weis, W., Brown, J.H., Cusack, S., Paulson, J.C., Skehel, J.J. and Wiley, D.C. (1988) Structure of the influenza virus hemagglutinin complexed with its receptor, sialic acid. Nature 333, 426–431.

Wiley, D.C. and Skehel, J.J. (1987) The structure and function of the hemagglutinin membrane glycoprotein of influenza virus. Ann. Rev. Biochem. 56, 365–394.

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