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# Newcastle Disease Virus Contains a Linkage-specific Glycoprotein Sialidase

## APPLICATION TO THE LOCALIZATION OF SIALIC ACID RESIDUES IN *N*-LINKED OLIGOSACCHARIDES OF $\alpha_1$-ACID GLYCOPROTEIN*

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**James C. Paulson‡§, Jasminder Weinstein§, Lambertus Dorland¶, Herman van Halbeek¶, and Johannes F. G. Vliegenthart¶**

*From the §Department of Biological Chemistry, UCLA School of Medicine, Los Angeles, California 90024 and the ¶Department of Bio-Organic Chemistry, University of Utrecht, Utrecht, The Netherlands*

**Newcastle disease virus sialidase was found to exhibit strict specificity for hydrolysis of the NeuAcα2→3Gal linkage contained in glycoprotein oligosaccharides both *N*-linked to asparagine and *O*-linked to threonine or serine under conditions that left oligosaccharides containing the NeuAcα2→6Gal and NeuAcα2→6GalNAc linkages intact. This was determined, in part, by examining the viral sialidase for its ability to hydrolyze glycoprotein oligosaccharides derivatized with purified sialyltransferases to contain the [$^{14}$C]NeuAcα2→3Gal, [$^{14}$C]NeuAcα2→6GalNAc, and [$^{14}$C]NeuAcα2→6Gal linkages. The viral sialidase was also tested for hydrolysis of the NeuAcα2→3Gal and NeuAcα2→6Gal linkages on the *N*-linked oligosaccharides of $\alpha_1$-acid glycoprotein. Selective hydrolysis of the NeuAcα2→3Gal linkage was shown by periodate oxidation and by 500-MHz $^1$H-NMR spectroscopy of native and sialidase-treated glycopeptides. The NMR spectra, together with composition data, further indicated that the NeuAcα2→3Gal and NeuAcα2→6Gal linkages were localized to specific branches of the major tri- and tetraantennary oligosaccharides of $\alpha_1$-acid glycoprotein. The results indicate that the Newcastle disease virus sialidase can initiate the selective degradation of *N*-linked oligosaccharide branches containing the NeuAcα2→3Gal linkage.**

Sialidases which can cleave sialic acids from soluble or cell-surface glycoproteins and glycolipids have been of great importance in the analysis of sialyloligosaccharide structure and function. Most sialidases in current use are bacterial in origin and are relatively nonspecific for the type of sialyloligosaccharides which serve as substrates. However, a number of viral sialidases have been shown to be quite specific (1, 2). In particular, Drzeniek (2), using milk oligosaccharides of defined structure, found that NDV[1] was extremely specific for hydrolysis of the NeuAcα2→3Gal and NeuAcα2→8NeuAc linkages. This report extends these studies to demonstrate directly the specificity of Newcastle disease virus sialidase towards sialyloligosaccharides of glycoproteins and illustrates its utility in analysis of the sialic acid substitutions of multiantennary oligosaccharides *N*-linked to asparagine.

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[1] The abbreviation used is: NDV, Newcastle disease virus.

## EXPERIMENTAL PROCEDURES[2,3]

Materials. The Clostridium perfringens sialidase (Type X, 140 units/mg) and Vibrio cholerae sialidase (0.11 units/ml) were purchased from Sigma Chemical Co. and Grand Island Biological Co., respectively. Streptococcus pneumoniae sialidase (1 unit/ml (3)) and ovine submaxillary mucin were gifts from Dr. Robert L. Hill, Duke University. Antifreeze glycoprotein (Mr = 2600), was purified from Dissostichus mawsoni serum, a gift of Dr. Arthur DeVries, University of Illinois and $\alpha_1$-acid glycoprotein (Mr = 40,000) was a gift of Dr. Karl Schmid, Boston University. Asialo ovine submaxillary mucin (4) was prepared as reported earlier. The β-galactoside α2→6 (6), the β-galactoside α2→3 (4) and the N-acetylgalactosaminide α2→6 (4,7) sialyltransferases were purified as previously described. Cytidine-5'-monophosphate-N-[$^{14}$C]-acetylneuraminic acid (CMP-[$^{14}$C]NeuAc, 1.6 mCi/mmol) was purchased from New England Nuclear Corp. Protein content was determined by the Amido-Schwarz method (8). Newcastle disease virus was obtained as a seed stock from the American Type Culture Collection, grown in 11 day old chick embryos, and partially purified as described earlier (9). Virus stocks typically had sialidase contents of 0.6-1.3 units/ml and had specific activities of about 50 milliunits/mg protein.

Preparation of [$^{14}$C]NeuAc labeled glycoprotein substrates. Sialidase substrates with glycoprotein oligosaccharides of defined sequence were obtained by sialylation of antifreeze glycoprotein (5 mg) with the β-galactoside α2→3 sialyltransferase (9 milliunits), ovine submaxillary asialo-mucin (5 mg) with the α-N-acetylgalactosaminide α2→6 sialyltransferase (180 milliunits) and asialo $\alpha_1$-acid glycoprotein (25 mg) with the β-galactoside α2→6 sialyltransferase (13 milliunits) to give the NeuAcα2→3Galβ1→3GalNAcαThr, NeuAcα2→6GalNAcαThr/Ser and NeuAcα2→6Galβ1→4GlcNAc-sequences, rspectively. Reaction mixtures also contained 3.9 μmol CMP-[$^{14}$C]NeuAc in 0.85 ml 60 mM sodium cacodylate pH 6.5 and were incubated for 15 h at 37°. Products obtained with $\alpha_1$-acid glycoprotein and ovine submaxillary mucin were separated from unreacted CMP-[$^{14}$C]NeuAc and [$^{14}$C]NeuAc by chromatography in 0.2 M NaCl on a column (0.9 x 13 cm) of Sephadex G-50, fine. The product obtained with antifreeze glycoprotein was similarly chromatographed on a column (1.5 x 28 cm) of Sephadex G-25, superfine. Finally, each product was dialyzed against deionized water and lyophilized. Yields based on the limiting substrate, CMP-[$^{14}$C]NeuAc, were 50-95 percent.

Sialidase assays. Sialidase activity was quantitated in an assay mixture containing 0.5 mg $\alpha_1$-acid glycoprotein (195 nmol sialic acid) and 0.25 mg bovine serum albumin in a final volume of 100 μl 0.1 M sodium cacodylate pH 6.5. Assays were incubated for 30 min at 37° and sialic acid released was measured by the thiobarbituric acid assay (10). One unit of activity for NDV sialidase and other enzymes used in this report is equal to 1 μmol product per minute.

To assess sialidase activity towards [$^{14}$C]NeuAc labeled glycoprotein substrates, reaction mixtures (60 μl) contained the substrate (3.5-5 nmol [$^{14}$C]NeuAc, 2800 cpm/nmol), neuraminidase (3-5 milliunits), and 12.5 mg bovine serum albumin in 50 mM sodium cacodylate pH 6.5. After incubation at 37° for the desired time, reaction mixtures with either [$^{14}$C]-labeled $\alpha_1$-acid glycoprotein or ovine submaxillary mucin as substrates were quenched with 1 ml 30 mM citric acid and applied to Pasteur pipet columns plugged with glass wool containing 1 ml Dowex-50 ($H^+$, 200-400 mesh). Each column was washed with an additional 1 ml 30 mM citric acid and effluents were collected directly into scintillation vials for counting. Under these conditions the [$^{14}$C]-labeled glycoprotein substrates quantitatively adsorb to the ion exchange resin and the released [$^{14}$C]NeuAc passes through unretarded. Reaction mixtures with [$^{14}$C]NeuAc labeled antifreeze glycoprotein were immediately applied to long tipped Pasteur pipet columns containing Sephadex G-25 (superfine, 0.5 x 7.5 cm) equilibrated with 0.2 M sodium chloride. The reaction mixture was washed into the column with 0.1 ml of 0.2 M NaCl and eluted with three 0.5 ml aliquots of the same solution while collecting the effluents from each application in separate scintillation counting vials. These small gel filtration columns conveniently resolved the sialylated antifreeze glycoprotein and free [$^{14}$C]NeuAc which emerged in the second and third 0.5 ml fractions, respectively.

Sialidase treatment of $\alpha_1$-acid glycoprotein. Asialo $\alpha_1$-acid glycoprotein was prepared by reaction of the native glycoprotein with C. perfringens sialidase as previously described (5). Partial removal of sialic acid with NDV sialidase was performed in a reaction mixture containing 25 mg $\alpha_1$-acid glycoprotein and 60 milliunits of the NDV sialidase in a final volume of 5 ml 0.1 M sodium cacodylate, pH 6.5. Incubation was allowed to proceed for 24 h at 37° and after addition of another 30 miliunits NDV sialidase, incubation was continued for another 24 h. Siaic acid released (10) by the C. perfringens and NDV sialidases was 172 and 390 nmol/mg protein ($\epsilon^{0.1\%}_{279}$ = 0.893), respectively.

[2] "Experimental Procedures" are presented in miniprint as prepared by the authors. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from The Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 82M-845, cite the authors, and include a check or money order for $1.00 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.

[3] K. Schmid, unpublished results.

500-MHz $^1$H-NMR Spectroscopy. Glycopeptides of native and NDV treated $\alpha_1$-acid glycoprotein were prepared by a modification of procedures previously described (11).[3] For $^1$H-NMR spectroscopic analysis the neutralized glycopeptides were repeatedly treated with $^2H_2O$ at room temperature with intermediate lyophylization. The 500-MHz $^1$H-NMR spectra were recorded on a Bruker WM-500 spectrometer, operating in the Fourier transform mode at probe temperatures of 25°C or 60°C, and equipped with a Bruker Aspect-2000 computer. Chemical shifts are given at 25°C, relative to sodium 4,4-dimethyl-4-silapentane-1-sulphonate (indirectly to acetone in $^2H_2O$ : $\delta$ = 2.225 ppm).

## RESULTS

*Specificity of NDV and Bacterial Sialidases*—Results in Table I compare the NDV sialidase and several commonly used bacterial sialidases for their hydrolysis of [$^{14}$C]NeuAc-labeled glycoprotein oligosaccharides. The substrates examined were the NeuAcα2→3Galβ1→3GalNAc and NeuAcα2→6GalNAc sequences, often found in oligosaccharides *O*-linked to threonine and serine, and the NeuAcα2→6Galβ1→4GlcNAc sequence found in oligosaccharides *N*-linked to asparagine (12). The NDV sialidase exhibited remarkable specificity for hydrolysis of the NeuAcα2→3Gal linkage, failing to hydrolyze the NeuAcα2→6GalNAc and NeuAcα2→6Gal linkages even after prolonged incubation. Under the same conditions, the three bacterial sialidases showed little specificity, quantitatively hydrolyzing all three substrates.

As indicated in Table I, hydrolysis of the NeuAcα2→3Gal linkage by the NDV sialidase was not complete (83%). Redigestion of the uncleaved product, after isolation by chromatography on a column (1.5 × 26 cm) of Sephadex G-25 superfine equilibrated in 0.1 M ammonium bicarbonate and concentration by lyophilization, gave no further release of sialic acid. The remaining sialic acid was judged to be in the NeuAcα2→3Gal linkage as evidenced by equimolar ratios of sialic acid and periodate-stable galactose (6). The reason for the failure of the NDV sialidase to hydrolyze the resistant oligosaccharides is unclear at present.

*Incomplete Hydrolysis of $\alpha_1$-Acid Glycoprotein by NDV*—The NeuAcα2→3Gal linkage is also found in *N*-linked oligosaccharides in addition to the *O*-linked oligosaccharide represented in Table I. $\alpha_1$-Acid glycoprotein was chosen to investigate further the specificity of NDV sialidase since its five *N*-linked oligosaccharides have been shown by methylation analysis to contain both of the most common sialic acid linkages, NeuAcα2→6Gal and NeuAcα2→3Gal (13). A comparison of the NDV and *S. pneumoniae* sialidases for their ability to desialylate $\alpha_1$-acid glycoprotein is shown in Fig. 1 as a time course of hydrolysis with equal amounts of the two enzymes. The NDV sialidase achieved only partial hydrolysis, rapidly releasing sialic acid to a plateau value equivalent to 44% of the total sialic acid content of $\alpha_1$-acid glycoprotein. In contrast, the *S. pneumoniae* sialidase gave nearly quantitative hydrolysis. Prolonged incubation times (24 h) with up to 10 times more NDV sialidase released no more than 55% of the total sialic acid.

To determine the nature of the sialic acid linkage cleaved by the NDV sialidase, periodate oxidation was employed since the NeuAcα2→3Gal and NeuAcα2→6Gal linkages can be distinguished, respectively, by the stability and lability of the penultimate galactose residues. As shown in Table II, about 40% or 7.3 mol of galactose/mol of $\alpha_1$-acid glycoprotein was stable to periodate oxidation, indicating 3′ substitution; and, of this, more than 90% appeared to be in the NeuAcα2→3Gal linkage as evidenced by the lack of periodate-stable galactose in asialo (*C. perfringens*-treated) $\alpha_1$-acid glycoprotein. NDV sialidase treatment, which removed 6.9 mol of NeuAc/mol, reduced the periodate-stable galactose content by 90% that value (6.2 mol/mol). These results suggested that most of the sialic acid removed from $\alpha_1$-acid glycoprotein by the NDV sialidase was in the NeuAcα2→3Gal sequence, and that sialic acids in other linkages were resistant to hydrolysis.

*Characterization of Sialic Acid Linkages in Native and NDV-treated Oligosaccharides by 400-MHz $^1$H-NMR Spectroscopy*—the specificity of the NDV sialidase for the sialic acid linkages present in $\alpha_1$-acid glycoprotein was confirmed by comparing the high resolution $^1$H-NMR spectra of the glycopeptides from native and NDV sialidase-treated $\alpha_1$-acid glycoprotein preparations. The spectra showed both preparations to be mixtures of mainly tri- and tetraantennary glycopeptides (14–16). Relevant parts of the 500-MHz $^1$H-NMR spectra of a glycopeptide mixture, derived from native $\alpha_1$-acid glycoprotein and a NDV sialidase-treated preparation, are shown in Fig. 2. The spectrum of the native preparation shows that

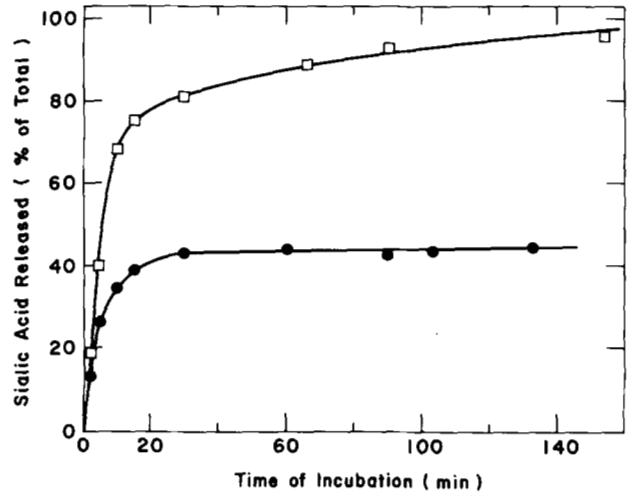


FIG. 1. **Incomplete release of sialic acid from $\alpha_1$-acid glycoprotein by NDV sialidase.** Reaction mixtures contained 0.25 mg of bovine serum albumin, 0.1 mg of $\alpha_1$-acid glycoprotein, and 5 μl of either the NDV (●) or *S. pneumoniae* (■) sialidases in a final volume of 100 μl of 0.1 M sodium cacodylate, pH 6.5. After incubation at 37 °C for the indicated times, reactions were stopped and assessed for sialic acid released (10), which is expressed as a per cent of the total sialic acid content of $\alpha_1$-acid glycoprotein (390 nmol/mg).

TABLE I

*The specificity of NDV and bacterial sialidases for hydrolysis of [$^{14}$C]NeuAc-labeled glycoprotein oligosaccharides*

Details of the sialidase reactions and assay procedures are described under "Experimental Procedures," as is the preparation of substrates A, B, and C which refer, respectively, to sialylated derivatives of antifreeze glycoprotein, asialo $\alpha_1$-acid glycoprotein, and ovine submaxillary asialomucin.

| Oligosaccharide sequence | Incubation time | [$^{14}$C]NeuAc released | | | |
|---|---|---|---|---|---|
| | | NDV | *S. pneumoniae* | *V. cholerae* | *C. perfringens* |
| | *h* | *% of total* | | | |
| A. NeuAcα2→3Galβ1→3GalNAc | 0.6 | 72 | 95 | 79 | 98 |
| | 18 | 83 | 98 | 91 | 100 |
| B. NeuAcα2→6Galβ1→4GlcNAc | 0.6 | 0 | 94 | 46 | 86 |
| | 18 | 1 | 95 | 94 | 97 |
| C. NeuAcα2→6GalNAc | 0.6 | 0 | 87 | 85 | 89 |
| | 18 | 0 | 93 | 93 | 94 |

sialic acid is present in two types of linkage, α2→3 to Gal and α2→6 to Gal. This conclusion is based on the chemical shift values for H-3eq ($\delta$ = 2.762 ppm for α2→3 and $\delta$ = 2.668 ppm for α2→6) and H-3ax ($\delta$ = 1.799 ppm for α2→3 and $\delta$ = 1.720 ppm for α2→6) of the NeuAc residues (15). Integration of these signals shows that the ratio of the two sialic acid linkage types is approximately 1:1. The spectrum of the NDV sialidase-treated preparation reveals only H-3 signals, stemming from NeuAc α2→6 linked to Gal (H-3eq = 2.669 ppm and H-3ax = 1.722 ppm). Thus, it appears that the NDV sialidase treatment gives rise to complete release of NeuAc residues α2→3 linked to Gal, while the remaining NeuAc is exclusively α2→6 linked to Gal.

Structure analysis of purified asialoglycopeptides has shown that the predominant oligosaccharides of $\alpha_1$-acid glycoprotein are tri- and tetraantennary structures with branch patterns as illustrated in Fig. 3 (11, 14, 17). Microheterogeneity arises, in part, from the substitution of fucose in the Fucα1→3GlcNAc linkage at the GlcNAc 7, GlcNAc 7′ and GlcNAc 5′ positions (18). Since the Fucα1→3GlcNAc linkage has been shown to prevent sialylation by a β-galactoside α2→6 sialyltransferase (5), the fucose content (2 mol/mol) may account for the presence of some nonsialylated galactose residues. However, most galactose residues are sialylated as evidenced by the sialic acid to galactose ratio of 0.91:1.00 (Table II). The NMR spectra also provide some information on the localization of the NeuAcα2→6Gal and NeuAcα2→3Gal linkages on specific branches of the multiantennary *N*-linked oligosaccharides. The assignments are made possible by the differential effects of the two linkages on the chemical shifts of other protons in the spectra which serve as reporter groups (15, 16, 19, 20). Thus, while the spectra of the mixed glycopeptides are complex, the chemical shifts and intensity of the reporter groups provide valuable information.

The proposed sialic acid linkages in the nonfucosylated oligosaccharides of $\alpha_1$-acid glycoprotein are shown in Fig. 3. The chemical shift of H-1 of Man **4** $\delta$ = 5.133 ppm in the spectra of the glycopeptides from both native and NDV-treated $\alpha_1$-acid glycoprotein indicates that Gal **6** bears a NeuAc residue at C-6. The chemical shifts of H-1 of Man **4′** are observed at $\delta$ = 4.927 ppm (triantenna) and $\delta$ = 4.871 ppm (tetraantenna) in both preparations, excluding the presence of the NeuAcα2→6Gal linkage at Gal **6′**. Moreover, they are consistent with a NeuAcα2→3Gal linkage at Gal **6′** since the chemical shifts of H-1 of Man **4′** of known structures are identical with and without sialic acid in this linkage (15, 16, 19). In the *N*-acetyl regions of both spectra, a small singlet is observed at $\delta$ = 2.103 ppm which is the *N*-acetyl signal of GlcNAc **7** when Gal **8** is bearing a NeuAc residue at C-6. The intensity of this signal, however, shows that the sialylation of Gal **8** at C-6 is less than 5%. Thus, given the high degree of sialylation of $\alpha_1$-acid glycoprotein (Table II), Gal **8** would appear to be primarily in the NeuAcα2→3 linkage. For the substitution of Gal **8′**, there are no reporter groups from which the sialic acid to galactose linkage can be directly obtained. However, there are about 8.7 mol of the NeuAcα2→6Gal

TABLE II

*Periodate-stable galactose contents of native and sialidase-treated derivatives of $\alpha_1$-acid glycoprotein*

| $\alpha_1$-Acid glycoprotein preparation[a] | NeuAc removed[a] | Galactose content[b] | |
|---|---|---|---|
| | | Total | Periodate-stable |
| | *mol/mol* | *mol/mol* | |
| Native | | 17.2 | 7.3 |
| NDV sialidase-treated | 6.9 | 17.1 | 1.1 |
| *C. perfringens* sialidase-treated | 15.6 | 17.2 | 0.6 |

[a] The procedure for NDV sialidase treatment of $\alpha_1$-acid glycoprotein is described under "Experimental Procedures," and the *C. perfringens* sialidase treatment, which quantitatively removed sialic acid, was done as reported earlier (5).

[b] Periodate oxidation and galactose quantitation were as previously described (7) except that hydrolysis was done in 1 ml of degassed 2 *N* HCl at 95 °C for 90 min. No destruction of galactose hydrolyzed in the presence of iodate ($IO_3^{2-}$) was observed under these conditions. Each value represents the mean of quadruplicate determinations which ranged no more than ±0.4 mol/mol.

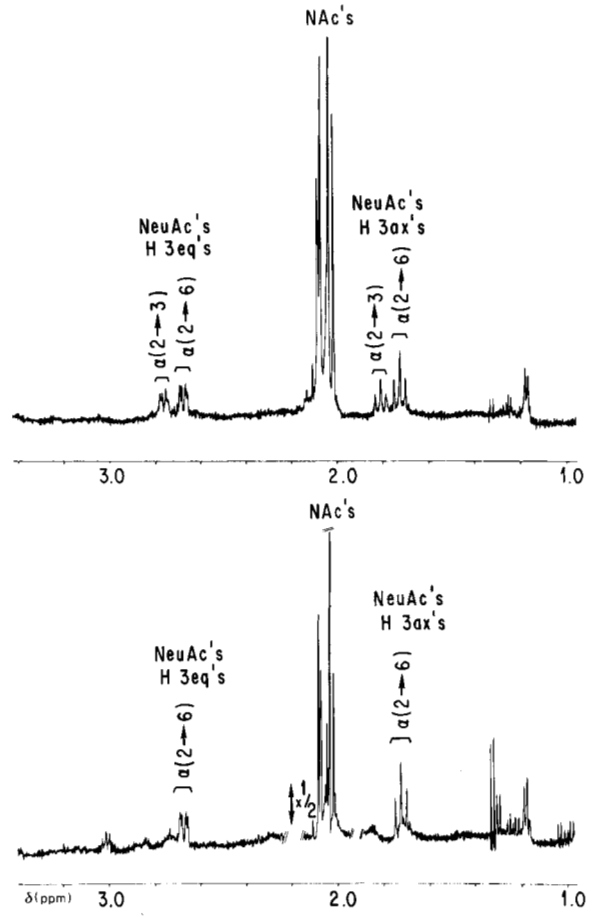


FIG. 2. **Characteristic resonance patterns of the NeuAc H-3 atoms and the *N*-acetylmethyl protons in the 500-MHz $^1$H-NMR spectra of a glycopeptide mixture derived from native $\alpha_1$-acid glycoprotein (*upper spectrum*) and a NDV sialidase-treated preparation (*lower spectrum*).**

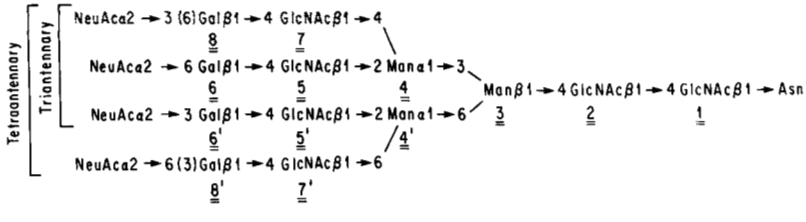


FIG. 3. **Proposed localization of NeuAcα2→3Gal and NeuAcα2→6Gal linkages in the major oligosaccharides of $\alpha_1$-acid glycoprotein.**

linkage as judged from the sialic acid content of the NDV-treated preparation (Table II and Fig. 2). Assuming that 100% of Gal **6** and 5% of Gal **8** are tied up in the NeuAcα2→6Gal linkage at each of the five glycosylation sites, this would account for a maximum of 5.25 mol. By difference, this leaves 3.4 mol unaccounted for. Thus, Gal **8'** is most likely in the NeuAcα2→6Gal linkage.

## DISCUSSION

Results in this report demonstrate the remarkable specificity of the NDV sialidase for the selective hydrolysis of the NeuAcα2→3Gal linkage in *N*-linked and *O*-linked oligosaccharides of glycoproteins. Preliminary evidence suggests that the NDV sialidase will also selectively degrade sialyloligosaccharides on the surface of intact cells. Indeed, of human asialoerythrocytes derivatized to contain the NeuAcα2→3Gal, NeuAcα2→6Gal, or NeuAcα2→6GalNAc linkage (23), the NDV sialidase hydrolyzed only the former (24).[4] These results extend the observations of Drzeniek (reviewed in Ref. 2) made using milk oligosaccharides as substrates. This investigator further found that the NeuAcα2→8 NeuAc and NeuAcα2→6 GlcNAc linkages were, respectively, sensitive and resistant to hydrolysis. Each of these sequences is also found in oligosaccharides of glycoproteins (13, 21, 22). The same specificity can be expected to hold for glycoprotein oligosaccharides. Schauer *et al.* (24) and Corfield *et al.* (25) have shown that the NDV sialidase also exhibits specificity towards various *N*- and *O*-acyl sialic acids found in nature. Compared to oligosaccharides containing *N*-acetylneuraminic acid, oligosaccharides containing the analogs *N*-glycolylneuraminic acid and 4-*O*-acetyl-*N*-acetylneuraminic acid are poor substrates.

Analysis of the NMR spectra of the mixed glycopeptides of native and NDV-treated $\alpha_1$-acid glycoprotein suggests that the NeuAcα2→3Gal and NeuAcα2→6Gal linkages are localized to specific branches of the predominant tri- and tetraantennary oligosacharide structures as depicted in Fig. 3. A number of *N*-linked triantennary oligosaccharides structures which contain both the NeuAcα2→3Gal and NeuAcα2→6Gal linkages are available for comparison (15, 26-32). The structure of the *N*-linked oligosaccharide proposed for fetuin by Nilsson *et al.* (29) is identical with the major triantennary structure proposed for $\alpha_1$-acid glycoprotein. In all other structures in which the sialic acid linkages have been established (15, 26, 27), only one branch contains the NeuAcα2→3Gal linkage; and, in each case, it is the branch linked β1→4 to the core mannose, also consistent with the structure in Fig. 3.

While analysis of NMR spectra has confirmed the location of NeuAcα2→3Gal and NeuAcα2→6Gal linkages on branches of several *N*-linked oligosaccharides (15, 16, 19, 20), direct chemical proof for these assignments is very difficult (26). To this end, the strict specificity of the NDV sialidase may prove valuable by allowing preferential desialylation only of those branches containing the NeuAcα2→3Gal linkage. Subsequent treatment with β-galactosidase and *N*-acetylglucosaminidase to remove the entire branch would alter the substitution pattern of the core mannose residues, allowing identification of the branches removed by methylation analysis. This general approach has been used successfully to identify the localization of NeuAcα2→3Gal and NeuAcα2→6Gal linkages on *N*-linked oligosaccharides of human ceruloplasmin.[5]

The functional significance of the diverse sialylation patterns of glycoprotein oligosaccharides is unknown at present. To date, sialic acids have been shown to be essential determinants in the maintenance of glycoproteins in blood (33), in the binding of toxins (34) and viruses (35) to cells, and in aspects of the cellular differentiation of slime molds (36). In view of the unique specificity of the NDV sialidase, it is anticipated that its use may, in the future, help provide further insights into the biological roles of the sialic acids.

*Acknowledgment*—We thank Dr. Karl Schmid for preparation of glycopeptides of $\alpha_1$-acid glycoprotein for the NMR analysis.

[4] L. D. Cahan and J. C. Paulson, unpublished results.

[5] J. Baenziger, personal communication.

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