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A Unique Sialidase That Cleaves the Neu5Gcα2→5-$O_{glycolyl}$Neu5Gc Linkage: Comparison of Its Specificity with That of Three Microbial Sialidases toward Four Sialic Acid Dimers

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We found that the hepatopancreas of oyster, *Crassostrea virginica*, contained a sialidase capable of releasing Neu5Gc from the novel polysialic acid chain (→5-$O_{glycolyl}$Neu5Gcα2→)$_n$ more efficiently than from the conventional type of polysialic acid chains, (→8Neu5Acα2→)$_n$, or (→8Neu5Gcα2→)$_n$. We have partially purified this novel sialidase and compared its reactivity with that of microbial sialidases using four different sialic acid dimers, Neu5Gcα2→5-$O_{glycolyl}$Neu5Gc (Gg2), Neu5Acα2→8Neu5Ac (A2), Neu5Gcα2→8Neu5Gc (G2), and KDNα2→8KDN (K2) as substrates. Hydrolysis was monitored by high performance anion-exchange chromatography with a CarboPac PA-100 column and pulsed amperometric detection, the method by which we can accurately quantitate both the substrate (sialiac acid dimers) and the product (sialic acid monomers). The oyster sialidase effectively hydrolyzed Gg2 and K2, whereas A2 and G2 were poor substrates. Neu5Ac2en but not KDN2en effectively inhibited the hydrolysis of Gg2 by the oyster sialidase. Likewise, the hydrolysis of K2 by the oyster sialidase was inhibited by a cognate inhibitor, KDN2en, but not by Neu5Ac2en. Using the new analytical method we found that Gg2 was hydrolyzed less efficiently than A2 but much more readily than G2 by *Arthrobacter ureafaciens* sialidase. This result was at variance with the previous report using the thiobarbituric acid method to detect the released free sialic acid [Kitazume, S., *et al.* (1994) *Biochem. Biophys. Res. Commun.* 205, 893–898]. In agreement with previous results, Gg2 was a poor substrate for *Clostridium perfringens* sialidase, while K2 was refractory to all microbial sialidases tested. Thus, the oyster sialidase is novel and distinct from microbial sialidases with regards to glycon- and linkage-specificity. This finding adds an example of the presence of diverse sialidases, in line with the diverse sialic acids and sialic acid linkages that exist in nature. The new sialidase should become useful for both structural and functional studies of sialoglycoconjugates.

Key Words: **sialidases; Neu5Gcα2→5-$O_{glycolyl}$Neu5Gc-specific sialidase; *Arthrobacter ureafaciens* sialidase; oyster sialidase.**

In addition to the three known oligo/polysialic acids, (→8Neu5Acα2→)$_n$, (→8Neu5Gcα2→)$_n$, and (→8KDNα2→)$_n$, a new polysialic acid (polySia) chain with Neu5Gc residues ketosidically linked to the glycolyl group of Neu5Gc, (→5-$O_{glycolyl}$Neu5Gcα2→)$_n$, has been recently found in the glycoprotein isolated from the sea urchin eggs and their jelly coat (1, 2). This novel type of oligo/polysialic acid has been implicated to be involved in complexing with Ca^{2+} in the egg jelly during fertilization and/or early development, and participate in initial binding of sperm to the receptor on the egg surface (1, 3). Very little is known about the enzymatic cleavage of this newly discovered Neu5Gcα2→5-$O_{glycolyl}$Neu5Gc linkage. While studying glycosidases in the hepatopancreas of oyster, *Crassostrea virginica* (4), we found a sialidase activity that effectively released NeuGc from (→5-$O_{glycolyl}$Neu5Gcα2→)$_n$. Since our preliminary report showed that the α2→5-$O_{glycolyl}$-linkage was highly resistant to bacterial sialidases (5), we have decided to purify and study the catalytic properties of this novel sialidase that is potentially useful for studying structure and function of oligo/polysialic acid having the new inter-residue linkage.

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TABLE 1

Purification of Neu5Gcα2→5-$O_{glycolyl}$Neu5Gc-Cleaving Enzyme from the Hepatopancreas of Oyster

Steps	Total protein (mg)	Total activity (units)	Specific activity (units/mg)	Recovery (%)	Purification (fold)
1. Acetone powder extract	15500	1111	0.071	100	1
2. Sephacryl S-200	426	1098	2.58	99	36
3. Octyl Sepharose	48	223	4.64	20	65
4. Red A	5	57	11.4	5	161

This paper describes the partial purification of a novel Neu5Gcα2→5-$O_{glycolyl}$Neu5Gc-cleaving sialidase from oyster hepatopancreas and comparison of its specificity with that of the sialidases isolated from *Arthrobacter ureafaciens*, *Clostridium perfringens*, and *Vibrio cholerae*. Disialic acids are the shortest units having the inter-residue ketosidic linkage of polySia chains, and most suitable substrate to clarify the linkage- and glycon-specificity of the enzyme. Hence, we have studied the hydrolysis of four distinct sialic acid dimers, Neu5Gcα2→5-$O_{glycolyl}$Neu5Gc, Neu5Acα2→8Neu5Ac, Neu5Gcα2→8Neu5Gc, and KDNα2→8KDN by sialidases. The hydrolyses of these sialic acid dimers were analyzed by using a high performance anion exchange chromatography (HPAEC) with a pulsed amperometric detector (PAD) (6, 7). The HPAEC-PAD method enabled us to analyze sialidase-catalyzed reactions more accurately and quantitatively than the previously used colorimetric and TLC methods.

MATERIALS AND METHODS

Materials. Neu5Acα2→8Neu5Ac was obtained from Nihon Gaishi (Handa, Japan) and also from E.Y. Labs, Inc. (San Mateo, CA). Other sialic acid dimers were prepared by controlled acid hydrolysis of the oligo/polysialosyl chains of parent glycoproteins, followed by anion-exchange fractionation of oligosialic acids on a DEAE-Sephadex A-25 column: KDNα2→8KDN, from the KDN-containing glycoproteins isolated from the rainbow trout ovarian fluid (8); Neu5Gcα2→8Neu5Gc, from the polysialoglycoproteins of rainbow trout eggs (9); and Neu5Gcα2→5-$O_{glycolyl}$Neu5Gc, from the (→5-$O_{glycolyl}$Neu5Gcα2→)$_n$-containing glycoprotein of the jelly coat of sea urchin eggs (1). Plastic backed precoated silica gel-60 TLC plates were from Merck (Darmstadt, Germany); octyl Sepharose SL-4B, Sephacryl S-200 HR, Amersham Pharmacia Biotech Inc. (Piscataway, NJ); Matrex gel Red A and Blue A and Centricon microconcentrators, Millipore (Bedford, MA). 2′(4-Methylumbelliferyl)-α-*N*-acetylneuraminic acid (MU-Neu5Ac), Sigma (St. Louis, MO). All other chemicals were from commercial sources and of the highest grade.

KDN-sialidase was isolated from oyster hepatopancreas (4). 2-Deoxy-2,3-didehydro-*N*-acetylneuraminic acid (Neu5Ac2en) was from Boehringer Mannheim (Indianapolis, IN). 2,3-Didehydro-2,3-dideoxy-D-*glycero*-D-*galacto*-2-nonulopyranosonic acid (KDN2en) was synthesized according to the published procedures (10). Sialidases from *C. perfringens* and *V. cholerae* were purchased from Calbiochem (La Jolla, CA) and Sigma (St. Louis, MO), respectively. *A. ureafaciens* sialidase was obtained from Nacalai Tesque Co. (Kyoto, Japan). One unit of bacterial sialidase activity is defined as the amount of enzyme that releases 1 μmol of Neu5Ac from sialyllactose per min at 37°C. One unit of oyster sialidase activity is defined as the amount of enzyme that releases 1 nmol of Neu5Ac from MU-Neu5Ac per min at 37°C.

Analysis of sialic acid monomers and dimers. A DX 500 ion chromatography system (Dionex, Sunnyvale, CA) equipped with an ED-40 electrochemical detector (HPAEC-PAD system) with a CarboPac PA-100 column was used to analyze mono- and disialic acids. The column was eluted with a programmed salt gradient: 10-10-20-20-1000-120-150-200 mM $NaNO_3$ in 0.1 M NaOH for 0-4-5-8-16-26-30 min.

Time course of the hydrolysis of sialic acid dimers. Time course analysis of the appearance and disappearance of the product (monoSia) and the substrate (diSia) catalyzed by different sialidases was performed for each substrate in the presence and absence of one of the two competitive inhibitors, KDN2en and Neu5Ac2en. The reaction mixture contained 1 μg of substrate and 1 μl of enzyme solution containing an appropriate amount of enzyme activity (or 1 μl of double deionized water to serve as a control) in 50 μl of 50 mM acetate buffer with the pH value optimal for each enzyme as listed in Table 2. After incubation at 37°C for a preset time, an aliquot (8 μl) of the reaction mixture was mixed with 152 μl of either 10 mM NaOH (for Neu5Acα2→8Neu5Ac and KDNα2→8KDN) or 0.1 M NaOH (for Neu5Gcα2→8Neu5Gc and Neu5Gcα2→5-$O_{glycolyl}$Neu5Gc). A 100 μl-aliquot of this mixture (containing 100 ng of sialic acid) was subsequently analyzed by the Dionex HPAEC-PAD system.

Conventional assay of the Neu5Gcα2→5-$O_{glycolyl}$Neu5Gc-cleaving activity. The enzyme from oyster was incubated with 7 μg of the polySia-glycoprotein containing about 3 μg of (→5-$O_{glycolyl}$Neu5Gcα2→)$_n$ in 30 μl of 50 mM sodium acetate buffer pH 4.1 at 37°C. After a set time, the reaction was terminated by adding 5 volumes of chloroform:methanol (2/1, v/v). The mixture was vortexed and briefly centrifuged to separate two phases. The aqueous phase was evaporated to dryness, redissolved in water, and analyzed by the Dionex HPAEC-PAD system or by TLC to detect the released free Neu5Gc. For TLC analysis, the plates were developed by 1-butanol:acetic acid:water (2/1/1, v/v/v), sprayed with diphenylamine reagent (11), and heated at 115°C for 20 min to visualize sialic acids. For convenience, the TLC analysis of the released Neu5Gc was used to locate the Neu5Gcα2→5-$O_{glycolyl}$Neu5Gc-cleaving activity in column fractions. After pooling the active fractions, the Neu5Gcα2→5-$O_{glycolyl}$Neu5Gc-cleaving activity was then quantitatively determined by the Dionex HPAEC-PAD system. One unit of this novel oyster sialidase is defined as the amount that liberates 1 nmol of Neu5Gc from (→5-$O_{glycolyl}$Neu5Gcα2→)$_n$ per min at 37°C.

Isolation of Neu5Gcα2→5-$O_{glycolyl}$Neu5Gc-cleaving sialidase from oyster hepatopancreas. The conditions for the preparation of the acetone powder (115 g) from oyster hepatopancreas and subsequent purification of Neu5Gcα2→5-$O_{glycolyl}$Neu5Gc-cleaving sialidase by Sephacryl S-200 (5 × 100 cm) filtration (Fig. 1A) and octyl Sepharose (1.5 × 36 cm) chromatography (Fig. 1B) were identical to that for the isolation of KDN-sialidase (4). 4-Methylumbelliferyl-KDN was used as substrate to assay KDN-sialidase (4). The enzyme preparation obtained from octyl Sepharose column as shown in Fig. 1B was subsequently applied onto a Matrex Gel Red A column (1.0 × 8.0 cm),

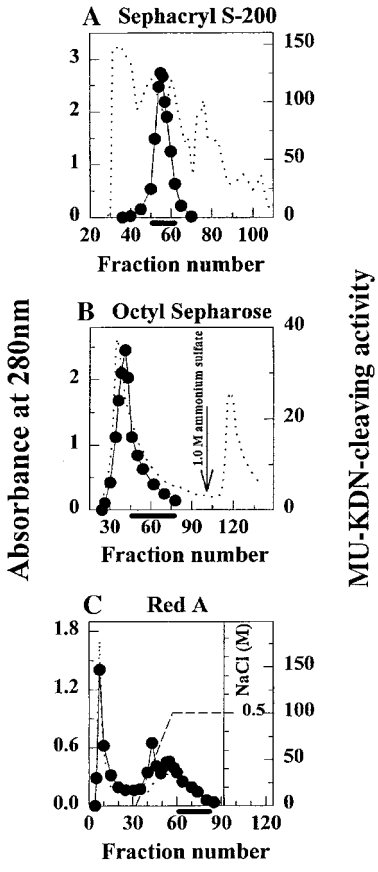

FIG. 1. Purification of a Neu5Gcα2→5-$O_{glycolyl}$Neu5Gc-cleaving sialidase from the acetone powder of oyster hepatopancreas. (A) Sephacryl S-200 gel filtration; (B) octyl Sepharose chromatography; (C) Matrex Red A dye chromatography. Dotted line, absorbance at 280 nm; dashed line, NaCl gradient; ●, MU-KDN-cleaving activity; horizontal bar, Neu5Gcα2→5-$O_{glycolyl}$Neu5Gc-cleaving activity. Detailed conditions are described under Materials and Methods.

equilibrated with 20 mM sodium phosphate buffer, pH 6.9. After washing with the same buffer to remove unadsorbed proteins, the column was eluted with a linear NaCl gradient from 0 to 0.5 M in the same buffer (total volume 100 ml) at 1.0 ml/min, and 2.5 ml-fractions were collected. The fractions containing the Neu5Gcα2→5-$O_{glycolyl}$Neu5Gc-cleaving activity, as shown in Fig. 1C were concentrated and desalted using an Amicon stirred cell with PM-10 membrane. This enzyme preparation was used for the studies presented in this report. Table 1 summarizes the results of a typical purification of Neu5Gcα2→5-$O_{glycolyl}$Neu5Gc-cleaving sialidase from 115 g of the acetone powder prepared from the hepatopancreas of oyster.

RESULTS

Partial purification of a Neu5Gcα2→5-$O_{glycolyl}$Neu5Gc-cleaving sialidase from oyster hepatopancreas. As shown in Fig. 1A, by Sephacryl S-200 filtration, the Neu5Gcα2→5-$O_{glycolyl}$Neu5Gc-cleaving activity overlapped completely with the KDN-cleaving activity. However, by octyl Sepharose chromatography the main Neu5Gcα2→5-$O_{glycolyl}$Neu5Gc-cleaving activity was eluted later than the main KDN-cleaving activity (Fig. 1B). By Matrex Red A chromatography, the Neu5Gcα2→5-$O_{glycolyl}$Neu5Gc-cleaving activity was further separated from the main KDN-cleaving activity, as shown by the horizontal bar in Fig. 1C. These results indicated that the Neu5Gcα2→5-$O_{glycolyl}$Neu5Gc-cleaving sialidase was different from the KDN-sialidase previously studied (4). The partially purified sialidase exhibited maximal activity toward the hydrolysis of Neu5Gcα2→5-$O_{glycolyl}$Neu5Gc at pH 4.0–4.2 in 50 mM sodium acetate buffer.

Hydrolysis of four sialic acid dimers by different sialidases. Table 2 shows that among four sialidases tested, the oyster sialidase was most active in cleaving Neu5Gcα2→5-$O_{glycolyl}$Neu5Gc and KDNα2→8KDN. Compared with these two substrates, Neu5Ac2→8Neu5Ac and Neu5Gcα2→8Neu5Gc were hydrolyzed less efficiently by the oyster sialidase. Among the four sialic acid dimers, Neu5Acα2→8Neu5Ac was the best substrate for the three sialidases isolated from *A. ureafaciens*, *C. perfringens*, and *V. cholerae*. KDNα2→8KDN was refractory to these three microbial sialidases. In contrast to our previous report (5), *Arthrobacter* sialidase was also found to cleave Neu5Gcα2→5-$O_{glycolyl}$Neu5Gc. This finding prompted us to re-examine the hydrolysis of four sialic dimers by *Arthrobacter* sialidase.

Reexamination of the reactivity of A. ureafaciens sialidase to Neu5Gcα2→5-$O_{glycolyl}$Neu5Gc by HPAEC-PAD. In our earlier studies (5), we used the thiobarbituric acid (TBA) method (12, 13) to analyze the hydrolysis of sialic acid dimers. To overcome the poor sensitivity and the lack of specificity of the TBA colorimetric method, we used the HPAEC-PAD method in the present work. The HPAEC-PAD method enabled us to simultaneously analyze the disappearance of the substrate (disialic acid) and the appearance of the

TABLE 2

Hydrolysis of Four Sialic Acid Dimers by Different Sialidases

Source of sialidase	A2	K2	G2	Gg2
	% hydrolysis			
Oyster (pH 4.1)[a]	24.1	91.5	28.4	73.9
Arthrobacter ureafaciens (pH 5.5)[b]	49.1	0	8.1	31.6
Clostridium perfringen (pH 5.5)[b]	18.8	0	5.9	3.5
Vibrio cholerae (pH 5.8)[c]	38.3	0	16.3	29.5

Note. Hydrolysis was conducted at 37°C for 30 min in 50 mM sodium acetate buffer. The pH of the buffer used is indicated in the parenthesis.

[a] 1 μg of each substrate was incubated in 50 μl with 10 mU of enzyme.

[b] 0.5 μg of each substrate was incubated in 10 μl with 1 mU of enzyme.

[c] 0.5 μg of each substrate was incubated in 10 μl with 0.5 U of enzyme. A2, Neu5Acα2→8Neu5Ac; K2, KDNα2→8KDN; G2, Neu5Gcα2→8Neu5Gc, and Gg2, Neu5Gcα2→5-$O_{glycolyl}$Neu5Gc.

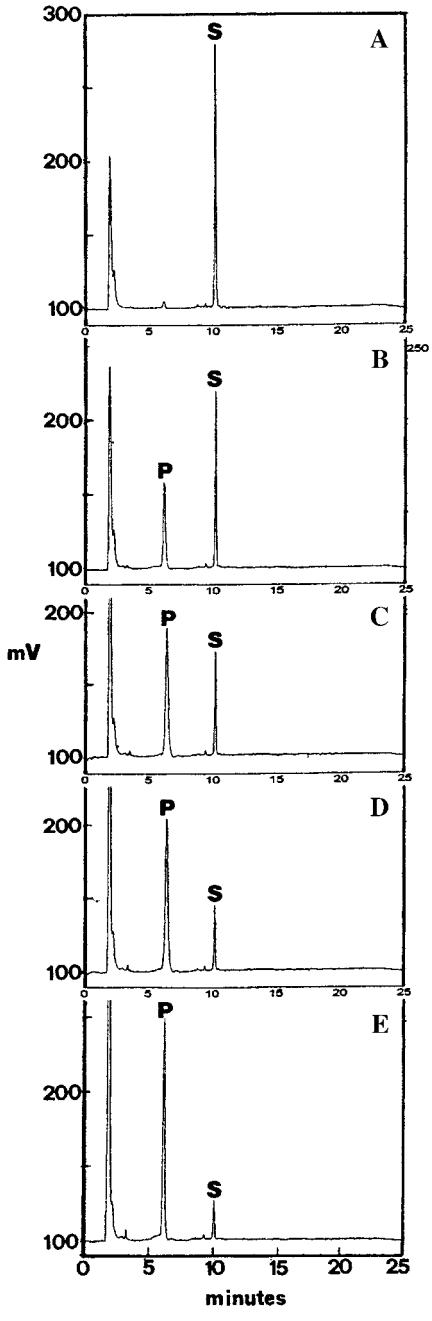

FIG. 2. Time-dependent changes of the concentrations of Neu5Gcα2→5-$O_{glycolyl}$Neu5Gc (substrate, s) and Neu5Gc (product, p) during the *A. ureafaciens* sialidase-catalyzed hydrolysis. Measurements were made by the HPAEC-PAD method under the conditions where the enzyme (42 mU) and the substrate (5 μg) in 185 μl of 50 mM acetate buffer (pH 5.5) were incubated at 37°C for (A) 0 min, (B) 10 min, (C) 30 min, (D) 60 min, (E) 120 min.

product (monosialic acid) as a function of time. Furthermore, this method can accurately quantitate ng quantities of monosialic acid and disialic acid. Figure 2 shows the use of the HPAEC-PAD method to follow the disappearance of Neu5Gcα2→5-$O_{glycolyl}$Neu5Gc, and the appearance of Neu5Gc mediated by the action of *A. ureafaciens* sialidase. As shown in Fig. 3A, the results of the time course hydrolysis indicated that Neu5Gcα2→5-$O_{glycolyl}$Neu5Gc was hydrolyzed about four times faster than Neu5Gcα2→8Neu5Gc by *A. ureafaciens* sialidase. However, the hydrolysis rates of Neu5Gcα2→8Neu5Gc and Neu5Gcα2→5-$O_{glycolyl}$Neu5Gc were much lower than that of Neu5Acα2→8Neu5Ac, i.e., 16 and 64%, respectively (data not shown).

Hydrolysis of four sialic acid dimers by the oyster sialidase. The reactivity of the partially purified oyster sialidase to four discrete sialic acid dimers was analyzed

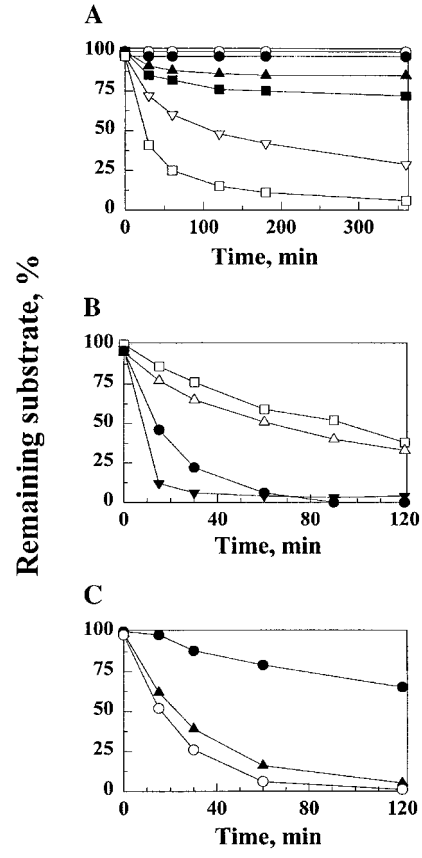

FIG. 3. (A) Hydrolysis of Neu5Gcα2→8Neu5Gc and Neu5Gcα2→5-$O_{glycolyl}$Neu5Gc by *A. ureafaciens* sialidase. ●, incubation of Neu5Gcα2→8Neu5Gc without enzyme; ▲, incubation of Neu5Gcα2→8Neu5Gc with 4 mU enzyme; ■, incubation of Neu5Gcα2→8Neu5Gc with 10 mU enzyme; ○, incubation of Neu5Gcα2→5-$O_{glycolyl}$Neu5Gc without enzyme; ▽, incubation of Neu5Gcα2→5-$O_{glycolyl}$Neu5Gc with 4 mU enzyme; □, incubation of Neu5Gcα2→5-$O_{glycolyl}$Neu5Gc with 10 mU enzyme. (B) Hydrolysis of Neu5Acα2→8Neu5Ac (□), Neu5Gcα2→8Neu5Gc (△), Neu5Gcα2→5-$O_{glycolyl}$Neu5Gc (●), and KDNα2→8KDN (▼) by the sialidase isolated from oyster hepatopancreas. The incubation mixture contained 1 μg of the substrate and 10 mU of the oyster sialidase in 50 μl of 50 mM sodium acetate buffer, pH 4.1. (C) Hydrolysis of Neu5Gcα2→5-$O_{glycolyl}$Neu5Gc by the oyster sialidase in the presence of Neu5Ac2en (●) or KDN2en (▲). ○, Hydrolysis of Neu5Gcα2→5-$O_{glycolyl}$Neu5Gc by the oyster sialidase in the absence of inhibitor. The incubation mixture contained 40 mM of the substrate, 1 mM of the inhibitor and 10 mU of the enzyme in 50 μl sodium acetate buffer, pH 4.1. Detailed assay conditions are described under Materials and Methods.

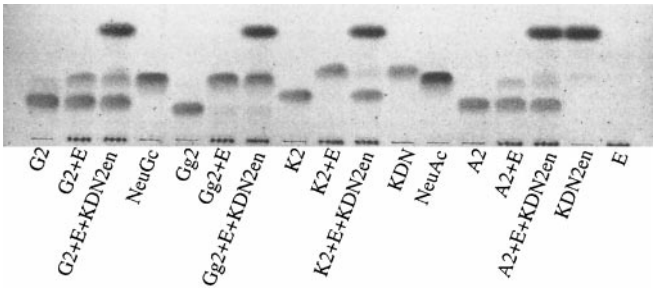

FIG. 4. TLC analysis showing the hydrolysis of Neu5Gcα2→8Neu5Gc (G2), Neu5Gcα2→5-$O_{glycolyl}$Neu5Gc (Gg2), KDNα2→8KDN (K2), and Neu5Acα2→8Neu5Ac (A2), by the oyster sialidase (E) in the presence or absence of KDN2en. Each sialic acid dimer (1 μmol) was incubated with 0.15 unit of the enzyme in 25 μl of 50 mM sodium acetate buffer, pH 4.1 in the presence or absence of 15 nmol of KDN2en at 37°C for 1 h. The detailed assay conditions are described under Materials and Methods.

by the HPAEC-PAD method. In contrast to microbial sialidases, the oyster sialidase cleaved Neu5Acα2→8Neu5Ac least efficiently among the sialic acid dimers, and cleaved Neu5Gcα2→5-$O_{glycolyl}$Neu5Gc about three times faster than Neu5Acα2→8Neu5Ac (Fig. 3B). This enzyme also efficiently hydrolyzed KDNα2→8KDN. It is interesting that the oyster sialidase hydrolyzed Neu5Gcα2→8Neu5Gc slightly faster than Neu5Acα2→8Neu5Ac, whereas, for the microbial sialidases tested, Neu5Gcα2→8Neu5Ac was always a preferred substrate.

Inhibitory effect of KDN2en and NeuAc2en on the hydrolysis of KDNα2→8KDN and Neu5Gcα2→5-$O_{glycolyl}$Neu5G by the oyster sialidase. Since the sialidase prepared from oyster also effectively cleaved KDNα2→8KDN in addition to cleaving Neu5Gcα2→5-$O_{glycolyl}$Neu5Gc, we have studied the effect of KDN2en and Neu5Ac2en on the hydrolysis of these two substrates. As shown in Fig. 3C, KDN2en did not appreciably inhibit the hydrolysis of Neu5Gcα2→5-$O_{glycolyl}$Neu5Gc. However, Neu5Ac2en was found to be a potent inhibitor for the hydrolysis of this sialic acid dimer. These results suggest that the Neu5Gcα2→5-$O_{glycolyl}$Neu5Gc-cleaving activity is distinct from the KDN-cleaving activity.

TLC-analysis showing the effect of KDN2en on the hydrolysis of four sialic acid dimers catalyzed by the oyster sialidase. Figure 4 shows the TLC analysis of the hydrolysis of four sialic acid dimers by the oyster sialidase in the presence or absence of KDN2en. In agreement with the results presented in Fig. 3B, among the four distinct sialic acid dimers, Neu5Gcα2→5-$O_{glycolyl}$Neu5Gc and KDNα2→8KDN were the best substrates for the oyster sialidase. As expected, KDN2en was found to be a potent inhibitor for the hydrolysis of KDNα2→8KDN. This inhibitor, on the other hand, did not appreciably affect the hydrolysis of Neu5Gcα2→8Neu5Gc, Neu5Gcα2→5-$O_{glycolyl}$Neu5Gc, and Neu5Acα2→8Neu5Ac. These results are constant with that shown in Fig. 3C.

DISCUSSION

Since ketosidic linkages are alkali stable and acid labile, the high-pH HPAEC-PAD method (3) is more reliable than the classical TBA reaction (12, 13) for the analysis of a reaction mixture that contains both free sialic acids and ketosidically bound sialic acids. All four sialic acid dimers used in this study were found to be stable under the alkaline elution condition for the HPAEC-PAD system (Fig. 2). A major advantage of the HPAEC-PAD method, shown in Fig. 2, is that ng quantities of both the product and the substrate are simultaneously quantitated more accurately than can be achieved with densitometry used for TLC method. Thus, this method should also be useful for monitoring reactions catalyzed by glycosidases and glycosyltransferases in general.

By using the TBA reaction to detect the release of free sialic acids, we previously reported that compared to Neu5Gcα2→8Neu5Gc, Neu5Gcα2→5-$O_{glycolyl}$Neu5Gc was highly resistant to *A. ureafaciens* sialidase and that this sialic acid dimer was slowly hydrolyzed by *C. perfringens* and *V. cholerae* sialidases (5). In the current study, we used the HPAEC-PAD method to compare the specificity of a novel oyster sialidase with that of three representative bacterial sialidases with respect to the reactivity towards four different sialic acid dimers. By this method, we found that Neu5Gcα2→5-$O_{glycolyl}$Neu5Gc was more susceptible to *A. ureafaciens* and *V. cholerae* sialidases than Neu5Gcα2→8Neu5Gc (Table 2 and Fig. 3A).

It is intriguing that the oyster sialidase cleaved Neu5Gcα2→5-$O_{glycolyl}$Neu5Gc much faster Neu5Acα2→8Neu5Ac that is the most preferable substrate for bacterial sialidases. Since the oyster sialidase hydrolyzed Neu5Gcα2→5-$O_{glycolyl}$Neu5Gc as efficiently as KDNα2→8KDN (Figs. 3B and 4), we have studied the inhibitory effects of two transition state analogues, KDN2en and Neu5Ac2en, on the hydrolysis of these two sialic acid dimers by this enzyme. The hydrolysis of KDNα2→8KDN was greatly inhibited by KDN2en, but less significantly by Neu5Ac2en, whereas the hydrolysis of Neu5Gcα2→5-$O_{glycolyl}$Neu5Gc was significantly inhibited by Neu5Ac2en, but not by KDN2en (Figs. 3C and 4). Further kinetic analysis of the enzyme reaction and structural analysis of enzyme protein using purified enzyme are the future goals of our study. Even at the partially purified stage, the Neu5Gcα2→5-$O_{glycolyl}$Neu5Gc-cleaving sialidase should be useful for the structural and functional studies of sialoglycoconjugates.

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