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Identification, characterization, and developmental expression of a novel α2 → 8-KDN-transferase which terminates elongation of α2 → 8-linked oligo-polysialic acid chain synthesis in trout egg polysialoglycoproteins

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A novel glycosyltransferase which catalyses transfer of deaminated neuraminic acid, KDN (2-keto-3-deoxy-D-*glycero*-D-*galacto*-nononic acid) from CMP-KDN to the non-reducing termini of oligo-polysialyl chains of polysialoglycoprotein (PSGP), was discovered in the ovary of rainbow trout (*Oncorhynchus mykiss*). The KDN-transferase activity was optimal at neutral pH, and stimulated 2 to 2.5-fold by 2–5 mM Mg^{2+} or Mn^{2+}. Expression of KDN-transferase was developmentally regulated in parallel with expression of the α2 → 8-polysialyltransferase, which catalyses synthesis of the oligo-polysialyl chains in PSGP. Incorporation of the KDN residues into the oligo-polysialyl chains prevented their further elongation, resulting in 'capping' of the oligo-polysialyl chains. This is the first example of a glycosyltransferase that catalyses termination of α2 → 8-polysialylation in glycoproteins.

Keywords: KDN-capping, KDN-transferase, termination of polysialylation

Abbreviations: KDN, 2-keto-3-deoxy-D-*glycero*-D-*galacto*-nononic acid or naturally occurring deaminated neuraminic acid; Neu5Ac, *N*-acetylneuraminic acid; Neu5Gc, *N*-glycolylneuraminic acid; CMP-KDN, cytidine 5′-(3-deoxy-D-*glycero*-D-*galacto*-2-nonulosonic phosphate) or cytidine 5′-KDN phosphate; CMP-NeuAc, cytidine 5′-Neu5Ac phosphate; oligo-polySia, oligo- and/or polysialic acid; PSGP, rainbow trout egg polysialoglycoprotein comprising α2 → 8-linked oligo- polyNeu5Gc; PSGP (low Sia), a precursor of PSGP present at early stages of oogenesis which contains mostly the disialyl group, Siaα2 → 8Siaα2 → 6-; ***K**-PSGP, [^{14}C]KDN-labelled PSGP obtained by incubating PSGP and CMP-[^{14}C]KDN with the immature cortical vesicle fraction P1 containing KDN-transferase; ***A**-PSGP, [^{14}C]Neu5Ac-labelled PSGP obtained by incubating PSGP and CMP-[^{14}C]Neu5Ac with the P1 fraction; **A**-***K**-PSGP and **K**-***K**-PSGP, the products obtained after incubating ***K**-PSGP with P1 fraction and unlabelled CMP-Neu5Ac or CMP-KDN, respectively; ***K**-$PSGP_{CHO}$, **A**-***K**-$PSGP_{CHO}$, and **K**-***K**-$PSGP_{CHO}$, mixture of oligosaccharide alditols obtained by alkaline borohydride treatment of ***K**-PSGP, **A**-***K**-PSGP, and **K**-***K**-PSGP, respectively; ***A**-$PSGP_{CHO}$, a mixture of oligosaccharide alditols obtained by alkaline borohydride treatment of [^{14}C]Neu5Ac-labelled PSGP; Endo-N, endo-N-acylneuraminidase; DP, degree of polymerization; GLC, gas-liquid chromatography; HPLC, high performance liquid chromatography; TLC, thin layer chromatography.

Introduction

The new deaminated neuraminic acid, KDN (2-keto-3-deoxy-D-*glycero*-D-*galacto*-nononic acid) was first reported as a minor component of rainbow trout egg polysialoglycoprotein (PSGP) in 1986 [1]. Since this discovery, reports on the occurrence of KDN-containing glycoconjugates have been increasing. Indeed, KDN residues have now been identified in glycoproteins, glycosphingolipids and a bacterial capsular polysaccharide, and may be ubiquitous components of glycoconjugates that range in evolutionary

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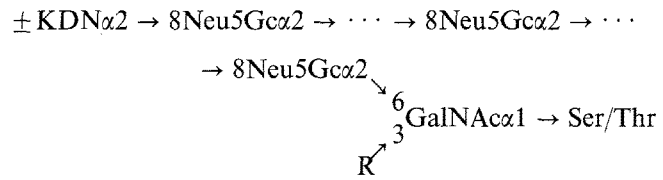
diversity from bacteria to vertebrates [2–14]. In rainbow trout PSGPs, the KDN residues were demonstrated to cap the nonreducing termini of the α2 → 8-linked oligo-polysialyl chains, as shown below:



where R → 3GalNAcα → represents five different core oligosaccharide chains [13, 15–19]. Terminal capping of the oligo-polysialic acid (polySia) chains by KDN is believed to protect them from the action of exosialidases [1]. Such modification would also provide a mechanism to prevent further chain elongation.

We have recently shown that synthesis of the oligo-polySia residues of PSGPs in trout oocytes requires three different sialyltransferase activities [20]. The first of these is an α-*N*-acetylgalactosaminide α2,6-sialyltransferase (α2,6-ST) that catalyses formation of the Sia residues α2,6-linked to the proximal GalNAc residue. The second is an α2,6-sialoside α2,8-sialyltransferase (α2,8-ST or 'initiase') that catalyses transfer of the first α2,8-Sia residue to the α2,6-linked Sia residue. The third activity is an α2,8-polysialyltransferase (α2,8-polyST or 'polymerase') that is responsible for synthesis of the α2,8-linked oligo-polySia chains. Expression of these enzymes is developmentally regulated and the activities increase in accordance with the developmental appearance of each PSGP [20]. Our current interest in biosynthesis of the carbohydrate moieties of fish egg PSGP stems from the recent finding of a CMP-KDN synthetase in trout testis and ovary [21]. This raises the question of the involvement of this enzyme in KDN-glycoconjugate synthesis and, importantly, in how KDN residues are transferred from CMP-KDN into KDN-glycan chains.

The aim of this study was to determine if a KDN-transferase activity was present in trout ovaries which may be involved in the capping reaction to form KDNα2 → 8(Neu5Gcα2 → 8)$_n$ chains. We report here our findings of such an enzyme in the Golgi-derived immature cortical vesicle fraction obtained from trout ovaries 2 months prior to ovulation. Appearance of the KDN-transferase at this stage of development coincides with the developmental expression of the α2,8-polysialyltransferase required for polySia chain polymerization.

Materials and methods

Materials

Rainbow trout ovaries were generously supplied every month between April and October during the 6 months of oogenesis by the Gunma Prefectural Fisheries Experimental Station at Kawaba. Ovaries were immediately frozen after dissection and stored at −80 °C until use. CMP-[^{14}C]KDN and CMP-KDN were synthesized as previously described [21]. CMP-[^{14}C]Neu5Ac was purchased from Amersham (UK). Unlabelled CMP-Neu5Ac was obtained from Wako Chemicals (Japan). PSGP, in which about 30% of the poly-oligosialyl chains were substituted by KDN residues at their nonreducing termini, was prepared from the unfertilized mature eggs of rainbow trout by methods previously described [22, 23], *Arthrobacter ureafaciens* exosialidase was obtained from Nacalai Tesque (Japan).

Preparation of KDN-transferase from rainbow trout ovaries

All procedures were carried out at 0 ~ 4 °C. Five grams of frozen rainbow trout ovaries were thawed and homogenized in 5 ml of Buffer A [50 mM MES (pH 7.0) containing 0.1 M NaCl, 10% (v/v) glycerol, and 0.1 mg ml^{-1} soybean trypsin inhibitor (SBTI)] in a porcelain mortar. The homogenate was filtered through Tetoron gauze and centrifuged at 10 000 × **g** for 20 min. The pellet (P1 fraction) was resuspended in Buffer A, centrifuged as above, and was resuspended in 1 ml of Buffer B [Buffer A devoid of NaCl and containing 6% (v/v) Tween 80]. The P1 fraction contains the Golgi-derived immature cortical vesicles. The supernatant (S1 fraction) was centrifuged again as described above to avoid any contamination from the pellet and then centrifuged at 100 000 × **g** for 60 min. The supernatant (S2 fraction) was removed, and the pellet was suspended in Buffer A, ultracentrifuged again as above, and resuspended in 0.5 ml of Buffer B. This pellet was designated the P2 fraction. A part of the S2 fraction was diluted with four volumes of Buffer B to reduce the salinity to 10 mM. When the pH profile of enzyme activity in the P1 fraction was determined, MES (pH 7.0) buffer in Buffer A (used for washing) and in Buffer B (used for assay) was replaced by 50 mM of MES (pH 5.5 ~ 7.0) or HEPES (pH 7.0 ~ 8.5).

Standard KDN-transferase assay procedure

Incubation mixtures (50 μl) containing PSGP (0.2 nmol) as exogenous acceptor, CMP-[^{14}C]KDN (0.075 nmol, ~53 000 cpm) as donor substrate, and a KDN-transferase fraction (P1, P2, or S2 fraction) were incubated at 25 °C for 3 h. Before and after the incubation 20 μl aliquots were removed and spotted on Whatman 3MM paper. The enzyme reactions were terminated quickly by adding 20 μl of absolute ethanol. The papers were chromatographed in 95% ethanol, 1 M ammonium acetate, pH 7.5 (7:3) for 5 h. After air drying, the amount of [^{14}C]KDN incorporated into the PSGP acceptor, which remained at the origin, was determined by a Bio-Imaging Analyzer (Fujix BAS 2000) or by an Aloka liquid scintillation system LSC-700 with ACS-II (Amersham) as scintillant. One unit of KDN-transferase activity was defined as the amount of enzyme required to incorporate 1 pmol of KDN per min into the exogenous acceptor.

Standard polysialyltransferase assay procedure

Activity of the α2,8-polysialyltransferase was determined as previously described, using PSGP as an exogenous acceptor [20]. CMP-[^{14}C]Neu5Ac (0.075 nmol, about 53 000 cpm) was used as donor substrate. One unit of polysialyltransferase activity was defined as the amount of enzyme required to incorporate 1 pmol of Neu5Ac per min into PSGP.

*Synthesis and characterization of [^{14}C]KDN-labelled PSGP, *K-PSGP*

The immature cortical vesicle (P1) fraction prepared from ovaries 2 months prior to ovulation was used as a source of KDN-transferase to synthesize [^{14}C]KDN-labelled PSGP. Incorporation of [^{14}C]KDN into PSGP was carried out as described above, except that the concentration of CMP-[^{14}C]KDN was four-fold higher, and a 15-fold larger scale incubation mixture (750 µl) was used. After a 24 h incubation period, the [^{14}C]KDN-containing PSGP thus obtained was designated ***K**-PSGP. ***K**-PSGP was purified by column chromatography on Sephacryl S-200 (1.3 × 100 cm, eluted with 0.1 M NaCl, 5 mM Tris-HCl, pH 8.0) and desalted by dialysis against water. ***K**-PSGP was treated with alkaline borohydride (1 M $NaBH_4$ in 0.1 N NaOH) at 37 °C for 48 h, and neutralized by the gradual addition of acetic acid in an ice bath. The oligosaccharide alditols released by β-elimination were purified by a Sephacryl S-200 column (1.3 × 100 cm, eluted with 0.1 M NaCl, 5 mM Tris-HCl, pH 8.0) and desalted on a Sephadex G-25 column (1.3 × 100 cm, eluted with 5% aqueous ethanol). The mixture of oligosaccharide alditols was pooled, lyophilized, and resolved by HPLC on an anion exchange Mono-Q (Pharmacia) column using an Irica HPLC system, as described previously [20, 24]. Fractions were collected and the radioactive elution patterns were determined by liquid scintillation counting, as described above.

The tetra-anionic oligosaccharide alditol fraction obtained from ***K**-PSGP was divided into two parts. One part was treated with *A. ureafaciens* exosialidase (1.25 mU) in 20 µl of 50 mM sodium acetate buffer (pH 5.5) at 37 °C for 6 h. The second part did not receive exosialidase and served as the control. Both fractions were spotted on a thin-layer chromatography sheet (0.2 mm thick silica gel plastic sheet; Kiesel gel 60, Merck) and developed with 1-propanol: 25% NH_4OH: water (6:1:2.5) for 12 h [18, 24]. Radioactive spots were visualized by a Bio-Imaging Analyser (Fujix BAS 2000).

*Incorporation of Neu5Ac or KDN into *K-PSGP*

K**-PSGP (1.2 nmol, 30 000 cpm) was incubated with an excess of CMP-Neu5Ac or CMP-KDN (75 nmol each) and the P1 enzyme fraction at 25 °C for 24 h. The reaction products obtained after incubation with CMP-Neu5Ac or CMP-KDN were designated **A**–K**-PSGP and **K**–***K**-PSGP, respectively. **A**-***K**-PSGP, **K**-***K**-PSGP, and ***K**-PSGP were separately treated with alkaline borohydride and the oligosaccharide alditols were isolated as described above. They were denoted as **A**-***K**-$PSGP_{CHO}$, **K**-***K**-$PSGP_{CHO}$, and ***K**-$PSGP_{CHO}$, respectively, and analysed by a Mono-Q HPLC.

*Endo-N-acylneuraminidase (Endo-N) depolymerization of *K-$PSGP_{CHO}$*

Endo-N was prepared from bacteriophage K1F as previously described [26]. To the freeze-dried sample of ***K**-$PSGP_{CHO}$, which had more than five sialyl residues (including [^{14}C]KDN) on each glycan chain, were added 10 µl of 20 mM Tris-HCl (pH 7.4) containing 25 mU of Endo-N. After incubation at 37 °C for 12 h, another 25 mU of Endo-N was added and incubation was continued for an additional 12 h at 37 °C. After Endo-N treatment, the oligosaccharides were analysed by HPLC, as previously described [20, 24].

Results and discussion

Localization and properties of the KDN-transferase found in rainbow trout ovaries

As shown in Table 1, KDN-transferase activity was found in the pellet (P1) fraction which contained the Golgi-derived immature cortical vesicles, and in the soluble (S2) fraction. The S2 fraction contains soluble enzymes released from the fragile cortical vesicles [20]. Little or no activity was detected in the membrane (P2) fraction which contains the Golgi apparatus. These results are nearly identical to our earlier localization studies of the α2,8-ST and the α2,8-polyST activities in trout ovaries [20]. These studies showed that more than 96% of these ST activities were found in either the cortical vesicles (P1) or the soluble (S2) fractions. Less than 4% of the activities were localized in the P2 membrane (Golgi) fraction [20]; these residual activities are likely to be due to carry-over from the P1 fraction as judged from the failure to detect α2,8-polyST activity in the P2 fraction after repeated centrifugation of the S1 fraction at 10 000 × **g** prior to ultracentrifugation (results not shown). Rather high total activity was observed in the S2 fraction (Table 1), but because of the presence, in this fraction, of more than 95% of total ovary proteins, which are most soluble yolk proteins, the specific activity was so low that the S2 fraction was not used in the subsequent experiments. The cortical vesicle fraction (P1) was used as the source of enzyme for further characterization because its specific activity was about 38-fold higher than that of the soluble (S2) fraction (17.7 *versus* 0.47 mU per mg protein).

Maximum activity of the KDN-transferase in the cortical vesicle (P1) fraction occurred between pH 6.0–7.0 (Fig. 1). There was a dramatic decrease in activity above pH 7.0. Both Mg^{2+} and Mn^{2+} at 2–5 mM stimulated the KDN-transferase two to 2.5-fold.

Table 1. Localization of the KDN-transferase activity in rainbow trout ovaries 2 months prior to ovulation (August). One unit of KDN-transferase activity is defined as the amount of enzyme required to incorporate 1 pmol of [^{14}C]KDN per min into the exogenous acceptor.

Fraction	*Total activity* (*unit per ovary*)	(%)	*Specific activity*[a] (*mU per mg protein*)
Immature cortical vesicle fraction (P1)	0.860	32.0	17.7
Soluble fraction (S2)	1.83	68.0	0.470
Membrane fraction (P2)	ND[b]	0	ND

[a] Determined by dividing the total activity by the amount of protein in each enzyme fraction. The amount of protein was estimated by the modified Lowry method (BCA, Pierce).
[b] ND = not detected.

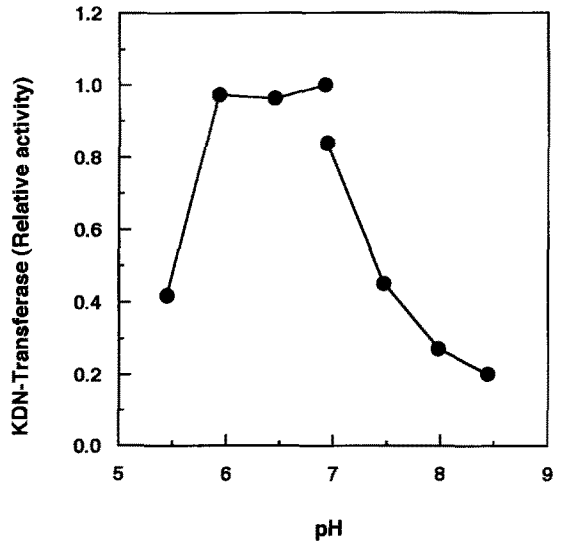


Figure 1. pH dependence of KDN-transferase activity in the immature cortical vesicles (P1) fraction of rainbow trout ovary. Activity values are expressed relative to that at pH 7.0 (assayed in MES buffer) with PSGP set to 1.0.

*Characterization of [^{14}C]KDN-labelled PSGP (*K-PSGP) and effect of exo- and endo-sialidase treatment of [^{14}C]KDN-capped oligosialylalditols, *K-PSGP$_{CHO}$*

[^{14}C]KDN residues were incorporated into PSGP as described under Materials and methods. HPLC analysis of the [^{14}C]KDN-labelled oligosaccharide alditols, ***K**-PSGP$_{CHO}$, revealed that [^{14}C]KDN residues were incorporated into a series of oligosialylalditols, (Neu5Gcα2 $\rightarrow$ 8)$_n$ $\rightarrow$ R, where R is the core oligosaccharide alditol, and n ranged from 1, 2, 3, . . . , up to more than 7 (see Fig. 2A). For product analysis, the tetra-anionic fraction of [^{14}C]KDN-labelled oligosialylalditols (***K**-PSGP$_{CHO}$) was treated with *A. ureafaciens* exosialidase. As shown in Fig. 3A, no significant change in migration of the glycan chains on a TLC plate was observed, indicating that the [^{14}C]KDN residues were incorporated into the non-reducing termini of the oligosialyl chains. As shown earlier, capping of these oligoNeu5Gc chains with KDN renders them resistant to hydrolysis by exosialidase [1].

A mixture of KDN-capped oligoNeu5Gc-glycan alditols (DP $\geqslant$ 5) was treated with Endo-N, and the reaction mixture was chromatographed on a Mono-Q HPLC column. The elution pattern (Fig. 3B) was nearly identical to that observed for the Endo-N treatment of ***A**-PSGP$_{CHO}$, where ***A**-PSGP$_{CHO}$ is a mixture of oligosaccharide alditols derived from [^{14}C]Neu5Ac-labelled PSGP [20]. These results thus indicate that the KDNα2 $\rightarrow$ 8(Neu5Gcα2 $\rightarrow$ 8)$_n$ sequence ($n \geqslant 4$) was partially sensitive to Endo-N digestion, although the (KDNα2 $\rightarrow$ 8)$_n$ structures present in KDN-rich glycoprotein were resistant [25]. While the detailed molecular mechanism by which Endo-N cleaves the α2 $\rightarrow$ 8 linked oligo-polySia chains is not known [27, 28], the present result shows that Endo-N can catalyse hydrolysis of some intersialyl linkages, even when the chains are substituted at C-8 of the nonreducing terminal residues with KDN.

Specificity of the KDN-transferase

To examine the specificity of the α2 $\rightarrow$ 8-KDN-transferase towards oligo-polySia structures, the immature cortical vesicle (P1) fraction from rainbow trout ovary cells was incubated with ***K**-PSGP as the acceptor and unlabelled CMP-Neu5Ac or CMP-KDN. After incubation, the oligosaccharide chains were released from the PSGP by β-elimination with alkaline borohydride, as described under Materials and methods. The released oligosaccharide alditols (denoted as **A-*K**-PSGP$_{CHO}$ and **K-*K**-PSGP$_{CHO}$) were fractionated by Mono-Q HR5/5 anion exchange HPLC. Fig. 2B and 2C show the elution profiles for **A-*K**-PSGP$_{CHO}$ and **K-*K**-PSGP$_{CHO}$, respectively. As shown in Figs. 2A–2C, no significant difference was observed in the amounts of the tri- through higher oligoanionic alditols. There was an evident increase in the dianionic fraction, however, that was concomitant with a decrease in the monoanionic fraction. These results clearly show that the

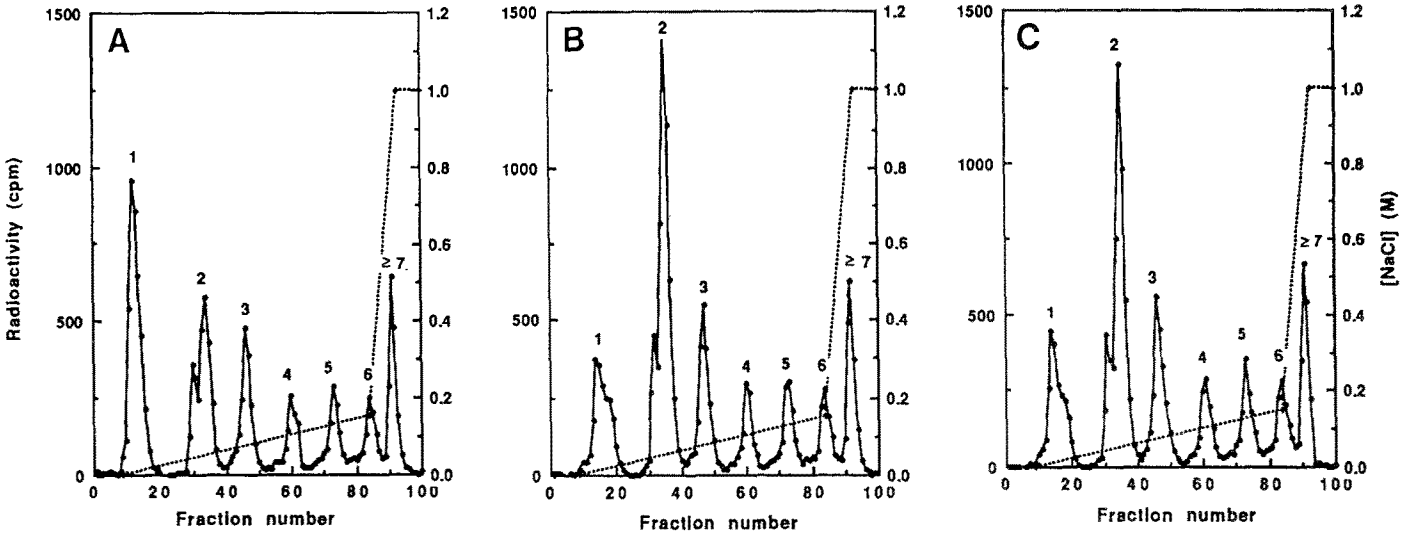


Figure 2. Analysis of [^{14}C]KDN-labelled oligo-polysialylglycan chains in PSGP by HPLC after release by β-elimination with alkaline borohydride. (**A**) Elution profile of ***K**-PSGP$_{CHO}$ by Mono-Q anion exchange HPLC. Numbers above each peak denote the degree of polymerization (DP) of oligo-polySia including the capped [^{14}C]KDN residue. The oligo-polySia chains capped by KDN at their non-reducing termini no longer can serve as an exogenous acceptor for incorporation of Neu5Ac (**B**) or KDN (**C**), since the elution patterns for DPs above 3 in **A**-***K**-PSGP$_{CHO}$ (**B**) and **K**-***K**-PSGP$_{CHO}$ (**C**) are identical with that in (**A**).

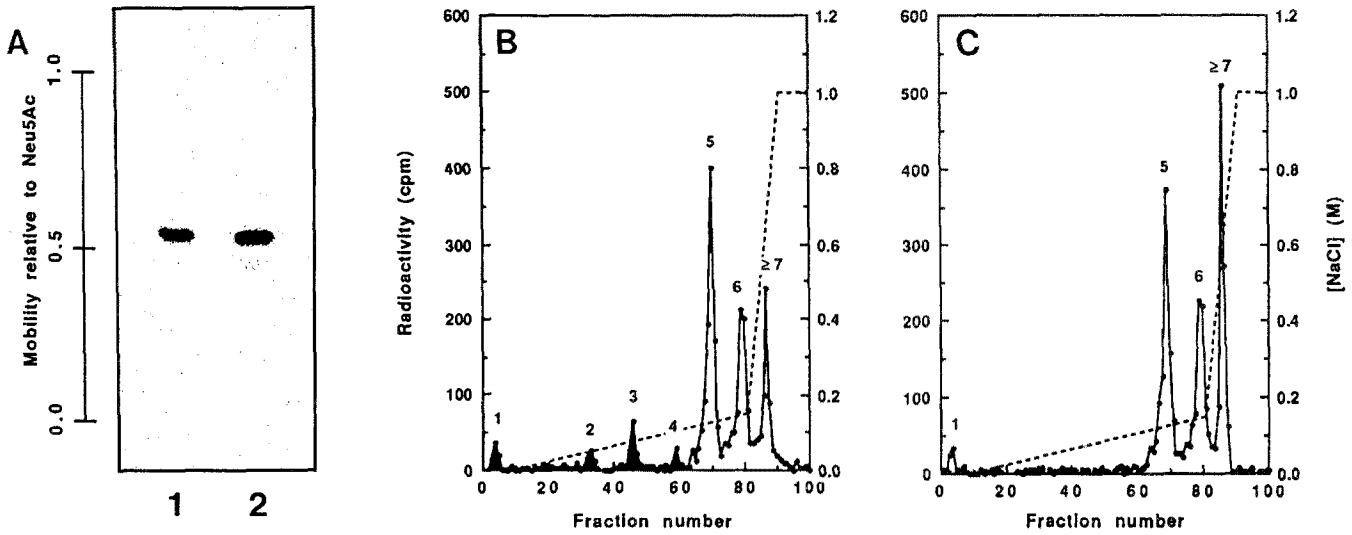


Figure 3. Susceptibility of the KDN-capped oligosialic acid chains in *K-PSGP$_{CHO}$ to *A. ureafaciens* exo-sialidase and endo-sialidase (Endo-N). (**A**) TLC analysis of ***K**-PSGP$_{CHO}$ (DP = 4) after incubation with (lane 1) and without (lane 2) *A. ureafaciens* exo-sialidase. HPLC anion exchange chromatography of ***K**-PSGP$_{CHO}$ (DP ⩾ 5) after incubation with (**B**) and without (**C**) Endo-N.

KDN-capped oligosialyl chains in ***K**-PSGP cannot serve as an exogenous acceptor for the trout ovary α2,8-polysialyl-transferase, or for the subsequent addition of KDN residues by the KDN-transferase. Contrary to this, viability of [^{14}C]Neu5Ac-incorporated oligosialyl chains in ***A**-PSGP as an acceptor for α2,8-polyST was demonstrated in our previous study [20].

The increase in the dianionic fraction at the expense of the monoanionic species following incubation of ***K**-PSGP with unlabelled sugar nucleotides is presumably attributed to the following two sequential reactions:

(**1**) GalNAcβ1 → 4GalNAcβ1 → 3Galβ1 → 4Galβ1 → 3GalNAcα1 → *O*-Ser/Thr + CMP-[^{14}**C]KDN** → GalNAcβ1 → 4([^{14}**C]KDNα2** → **3**)GalNAcβ1 → 3Galβ1 → 4Galβ1 → 3GalNAcα1 → *O*-Ser/Thr;

(**2**) GalNAcβ1 → 4([^{14}**C]KDNα2** → **3**)GalNAcβ1 → 3Galβ1 → 4Galβ1 → 3GalNAcα1 → *O*-Ser/Thr + CMP-***Neu5Ac*** (or CMP-***KDN***) → GalNAcβ1 → 4([^{14}**C]KDNα2** → **3**)GalNAcβ1 → 3Galβ1 → 4Galβ1 → 3(***Neu5Acα2*** → ***6*** or ***KDNα2*** → **6**)GalNAcα1 → *O*-Ser/Thr.

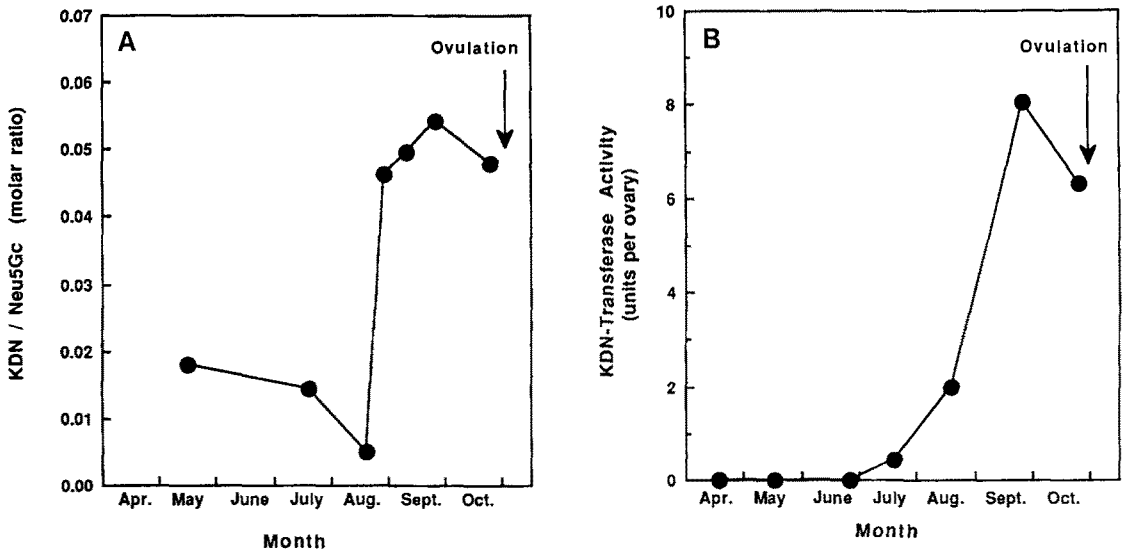


Figure 4. Developmental changes in KDN and KDN-transferase expression during oogenesis. (**A**) Developmental changes in the KDN/Neu5Gc ratio in PSGP. Values are expressed as the molar ratios of KDN to Neu5Gc in PSGP. Isolation and carbohydrate analysis were carried out as described under 'Materials and methods'. (**B**) Developmental expression of the α2 → 8-KDN-transferase in P1 fraction. Activities were assayed using PSGP as exogenous acceptor, as described under 'Materials and methods'.

These two reactions account for synthesis of the 'KDN-containing long core unit' in trout egg PSGP [2, 13].

Developmental changes in the level of the KDN residues in PSGP during oogenesis

Developmental expression of the KDN residues in PSGP in oocytes and eggs between April and October is shown in Fig. 4A. The ratio of KDN to Neu5Gc showed little increase during the first 3 months of oogenesis. In contrast, there was a dramatic increase in KDN expression between August and September, 2 months prior to ovulation. KDN was nearly undetectable before May, although a small amount was found in the PSGP(low Sia) fraction from oocytes harvested on May 16. PSGP(low Sia) is a precursor PSGP that contains mostly disialyl (Siaα2 → 8Siaα2 → 6-) side chains [20]. The KDN residues in PSGP(low Sia) probably represents KDN linked α2 → 3 and α2 → 6 to the internal GalNAc and the proximal GalNAc residues, respectively [2]. The KDN-transferase(s) responsible for synthesis of these KDN residues are enzymes presumably different from the KDN-transferase studied here that is responsible for the terminal capping of the oligo-polySia chains in PSGP.

Developmental expression of the KDN-transferase during oogenesis

Expression of the KDN-transferase activity was temporally correlated with expression of KDN (Fig4A, B). Expression was also developmentally regulated (Fig. 4B), and in parallel with the temporal expression of the α2 → 8-polysialyltransferase which we previously showed was responsible for synthesis of the α2 → 8-linked oligo-polySia chains in PSGP [20]. The observed change in KDN-transferase activity was not simply the result of an increase in ovary weight, but its expression was shown to increase because the activity per 1 g of ovary also increased during oocyte maturation in a parallel fashion to the activity per whole ovary shown in Fig. 4B (data not shown).

Concluding remarks

A new glycosyltransferase activity which catalyses the transfer of KDN from CMP-KDN to the nonreducing termini of oligo-polySia chains of polysialoglycoprotein was identified in the ovary of rainbow trout. The KDN-transferase activity has a pH optimum around 7 and requires a divalent cation (Mg^{2+} or Mn^{2+}) for maximal activity. Incorporation of KDN residues into the oligo-polySia chains at their nonreducing termini prevented the subsequent elongation of the chains. Therefore, we conclude that biosynthesis of oligo-polySia chains precedes incorporation of KDN residues during maturation, and that it is the KDN-capping reaction which terminates oligo-polySia chain elongation in PSGPs. Identification of the KDN-transferase is the first step in unravelling the biogenetic mechanism for synthesis of the different types of KDN-glycan chains in the various KDN-containing glycoconjugates. This is the first example of a glycosyltransferase that catalyses the termination of α2 → 8-polysialylation in glycoproteins. It will be of interest to see if a similar mechanism may terminate elongation of the α2 → 8-linked polySia chains in synthesis of the capsular polysaccharide in neurotropic *E. coli* K1 and *N. meningitidis* group B strains, and in the embryonic form of the neural cell adhesion molecule.

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References

1. Nadano D, Iwasaki M, Endo S, Kitajima K, Inoue S, Inoue Y (1986) *J Biol Chem* **261**:11550–57.
2. Iwasaki M, Inoue S, Nadano D, Inoue Y (1987) *Biochemistry* **26**:1452–57.
3. Inoue S, Kanamori A, Kitajima K, Inoue Y (1988) *Biochem Biophys Res Commun* **153**:172–76.
4. Kanamori A, Kitajima K, Inoue S, Inoue Y (1989) *Biochem Biophys Res Commun* **164**:744–49.
5. Iwasaki M, Inoue S, Troy FA (1990) *J Biol Chem* **265**: 2596–602.
6. Kanamori A, Inoue S, Iwasaki M, Kitajima K, Kawai G, Yokoyama S, Inoue Y (1990) *J Biol Chem* **265**:21811–19.
7. Strecker G, Wieruszeski J-M, Michalski J-C, Alonso C, Boilly B, Montreuil J (1992) *FEBS Lett* **298**:39–43.
8. Strecker G, Wieruszeski J-M, Michalski J-C, Montreuil J (1992) *Biochem J* **287**:905–9.
9. Strecker G, Wieruszeski J-M, Michalski J-C, Alonso C, Leroy Y, Boilly B, Montreuil J (1992) *Eur J Biochem* **207**:995–1002.
10. Yu S, Kitajima K, Inoue S, Inoue Y (1991) *J Biol Chem* **266**:21929–35.
11. Yu S, Kitajima K, Inoue S, Muto Y, Kasama T, Handa S, Inoue Y (1993) *Biochemistry* **32**:9221–29.
12. Knirel YA, Kocharova NA, Shashkov AS, Kochetkov NK, Mamontova VA, Solov'eva TF (1989) *Carbohydrate Res* **188**:145–55.
13. Inoue Y (1993) In *Polysialic Acid: From Microbes to Man* (Roth J, Rutishauser U, Troy FA, eds) pp 171–81. Basel: Birkhäuser Verlag.
14. Troy FA (1992) *Glycobiology* **2**:5–23.
15. Nomoto H, Iwasaki M, Endo T, Inoue S, Inoue Y, Matsumura G (1982) *Arch Biochem Biophys* **218**:335–41.
16. Iwasaki M, Inoue S, Kitajima K, Nomoto H, Inoue Y (1984) *Biochemistry* **23**:305–10.
17. Iwasaki M, Inoue S, Kitajima K, Nomoto H, Inoue Y (1984) *Biochem Int* **8**:573–79.
18. Iwasaki M, Inoue S (1985) *Glycoconjugate J* **2**:209–28.
19. Kitajima K, Inoue Y, Inoue S (1986) *J Biol Chem* **261**:5262–69.
20. Kitazume S, Kitajima K, Inoue S, Troy FA, Inoue Y (1994) *J Biol Chem* **269**:10330–40.
21. Terada T, Kitazume S, Kitajima K, Inoue S, Ito F, Troy FA, Inoue Y (1993) *J Biol Chem* **268**:2640–48.
22. Inoue S, Inoue Y (1986) *J Biol Chem* **261**:5256–61.
23. Inoue S, Matsumura G (1979) *Carbohydrate Res* **74**:361–68.
24. Kitazume S, Kitajima K, Inoue S, Inoue Y (1992) *Anal Biochem* **202**:25–34.
25. Sato C, Kitajima K, Tazawa I, Inoue Y, Inoue S, Troy FA (1993) *J Biol Chem* **268**:23675–84.
26. Hallenbeck, PC, Vimr ER, Yu F, Bassler B, Troy FA (1987) *J Biol Chem* **262**:3553–61.
27. Kwiatkowski B, Boscheck B, Thiele H, Stirm S (1982) *J Virol* **43**:697–704.
28. Pelkonen S, Pelkonen J, Finne J (1989) *J Virol* **63**:4409–16.