HNK-1 Glyco-epitope: COMPLEX Machinery for Biosynthesis

67

Naoki Nakagawa and Shogo Oka

Contents

Abstract

The human natural killer-1 (HNK-1) carbohydrate is a well-known glyco-epitope that is predominantly expressed in the central and peripheral nervous systems and plays an essential role in higher brain functions such as synaptic plasticity, spatial learning, and memory. HNK-1 has a unique sulfated trisaccharide structure, HSO_3-3GlcAβ1-3Galβ1-4GlcNAc, and is expressed under the control of highly sophisticated machineries that cannot simply be explained by quantitative changes in a single synthetic enzyme, such as the selective expression of this epitope on limited types of proteins. Recent studies investigating the regulation of HNK-1 at cellular and molecular levels demonstrated that HNK-1-synthesizing enzymes physically and functionally interacted with each other to form a heteromeric enzyme complex. This enzyme complex then facilitated the biosynthesis of HNK-1 by increasing production efficacy and has also been implicated in the sorting of carrier molecules. While the biological significance of the enzyme complex in regulating the HNK-1 epitope has begun to be

N. Nakagawa • S. Oka (✉)
Department of Biological Chemistry, Human Health Sciences, Graduate School of Medicine, Kyoto University, Sakyo-ku, Kyoto, Japan
e-mail: nakagawa.naoki.33m@st.kyoto-u.ac.jp; shogo@hs.med.kyoto-u.ac.jp

N. Taniguchi et al. (eds.), *Glycoscience: Biology and Medicine*,
DOI 10.1007/978-4-431-54841-6_120

clarified, HNK-1ST also possesses distinct functions independent of the canonical synthesis of HNK-1, which has provided an insight into the diversity of the regulatory mechanisms responsible for mammalian glycosylation.

Keywords
HNK-1 • Enzyme complex • GlcAT-P • GlcAT-S • HNK-1ST • β4GalT-II

Introduction

Glycans are linear or branched polysaccharide structures that are covalently bound to proteins or lipids and play pivotal roles in various molecular and cellular events by modulating the biological activities of their carriers. In most cases, unique terminal moieties attached to glycans, such as polysialic acid, Lewis X epitope, and sulfated glycosaminoglycans (GAGs), serve as functional motifs. Among these, the human natural killer-1 (HNK-1) carbohydrate is a well-known glyco-epitope with a characteristic structure that is composed of a trisaccharide with a terminal sulfate group, HSO_3-3GlcAβ1-3Galβ1-4GlcNAc (Fig. 1) (Kizuka and Oka 2012). The HNK-1 epitope is expressed on several subsets of adhesion molecules (NCAM, L1, P0), extracellular matrix proteins (phosphacan, tenascin-C, tenascin-R), and glycolipids and is synthesized on the nonreducing end of an *N*-acetyllactosamine residue by one of two glucuronyltransferases (GlcAT-P or GlcAT-S) and a sulfotransferase (HNK-1ST) in the distal part of the Golgi apparatus (Fig. 1). A recent study demonstrated that the galactose in the *N*-acetyllactosamine residue of HNK-1 was mainly synthesized by β-1, 4-galactosyltransferase (β4GalT)-II (Fig. 1) (Yoshihara et al. 2009). Phenotypic analysis of the gene-deficient mouse for GlcAT-P, a rate-limiting enzyme in the HNK-1 biosynthesis in the brain, revealed that the HNK-1 glycan was required for the development of higher brain functions including synaptic plasticity and memory formation (Yamamoto et al. 2002). One prominent feature is that the expression level of the HNK-1 glycan is strictly controlled in spatially and temporally regulated manners. For example, the gross expression of HNK-1 in the mouse hippocampus has been shown to rapidly increase after birth, reaching a peak after approximately 2–3 weeks, which coincides with the period when the neural circuit is actively formed, and then gradually decreases in adulthood (Morita et al. 2009). These developmental changes in HNK-1 correspond well to the specific activity of GlcAT-P, which also peaks after approximately 2–3 weeks in the mouse brain. Hence, the amount of GlcAT-P is generally a key determinant for the regulation of HNK-1 expression. The nervous system-specific expression of HNK-1 can also be explained by the limited distribution of GlcAT-P to those tissues. However, it is more complex, with evidence to support the presence of a more complicated regulation system. For example, the synthesis of HNK-1 is very efficient because its non-sulfated form has rarely been detected in neural tissues. Moreover, expression of the HNK-1 epitope is known to be highly selective and only occurs on limited types of proteins. These characteristics cannot be explained solely by quantitative changes in GlcAT-P and their underlying

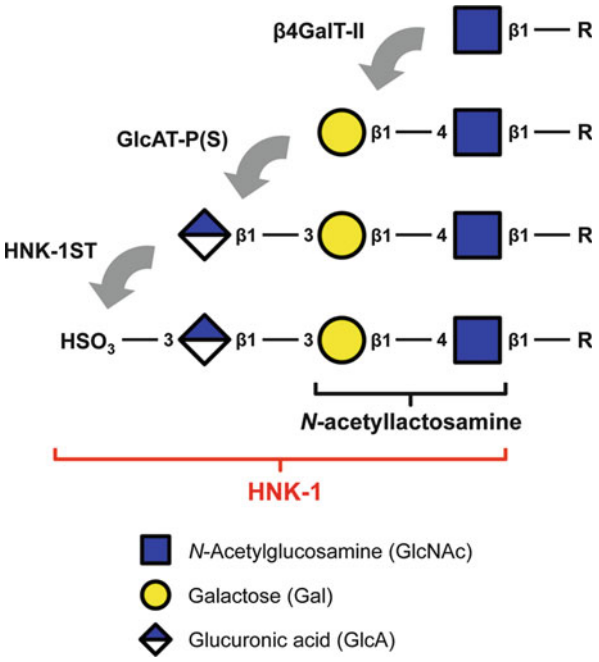

Fig. 1 Structure and biosynthetic enzymes of the HNK-1 glyco-epitope. The structure of the HNK-1 epitope is comprised of a sulfated glucuronic acid attached to an *N*-acetyllactosamine residue and synthesized by β4GalT-II, GlcAT-P (or GlcAT-S), and HNK-1ST in a stepwise manner

mechanisms still remain unclear. Recent evidence has revealed the existence and biological significance of the specific enzyme complex formed by HNK-1-synthesizing enzymes, which may account for the sophisticated expression of HNK-1.

Enzyme Complex of GlcAT-P and HNK-1ST and Its Biological Significance

Previous structural analyses demonstrated that the glucuronic acid in the HNK-1 epitope was always 3-*O*-sulfated, and HNK-1 without the terminal sulfate group was rare, especially in the brain. Moreover, mice lacking HNK-1ST, which catalyzes the 3-*O*-sulfation of glucuronic acid in HNK-1, exhibited similar defects to those reported in GlcAT-P-deficient mice, including reduced long-term potentiation (LTP) in the hippocampal CA1 region and poor performances in spatial learning tasks. These two findings indicated that the sulfate group attached to glucuronic acid was a prerequisite for the HNK-1 epitope to fully exert its biological activity. Therefore, efficient regulatory systems that facilitate the immediate production of a sulfated glucuronic acid residue, the functional motif of HNK-1 epitope, were assumed to exist in the Golgi apparatus.

One possible explanation for this is GlcAT-P (or GlcAT-S) and HNK-1ST being located close to each other in the Golgi apparatus, which enables HNK-1ST to act immediately following glucuronylation by GlcATs. It has been consistently demonstrated that GlcAT-P (or GlcAT-S) and HNK-1ST physically associate with each

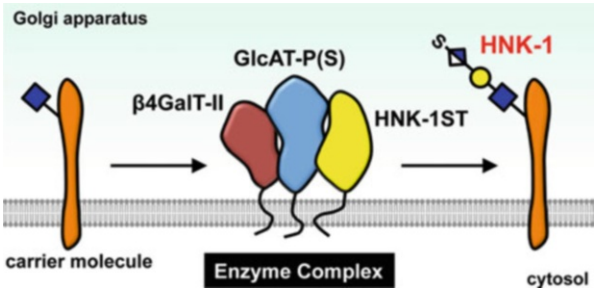

Fig. 2 Enzyme complex for the efficient biosynthesis of HNK-1. The HNK-1-synthesizing enzymes, β4GalT-II, GlcAT-P (or GlcAT-S), and HNK-1ST, specifically associate with each other to form a large enzyme complex in the Golgi apparatus. This complex contributes to the efficient production of the HNK-1 epitope

other to form an enzyme complex (Kizuka et al. 2006). This hetero-enzyme complex comprised by GlcAT-P (or GlcAT-S) and HNK-1ST is a specifically assembled complex because GlcAT-P (and GlcAT-S) interacts with neither C4ST-1 nor GalNAc4ST-1, both of which are highly homologous sulfotransferases belonging to the same sulfotransferase family as HNK-1ST. HNK-1ST also does not interact with ST3GalIV, a sialyltransferase that utilizes *N*-acetyllactosamine residues as its acceptor substrate as with GlcAT-P. Another feature of the enzyme complex of GlcAT-P (or GlcAT-S) and HNK-1ST is that the sulfotransferase activity of HNK-1ST is upregulated by approximately 2-fold in the presence of GlcAT-P (or GlcAT-S), which was confirmed using an in vitro enzyme assay. Since HNK-1ST and GlcATs bind mutually through their C-terminal catalytic domains, this interaction may cause conformational changes in the catalytic domain of HNK-1ST, resulting in an enhancement in enzyme activity. These physical and functional associations between GlcATs and HNK-1ST represent a beneficial system that avoids the accumulation of the non-sulfated intermediate, leading to the efficient production of the HNK-1 epitope (Fig. 2).

Enzyme Complex of GlcAT-P and β4GalT-II and Its Biological Significance

Another finding that exemplifies the complicated regulation of HNK-1 expression is that the HNK-1 epitope has only been detected on limited types of glycoproteins. Most cell-surface and extracellular proteins are known to be glycosylated during their maturation processes in the secretory pathway. However, a number of proteins do not carry the HNK-1 epitope even though they possess *N*-acetyllactosamine residues on their *N*-glycan, which could be potentially modified with HNK-1. For example, α-amino-3-hydroxy-5-methylisoxazole propionate (AMPA)-type and *N*-methyl-D-aspartate (NMDA)-type glutamate receptors are expressed on the dendritic spine of almost all excitatory neurons, but the HNK-1 epitope is selectively

expressed on AMPA-type glutamate receptors even though subunits comprising these receptors show highly homologous amino acid sequences and possess a similar number of potential *N*-glycosylation sites. Another example is NCAM and *N*-cadherin, both of which have essential functions as cell adhesion molecules through their hemophilic interactions and are expressed in almost all neurons. However, in the mouse brain, NCAM carries the HNK-1 epitope on its *N*-glycan, whereas *N*-cadherin does not. These findings suggest the presence of some sorting systems to determine the molecule to be modified with HNK-1, depending on the requirement of the HNK-1 epitope for their functions. In vivo sorting of the HNK-1 attachment appears to be very elaborate so that it can discriminate the correct target from various proteins showing high similarities in their tertiary structures or functions as described above.

Recent studies on gene-deficient mice for β4GalT-I and II have provided an important insight into the sorting machinery of the HNK-1 attachment (Yoshihara et al. 2009; Kouno et al. 2011). The β4GalT family is comprised of seven members (β4GalT-I to VII), some of which have been shown to synthesize *N*-acetyllactosamine residues on glycoproteins, which could be further modified to the HNK-1 epitope. Among these, β4GalT-II-deficient mice exhibited a marked reduction in HNK-1 expression in the brain as well as similar behavioral phenotypes to GlcAT-P knockout mice including impaired spatial learning and memory. On the other hand, β4GalT-I-deficient mice did not exhibit phenotypes caused by a disruption in synaptic plasticity and retained normal expression levels of the HNK-1 epitope. Importantly, the reduction in HNK-1 in β4GalT-II-deficient mice was not due to the general loss of *N*-acetyllactosamine residues on carrier glycoproteins because the amount of RCA120 lectin-reactive βGal moieties on *N*-glycans remained unchanged in the β4GalT-II-deficient mouse brain. This finding indicated that GlcAT-P transferred glucuronic acid only to the *N*-acetyllactosamine residue generated by β4GalT-II, suggesting the presence of a functional association between GlcAT-P and β4GalT-II. The specific interaction between GlcAT-P and β4GalT-II, but not β4GalT-I, was demonstrated by cell-based co-immunoprecipitation and endoplasmic reticulum (ER)-retention assays (Kouno et al. 2011). In the latter experiment, an ER-retained mutant of GlcAT-P could redistribute β4GalT-II from the Golgi apparatus to the ER, which indicated that the physical interaction between GlcAT-P and β4GalT-II was sufficient to deploy them into the same trafficking pathway. Therefore, GlcAT-P and β4GalT-II may move together and could accumulate in specialized compartments of the Golgi apparatus in which only defined acceptor molecules can enter, thereby serving as the sorting machinery responsible for the selective addition of the HNK-1 epitope. Moreover, GlcAT-P increased its glucuronyltransferase activity when it formed a complex with β4GalT-II, similar to the enhancement in HNK-1ST activity in the GlcAT-P and HNK-1ST complex.

These findings demonstrated that β4GalT-II, GlcAT-P, and HNK-1ST form a large heteromeric enzyme complex and act in concert with each other, contributing to the strictly regulated expression of HNK-1 in multiple ways such as production efficiency and sorting of the target protein (Fig. 2).

Function of HNK-1ST Independent of the HNK-1-Synthesizing Complex

The expression of the HNK-1 epitope is highly restricted to the nervous system because of the limited tissue distributions of GlcAT-P and GlcAT-S. Therefore, the abovementioned enzyme complex exists and functions only in the nervous system. However, HNK-1ST is expressed more ubiquitously and has been detected in several tissues in which GlcAT-P and GlcAT-S are not found, including skeletal muscle, the heart, spleen, and reproductive organs. These findings suggest that HNK-1ST has another function independent of HNK-1 biosynthesis. To date, the following two novel roles of HNK-1ST have been reported.

First, HNK-1ST may act as a modulator of GAG synthesis. GAG is a linear polysaccharide comprised of repeating disaccharide units such as [(-4GlcAβ1-3GalNAcβ1-)$_n$] for the chondroitin sulfate (CS) chain and [(-4GlcAβ1-4GlcNAcα1-)$_n$] for the heparan sulfate (HS) chain. The polymerization of GAGs is primed on the nonreducing end of the common GAG-protein linkage tetrasaccharide, GlcAβ1-3Galβ1-3Galβ1-4Xyl-Ser. Previous studies demonstrated, using a synthetic linkage tetrasaccharide or thrombomodulin (a CS-carrying proteoglycan), that HNK-1ST transferred a sulfate group to the terminal glucuronic acid of the linkage region to generate a sulfated tetrasaccharide, HSO_3-3GlcAβ1-3Galβ1-3Galβ1-4Xyl-Ser (Hashiguchi et al. 2011; Nakagawa et al. 2011). The sulfated tetrasaccharide was then no longer utilized as a substrate for CS-polymerizing enzymes (Nakagawa et al. 2011). Several types of proteoglycans, including thrombomodulin and neuroglycan C, are known to exist as both GAG-carrying proteoglycans and unmodified core proteins, called part-time proteoglycans. Therefore, the action of HNK-1ST on linkage tetrasaccharides may be one of essential determinants for the initiation of GAG polymerization in part-time proteoglycan synthesis in vivo. In supporting this proposal, the sulfated tetrasaccharide structure had been found on thrombomodulin obtained from human urine.

The second implication of the distinct role of HNK-1ST is in regulating the glycosylation of α-dystroglycan (α-DG). α-DG is a cell-surface glycoprotein that serves as a receptor for extracellular matrix molecules including laminin. α-DG carries unique disaccharide repeats composed of glucuronic acid and xylose, elongated via a phosphodiester linkage on the *O*-mannosyl glycan, which act as a ligand-binding motif. HNK-1ST has been shown to transfer a sulfate group onto the phosphodiester-linked structure on α-DG to compete with elongation of the ligand-binding glycan, resulting in the suppression of α-DG function (Nakagawa et al. 2012, 2013). Although the biological significance of the α-DG-regulating function of HNK-1ST has yet to be clarified in vivo, that in living cells has been demonstrated by experiments using melanoma cells. In several melanoma cell lines, the expression of HNK-1ST was upregulated and the invasive potential was reduced with retinoic acid treatments. HNK-1ST induced by retinoic acid was shown to suppress the ligand-binding activity of α-DG, leading to an attenuation of the α-DG-dependent migration of melanoma cells (Nakagawa et al. 2012).

Although the abnormal glycosylation of α-DG causes several types of congenital muscular dystrophies, the fine structure of the ligand-binding glycan on α-DG and its biosynthetic machinery are still poorly understood. Therefore, an investigation of HNK-1ST function in α-DG could be a powerful means to uncover the entire regulatory system of α-DG glycosylation.

These newly emerged aspects of HNK-1ST function are distinct from those originally reported and imply that a single enzyme can have multiple roles that affect the synthesis of different glycans, highlighting an important part in the complicated regulation of mammalian glycosylation.

References

Hashiguchi T, Mizumoto S, Nishimura Y, Tamura J, Yamada S, Sugahara K (2011) Involvement of human natural killer-1 (HNK-1) sulfotransferase in the biosynthesis of the GlcUA(3-*O*-sulfate)-Gal-Gal-Xyl tetrasaccharide found in α-thrombomodulin from human urine. J Biol Chem 286:33003–33011

Kizuka Y, Oka S (2012) Regulated expression and neural functions of human natural killer-1 (HNK-1) carbohydrate. Cell Mol Life Sci 69:4135–4147

Kizuka Y, Matsui T, Takematsu H, Kozutsumi Y, Kawasaki T, Oka S (2006) Physical and functional association of glucuronyltransferases and sulfotransferase involved in HNK-1 biosynthesis. J Biol Chem 281:13644–13651

Kouno T, Kizuka Y, Nakagawa N, Yoshihara T, Asano M, Oka S (2011) Specific enzyme complex of β-1,4-galactosyltransferase-II and glucuronyltransferase-P facilitates biosynthesis of *N*-linked human natural killer-1 (HNK-1) carbohydrate. J Biol Chem 286:31337–31346

Morita I, Kakuda S, Takeuchi Y, Itoh S, Kawasaki N, Kizuka Y, Kawasaki T, Oka S (2009) HNK-1 glyco-epitope regulates the stability of the glutamate receptor subunit GluR2 on the neuronal cell surface. J Biol Chem 284:30209–30217

Nakagawa N, Izumikawa T, Kitagawa H, Oka S (2011) Sulfation of glucuronic acid in the linkage tetrasaccharide by HNK-1 sulfotransferase is an inhibitory signal for the expression of a chondroitin sulfate chain on thrombomodulin. Biochem Biophys Res Commun 415:109–113

Nakagawa N, Manya H, Toda T, Endo T, Oka S (2012) Human natural killer-1 sulfotransferase (HNK-1ST)-induced sulfate transfer regulates laminin-binding glycans on α-dystroglycan. J Biol Chem 287:30823–30832

Nakagawa N, Takematsu H, Oka S (2013) HNK-1 sulfotransferase-dependent sulfation regulating laminin-binding glycans occurs in the post-phosphoryl moiety on α-dystroglycan. Glycobiology 23:1066–1074

Yamamoto S, Oka S, Inoue M, Shimuta M, Manabe T, Takahashi H, Miyamoto M, Asano M, Sakagami J, Sudo K, Iwakura Y, Ono K, Kawasaki T (2002) Mice deficient in nervous system-specific carbohydrate epitope HNK-1 exhibit impaired synaptic plasticity and spatial learning. J Biol Chem 277:27227–27231

Yoshihara T, Sugihara K, Kizuka Y, Oka S, Asano M (2009) Learning/memory impairment and reduced expression of the HNK-1 carbohydrate in β4-galactosyltransferase-II-deficient mice. J Biol Chem 284:12550–12561