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Review

Membrane oligo- and polysialic acids

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ABSTRACT

Polysialic acid (polySia) and oligosialic acid (oligoSia) chains are linear polysaccharides composed of sialic acid monomers. The majority of biological poly/oligoSia chains are bound to membranes. There is a large diversity of membrane poly/oligoSia in terms of chain length, occurrence, biological function, and the mode of membrane attachment. Poly/oligoSia can be anchored to a membrane via a phospholipid (polySia in bacteria), a glycosphingolipid (oligoSia in gangliosides), an integral membrane glycoprotein, or a glycoprotein attached to a membrane via glycosylphosphatidylinositol. In eukaryotic cells, the attachment of a poly/oligoSia chain to the membrane anchor is usually through α-2,3-glycosidic linkage to a galactose. In prokaryotic cells this attachment is proposed to occur through glycosidic linkage to the phosphate group of a phospholipid. Both long polySia chains attached to membrane proteins and short oligoSia attached to glycosphingolipids or membrane proteins are frequently found in neural membranes. In humans, poly/oligoSia is involved in development and plasticity of the brain, pathophysiology of schizophrenic brains, cancer metastasis, neuroinvasive potential of pathogenic bacterial strains, and the immune response. Biological roles of poly/oligoSia are based on its ability to modulate repulsive and attractive interactions between two molecules, and its ability to modulate membrane surface charge density, pH at the membrane surface, and membrane potentials.

1. Introduction

Polysialic acid (polySia) and oligosialic acid (oligoSia) chains are linear polysaccharides composed of sialic acid monomers: N-acetylneuraminic acid (Neu5Ac), N-glycolylneuraminic acid (Neu5Gc) or deaminated neuraminic acid (KDN; 3-deoxy-D-glycero-D-galacto-nonulosonic acid) (Fig. 1), joined internally by α-2,8-, α-2,9-, or α-2,8/α-2-9-ketosidic linkages [1–11]. In the case of oligoSia, there are also α-2,4-ketosidic linkages (in gangliosides, [12]) and α-2,5-ketosidic linkages (in glycoproteins, [13]). Poly/oligoSia monomers have a common neuraminic acid (Neu) or KDN backbone (Fig. 1). Although humans are genetically unable to produce Neu5Gc, human embryonic stem cells express this immunogenic nonhuman Sia [14] and an accumulation of Neu5Gc in human cancer cells is observed [15]. The expression level of KDN in humans is as low as <0.01 mol% of Neu5Ac [15]. Neither poly/oligo-Neu5Gc nor poly/oligo-KDN chains were detected in humans. Sia monomers within a poly/oligoSia chain are negatively charged at physiological pH due to the presence of a carboxylic group with a pK_a ~2.6. Poly/oligoSia chains usually consists of only one kind of monomers (thus forming a homopolymer), *e.g.* $(\alpha\text{-2,8-Neu5Ac})_n$ or $(\alpha\text{-2,8-Neu5Ac/}\alpha\text{-2,9-Neu5Ac})_n$. The majority of poly/oligoSia biopolymers discovered so far are connected to a membrane.

There is a large diversity of membrane poly/oligoSia in terms of:

- *chain length* (DP, degree of polymerization) — starting from a few monomers and extending to DP ~ 400 [16,17];
- *occurrence* — for example, they can be found in neural cells in vertebrates, human dendritic cells, human leukocytes, human milk fat globules, neural stem cell-derived progenitors, human cancer cells, bacterial cells, or in invertebrates — *e.g.* sea urchin sperm flagellum;
- *biological function* — for example, they can influence fertilization, induce neuronal plasticity of human nervous system, or enhance the metastatic potential of tumor cells;
- *mode of membrane attachment* — they can be joined to a membrane lipid (*e.g.* in the case of oligosialo-gangliosides) or a membrane protein (*e.g.* in the case of polysialylated neural cell adhesion molecule, NCAM) (see references in the next chapters).

The action of poly/oligoSia results from a combination of:

- *repulsive interactions* — between polySia chains or between polySia chains and membrane proteins;
- *attractive interactions* — between poly/oligoSia chains and membrane proteins, membrane lipids, or soluble proteins/peptides/proteoglycans;
- *modulation of surface charge density* — because of the negative charge of the poly/oligoSia monomers;

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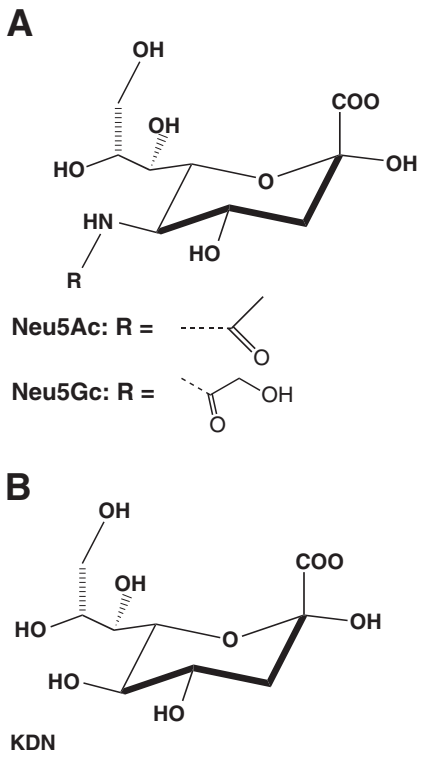

Fig. 1. The chemical structure of sialic acids: N-acetylneuraminic acid (Neu5Ac), N-glycolylneuraminic acid (Neu5Gc), deaminated neuraminic acid (KDN). A — neuraminic acid backbone, B — KDN backbone.

- *modulation of pH at the membrane surface* — because of the carboxylic groups present in poly/oligoSia chains;
- *modulation of membrane potentials* (*transmembrane potential and surface potential*) — arising from poly/oligoSia transmembrane translocation or from the changes of surface charge density and surface pH (see references in the next chapters).

In bacteria, the proteins involved in polySia synthesis and translocation are coded by a gene cluster designated the *kps* gene cluster (*Escherichia coli*) or the *cps* gene cluster (*Neisseria meningitidis*) [18]. In eukaryotic cells, there are two polysialyltransferases in the Golgi complex involved in biosynthesis of long polySia chains: ST8SiaII (STX) and ST8SiaIV (PST). There are also several other sialyltransferases, located in the ER or Golgi membranes, involved in biosynthesis of oligoSia chains (attached to lipids or to proteins): ST8SiaIII and ST8SiaVI that catalyze di- and tri-sialylation of glycoproteins, and ST8SiaI and ST8SiaV that catalyze di- and tri-sialylation of gangliosides [19].

It was proposed [20] that di-, oligo- and polySia could be defined as polymerized forms containing DP 2, DP 3–7, and DP 8 or more Sia residues, respectively. This classification is based on the recognition of each class by anti-di/oligo/polySia antibodies. The antibodies recognizing di/oligo/polySia glycotope are classified into three groups [20]: group I antibodies recognize chains of DP 8 or greater and the nonreducing terminal residues do not participate in the polySia recognition, group II antibodies recognize both oligoSia with DP 3–7 and longer polySia chains, and the nonreducing terminal residues are involved in antigen recognition, group III antibodies recognize chains of DP 2–4 but they do not bind to polySia, a subgroup within group III recognizes only diSia structures.

2. Structure, occurrence, and function of membrane polySia

The majority of poly/oligoSia is membrane-bound, although there are few examples of non-membrane polySia (or polySia for which membrane attachment has not been yet reported), *e.g.* polySia on a soluble polysialoglycoprotein (PSGP) in rainbow trout eggs cortical alveoli [21]. Fig. 2 shows the chemical structure of poly-α-2,8-Neu5Ac. A non-reducing residue (left side of Fig. 2) is followed by a linear homopolymer ending with a reducing end (right side of Fig. 2). Neu5Ac monomers are joined internally by α-2,8-ketosidic linkages. The reducing end is usually conjugated to a membrane anchor. PolySia chain growth occurs by the addition of Sia to the non-reducing terminus of the growing chain (tail growth). There are several types of cell type-specific poly/oligoSia membrane molecular anchors, such as which can be a phospholipid molecule (in the case of polySia in bacteria), a glycosphingolipid molecule (in the case of oligoSia in gangliosides), or a membrane protein molecule (see Fig. 3).

2.1. PolySia on phospholipids in the outer membrane of some capsule-expressing bacteria

PolySia chains attached to the outer bacterial membrane form a capsule surrounding neuroinasive *E. coli* K1, *N. meningitidis* serogroups B and C, and some other gram-negative bacterial strains, *e.g. Pasteurella haemolytica* A2, and *Moraxella nonliquefaciens* [3,22–27]. PolySia chains are proposed to be glycosidically linked to the phosphate moiety of phospholipid molecules in the outer layer of the outer bacterial membrane [28]. The polyanion capsule conceals surface structures that can activate complement, thus rendering bacterial cells resistant to phagocytosis by neutrophils [25]. *E. coli* K1 capsule may be vital for intracellular survival and for crossing the blood–brain barrier (BBB) [29], the capsule may also adversely affect bacterial ability to colonize as it can sterically hinder the surface-expressed adhesins [30] therefore it may be hypothesized that the K1 capsule may be lost within the BBB in some cases. PolySia chains are poorly immunogenic because of structural identity of bacterial polySia and human polySia on neural cells [22]. It was suggested that polySia in the inner membrane of *E. coli* K1 can be anchored to a 20 kDa membrane protein acceptor and also to undecaprenyl phosphate (P-C_{55}) [31,32]. Thus polySia chains may be translocated across the inner membrane while linked to P-C_{55} [32].

2.2. PolySia on NCAM in the plasma membrane of neural cells, neural stem cell-derived progenitors/precursors cells, cancer cells, and leukocytes

The major membrane protein carrier of polySia in mammalian cells is NCAM. There are several isoforms of NCAM, three of which can carry polySia: NCAM-180 and NCAM-140 (integral membrane isoforms), and NCAM-120 (isoform that is anchored to the plasma membrane via a glycosylphosphoinositol (GPI)) [5,33–35]. No more than 35% colocalization of polysialylated and non-polysialylated NCAM with the lipid raft marker (cholera toxin subunit B) is observed [36]. The polySia-modification of NCAM is correlated with brain development. In the adult brain, the polysialylated NCAM is expressed only in some regions that are associated with plasticity (*e.g.* hippocampus). PolySia on NCAM regulates neural cell migration and differentiation during brain development, and is associated with neurogenesis and synaptic plasticity in the adult brain. The loss of polySia on NCAM converts NCAM from a molecule that induces plasticity during neural development to one that mediates stability in adult brain [37] due to polySia-mediated modulation of cell-cell interactions. PolySia chains can be attached to both N- and O-linked oligosaccharides at the extracellular domain of NCAM [33,38–40].

Polysialylated NCAM is found on several neural stem cell-derived progenitors/precursor cells, including oligodendrocytes, astrocyte precursor cells, astroblasts, neuroblasts, and glial precursors [41–43]. PolySia on NCAM is also expressed in several tumor cells where it can function as an oncodevelopmental antigen and contribute to cancer metastasis [1,44]. Polysialylated NCAM is also present on the surface of immune system cells, such as human and murine leucocytes, to modulate immune responses [17].