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Complex oligosaccharides are *N*-linked to Kv3 voltage-gated K^+ channels in rat brain

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Abstract

Neuronal Kv3 voltage-gated K^+ channels have two absolutely conserved *N*-glycosylation sites. Here, it is shown that Kv3.1, 3.3, and 3.4 channels are *N*-glycosylated in rat brain. Digestion of total brain membranes with peptide N glycosidase F (PNGase F) produced faster migrating immunobands than those of undigested membranes. Additionally, partial PNGase F digests showed that both sites are occupied by oligosaccharides. Neuraminidase treatment produced a smaller immunoband shift relative to PNGase F treatment. These results indicate that both sites are highly available and occupied by *N*-linked oligosaccharides for Kv3.1, 3.3, and 3.4 in rat brain, and furthermore that at least one oligosaccharide is of complex type. Additionally, these results point to an extracytoplasmic S1–S2 linker in Kv3 proteins expressed in native membranes. We suggest that *N*-glycosylation processing of Kv3 channels is critical for the expression of K^+ currents at the surface of neurons, and perhaps contributes to the pathophysiology of congenital disorders of glycosylation.

Keywords: Glycosylation; Topology; K^+ channel; Brain; Congenital disorder of glycosylation

1. Introduction

The Kv3 channel family is part of the large super gene family of voltage gated K^+ (Kv)[1] channels. These channels play an essential role in enabling neurons to fire repetitively at high frequencies [1]. There are four different Kv3 genes: Kv3.1, 3.2, 3.3 and 3.4, and by alternative splicing encode for multiple protein isoforms with divergent C termini [2–6]. Hydropathy plots predict that Kv3 channels possess six transmembrane segments (S1–S6) with cytoplasmic amino- and carboxyl-termini (Fig. 1A). The Kv3 multiple protein isoforms have two absolutely conserved sites for *N*-glycosylation which lie within the S1–S2 extracytoplasmic loop. Amino acid sequence identity of the S1–S2 linker is 100% identical for the Kv3.1 protein of: rat, human, mouse, rabbit and cattle, and greater than 95% for the Kv3.4 protein (Fig. 1B). Sequence identity is also highly conserved for Kv3.2 and Kv3.3 proteins.

Attachment of *N*-linked oligosaccharides to newly synthesized membrane proteins is the most ubiquitous protein co-translational modification in the lumen of the endoplasmic reticulum (ER) [7]. The required protein consensus sequence is AsnXxxSer/Thr, where Xxx can be any amino acid except for proline. Maturation of the glycoprotein occurs in the lumen of the ER and Golgi apparatus. The importance of the *N*-glycosylation process has been indicated in congenital disorders of glycosylation (CDG) [8] and mice mutants [9–12]. Of recent, endoplasmic reticulum (ER) stress has been suggested to be associated with neurodegenerative diseases [13].

N-Glycosylation of K^+ channels has been shown to influence: folding, trafficking, and function [14–18]. It has also been used as an extracellular marker to verify topological K^+ channel structure [18–22]. Here we have evaluated utilization of the *N*-glycosylation consensus sequences of Kv3.1, 3.3, and 3.4 in rat brain. Western blots of total brain membranes demonstrated that Kv3.1, 3.3, and 3.4 proteins were expressed in rat brain. When total brain membranes were treated with peptide N glycosidase F (PNGase F) or neuraminidase, immunobands of Kv3.1, 3.3, and 3.4 migrated faster than those from untreated membranes. Endoglycosidase H (Endo H)

Abbreviations: Kv, voltage-gated K^+ channel; Endo H, Endoglycosidase H; CDG, Congenital disorders of glycosylation; PNGase F, peptide N glycosidase F

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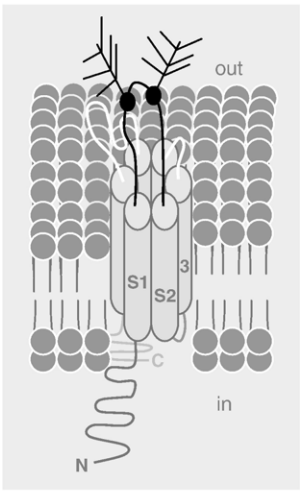

B

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Kv3.1  Rat    210 ETHERFNPIVNKTEIENVRNGTQVRYYREAETEAFLTY
Kv3.1  Human  210 ETHERFNPIVNKTEIENVRNGTQVRYYREAETEAFLTY
Kv3.1  Mouse  210 ETHERFNPIVNKTEIENVRNGTQVRYYREAETEAFLTY
Kv3.1  Rabbit 210 ETHERFNPIVNKTEIENVRNGTQVRYYREAETEAFLTY
Kv3.1  Cattle 210 ETHERFNPIVNKTEIENVRNGTQVRYYREAETEAFLTY

Kv3.2  Rat    249 ETHEAFNIVKNKTEPVINGTSAVLQYEIETDPALTY
Kv3.2  Human  249 ETHEAFNIVKNKTEPVINGTSVVLQYEIETDPALTY
Kv3.2  Mouse  253 ETHEAFNIVKNKTEPVINGTSPVLQYEIETDPALTY

Kv3.3  Rat    311 ETHEGFIHISNKTVTQASPIPGAPPENITNVEVETEPFLTY
Kv3.3  Human  310 ETHEGFIHISNKTVTQASPIPGAPPENITNVEVETEPFLTY
Kv3.3  Mouse  310 ETHEGFIHISNKTVTQASPIPGAPPENITNVEVETEPFLTY
Kv3.3  Rabbit 312 ETHEGFIHISNKTVTQASPIPGAPPENVTNVEVETEPFLTY

Kv3.4  Rat    249 ETHEAFNIDRNVTEIHRVGNITSVRFRREVETEPILTY
Kv3.4  Human  248 ETHEAFNIDRNVTEILRVGNITSVHFRREVETEPILTY
Kv3.4  Mouse  252 ETHEAFNIDRNVTEIHRVGNITSVRFRREVETEPILTY
Kv3.4  Rabbit  72 ETHEAFNIDRNVTEIHRVGNITSVRFRREVETEPILTY
Kv3.4  Cattle 243 ETHEAFNIDRNVTEIHRVGNTTSVRFRREVETEPILTY
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Fig. 1. Topological model of Kv3 channels and amino acid sequence alignments of the S1–S2 linker of Kv3 channels. (A) The Kv3 monomeric unit in a lipid bilayer. Transmembrane segments (S1–S6) are represented by cylinders in the lipid bilayer. The first transmembrane segment (S1) to the sixth transmembrane segment (S6) proceeds counterclockwise. Black circles stand for the Asn of the two native *N*-glycosylation consensus sequences for Kv3 channels. Branched structures represent *N*-linked oligosaccharides. (B) Alignments of available amino acid sequences of the S1–S2 linker for Kv3 channels in: rat, human, mouse, rabbit, and cattle. Conserved, native *N*-glycosylation sites are shown as underlined, enlarged font. The italicized number indicates the first residue of the S1–S2 linker. The shaded regions represent amino acids which are non-identical. Accession numbers are as follows: Kv3.1 Rat P25122, Human P48547, Mouse P15388, Rabbit AAX23602, Cattle AAX23601; Kv3.2 Rat P22462, Human AAO89503, Mouse NP_001020752; Kv3.3 Rat Q01956, Human Q14003, Mouse Q63959, Rabbit AAN15930; Kv3.4 Rat Q63734, Human Q03721, Mouse NP_666034, Rabbit AAM46839, Cattle XP_613047.

treatment of total membranes did not alter the immunoband patterns of Kv3.1, 3.3, and 3.4 from those of untreated samples. Based on enzyme specificities and electrophoretic migration patterns, our results indicate that both *N*-glycosylation sites are highly available and occupied by *N*-linked oligosaccharides of either hybrid or complex types. In addition, these results indicate that the S1–S2 loop is extracytoplasmic in native membranes. Given the above observations, the high conservation of the *N*-glycosylation sites, and CDG diseases, we suggest that *N*-glycosylation processing of the Kv3 channels is critical in regulating the expression of K^+ currents at the surface of neurons.