Carbohydrate Research 344 (2009) 1079–1084

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

Enzymatic characterization and inhibition of the nuclear variant of human *O*-GlcNAcase

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ARTICLE INFO

Article history:
Received 14 February 2009
Received in revised form 15 April 2009
Accepted 15 April 2009
Available online 20 April 2009

Keywords:
O-GlcNAcase
OGA isoform
MGEA5
Nuclear variant
β-*N*-Acetylglucosaminidase
O-GlcNAc

ABSTRACT

Increasing cellular *O*-GlcNAc levels through pharmacological inhibition of *O*-GlcNAcase, the enzyme responsible for removal of the *O*-GlcNAc post-translational modification, is being increasingly used to aid in discerning the roles played by this form of intracellular glycosylation. Interestingly, two forms of *O*-GlcNAcase have been studied; a full-length isoform that is better characterized, and a shorter nuclear-localized variant, arising from failure to splice out one intron, which has not been as well characterized. Given the increasing use of *O*-GlcNAcase inhibitors as research tools, we felt that a clear understanding of how these inhibitors affect both isoforms of *O*-GlcNAcase is important for proper interpretation of studies making use of these inhibitors in cell culture and in vivo. Here we describe an enzymatic characterization of the nuclear variant of human *O*-GlcNAcase. We find that this short nuclear variant of *O*-GlcNAcase, which has the identical catalytic domain as the full-length enzyme, has similar trends in a pH-rate profile and Taft linear free energy analysis as the full-length enzyme. These findings strongly suggest that both enzymes use broadly similar transition states. Consistent with this interpretation, the short isoform is potently inhibited by several previously described inhibitors of full-length *O*-GlcNAcase including PUGNAc, NAG-thiazoline, and the selective *O*-GlcNAcase inhibitor NButGT. These findings contrast with earlier studies and suggest that studies using *O*-GlcNAcase inhibitors in cultured cells or in vivo can be interpreted with the knowledge that both these forms of *O*-GlcNAcase are inhibited when present.

1. Introduction

Within higher eukaryotes, numerous nucleocytoplasmic proteins are post-translationally modified with *N*-acetylglucosamine (GlcNAc) residues O-linked to certain serine or threonine residues (*O*-GlcNAc).[1,2] Unlike glycosylation occurring within the secretory pathway, *O*-GlcNAc is a dynamic modification similar in some ways to phosphorylation; the modification can be cleaved off and reinstalled from the polypeptide backbone more quickly then the protein is turned over.[3,4] Two enzymes are involved in regulating the dynamic cycling of *O*-GlcNAc. *O*-GlcNAc transferase (OGT)[5] installs *O*-GlcNAc at appropriate serine or threonine residues of target proteins, whereas *O*-GlcNAcase (OGA)[6] acts to remove the modification and returns residues to their unmodified state. The catalytic mechanism of FL-OGA has been shown to proceed in two steps, via an oxazoline intermediate, to yield the β-anomer of the free GlcNAc hemiacetal.[7] This catalytic mechanism, and formation of the oxazoline intermediate, relies on the involvement of the 2-acetamido group of the substrate (Fig. 1a). In the first step of the reaction, this group acts as a nucleophile to attack the anomeric center and displace the leaving group. One enzymic carboxylate (Asp174) likely acts as a general base to enhance nucleophilicity of the acetamido group while another enzymic carboxylic acid (Asp175) acts as a general acid catalyst to facilitate departure of the leaving group.[8,9] The second step of the reaction is the near microscopic reverse of the first step; attack of water at the anomeric center is facilitated by an enzymic base (Asp175) and the acetamido group is expelled from the anomeric center with Asp174 likely acting as a general acid. How these catalytic activities of OGT and OGA are regulated within cells is slowly emerging[10,11] and shorter isoforms of both enzymes that stem from alternative splicing have been described.[12–14] Although these shorter isoforms have only been partially characterized, they have been reported to have altered properties such as catalytic activity, substrate specificity, and cellular localization.[14–17]

The gene encoding *O*-GlcNAcase (*MGEA5*) was shown by Comptesse et al. to undergo alternative splicing to generate a truncated protein.[14] When intron 10 fails to be spliced out, a transcript is generated that encodes a protein having a 662 amino acid N-terminal section identical to the full-length protein (OGA-FL) and an additional 15 amino acid C-terminal piece arising from the start of intron 10 (Fig. 1b). Such a protein (OGA-NV) therefore lacks the C-terminal domain of OGA. This C-terminal domain has been

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doi:10.1016/j.carres.2009.04.017

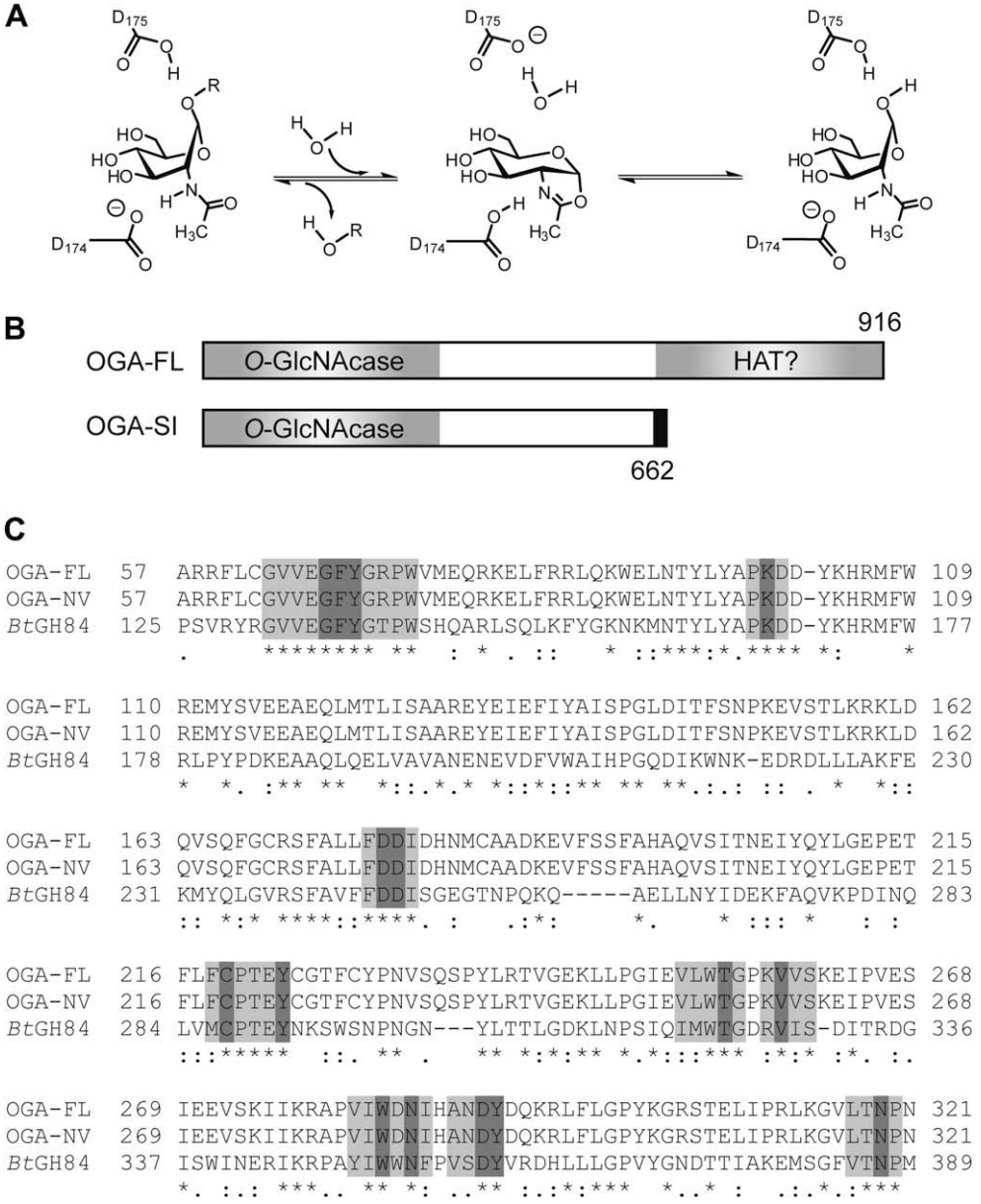

Figure 1. The catalytic domain responsible for *O*-GlcNAcase activity is present in both the nuclear variant (OGA-NV) and the full-length form of OGA (OGA-FL). (A) The proposed catalytic mechanism of OGA involves a two-step double displacement mechanism involving the transient formation of an oxazoline intermediate. Asp174 likely acts in the first step as a general base, orienting and enhancing the nucleophilicity of the 2-acetamido group toward the anomeric center. Departure of the leaving group is aided by Asp175, which acts as a general acid catalyst. The second step of the reaction is the near microscopic reverse of the first step; water acts as a nucleophile to attack the anomeric center and open the oxazoline ring. (B) Schematic showing the architecture of OGA-NV. OGA-NV lacks a putative histone acetyl transferase (HAT) C-terminal domain. In addition, the OGA-NV contains an additional 15 amino acids (filled black box) encoded by part of intron 10, which is not spliced out in this isoform. (C) Sequence alignment of the GH84 catalytic domain of OGA-FL (meningioma expressed antigen 5, NP_036347), OGA-NV (meningioma expressed antigen 5, NP_036347), and a bacterial homologue of *O*-GlcNAcase from *B. thetaiotamicron* (*Bt*GH84) (hyaluronoglucosaminidase precursor, NP_813306). Highlighted in light gray are residues that are within 8 Å of NAG-thiazoline in a structure with *Bt*GH84[22] (PDB 2CHO) and residues highlighted in darker gray are those that make contacts (<4 Å) with NAG-thiazoline. Notably, the active site residues are completely conserved between the three enzymes. Sequence alignment was performed using ClustalW.[33]

reported to have histone acetyl transferase (HAT) activity,[18] although more recent efforts have failed to support these findings.[19] Cell fractionation studies provided evidence that OGA-NV resides in the nucleus,[14] whereas OGA-FL is predominantly cytosolic.[20] Unexpectedly, OGA-NV was reported to have no detectable glycoside hydrolase activity toward the chromogenic substrate *p*-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside (*p*NP-GlcNAc)[17] but could be assayed with a more sensitive fluorogenic substrate.[17] Furthermore, OGA-NV was shown to be active on *O*-GlcNAc-modified proteins, albeit at a diminished rate compared to OGA-FL.[17] More recently it was reported that OGA-NV was not inhibited by two inhibitors of OGA-FL; *O*-(2-acetamido-2-deoxy-D-glycopyranosylidene)amino-*N*-phenylcarbamate (PUG-NAc, Fig. 2a) and 1,2-dideoxy-2′-methyl-α-D-glucopyranoso-[2,1-*d*]-Δ2′-thiazoline (NAG-thiazoline, Fig. 2b).[21] While these two inhibitors have nanomolar potencies against OGA-FL,[7] it was reported that OGA-NV was only inhibited 50% when they were used at a concentration of 0.5 mM.[21] Based on these studies it was proposed that the active site of OGA-NV may differ from that of OGA-FL.[21] These discrepancies between OGA-FL and OGA-NV in activity and susceptibility to inhibition are important in the context of defining the functional roles of these two isoforms in biological systems. Because these isoforms of OGA may have different, or compensatory, functional roles, the inhibition of only one of them