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Structures of Human *N*-Acetylglucosamine Kinase in Two Complexes with *N*-Acetylglucosamine and with ADP/Glucose: Insights into Substrate Specificity and Regulation

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N-Acetylglucosamine (GlcNAc), a major component of complex carbohydrates, is synthesized *de novo* or salvaged from lysosomally degraded glycoconjugates and from nutritional sources. The salvage pathway requires that GlcNAc kinase converts GlcNAc to GlcNAc-6-phosphate, a component utilized in UDP-GlcNAc biosynthesis or energy metabolism. GlcNAc kinase belongs to the sugar kinase/Hsp70/actin superfamily that catalyze phosphoryl transfer from ATP to their respective substrates, and in most cases catalysis is associated with a large conformational change in which the N-terminal small and C-terminal large domains enclose the substrates. Here we report two crystal structures of homodimeric human GlcNAc kinase, one in complex with GlcNAc and the other in complex with ADP and glucose. The active site of GlcNAc kinase is located in a deep cleft between the two domains of the V-shaped monomer. The enzyme adopts a "closed" configuration in the GlcNAc-bound complex and GlcNAc interacts with residues of both domains. In addition, the *N*-acetyl methyl group contacts residues of the other monomer in the homodimer, a unique feature compared to other members of the sugar kinase/Hsp70/actin superfamily. This contrasts an "open" configuration in the ADP/glucose-bound structure, where glucose cannot form these interactions, explaining its low binding affinity for GlcNAc kinase. Our results support functional implications derived from apo crystal structures of GlcNAc kinases from *Chromobacter violaceum* and *Porphyromonas gingivalis* and show that Tyr205, which is phosphorylated in thrombin-activated platelets, lines the GlcNAc binding pocket. This suggests that phosphorylation of Tyr205 may modulate GlcNAc kinase activity and/or specificity.

Keywords: *N*-acetylglucosamine kinase; crystal structure; domain rotation; induced fit; sugar kinase

Introduction

N-Acetylglucosamine (GlcNAc) is a major component of oligosaccharide chains of N- and O-glycans and of glycolipids.[1–3] Furthermore, it is part of glycosaminoglycans and of the glycosyl phosphatidylinositol anchor of membrane-bound glycoproteins.[4,5] In mammalian cells, GlcNAc is synthesized *de novo* as part of the cytosolic amino sugar metabolism and is used as a substrate for UDP-GlcNAc biosynthesis. This activated sugar nucleotide is a substrate for glycoconjugate and sialic acid biosynthesis.[6,7] In addition, UDP-GlcNAc

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Abbreviations used: GlcNAc, *N*-acetylglucosamine; SeMet, selenomethionine; GST, glutathione-*S*-transferase; PEG, polyethylene glycol.

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serves in cytosolic or nuclear O-GlcNAc modification of proteins that is associated with diverse regulatory functions ranging from transcription, translation, cell signalling and stress response to carbohydrate metabolism.[6,8]

UDP-GlcNAc can also be provided by a salvage pathway, which requires the phosphorylation of GlcNAc by GlcNAc kinase (EC 2.7.1.59). GlcNAc is thereby recycled from lysosomal degradation of oligosaccharides or aquired from nutritional sources. Evidence suggests that this pathway complements *de novo* synthesis and may provide high-energy intermediates for glycolysis. For example, in tissues with high-energy requirements like neuronal cells, in sperm cells or in the apical zone of transporting epithelia, glucosamine-6-phosphate is converted to fructose-6-phosphate.[9] In addition, fibroblasts derived from mice lacking glucosamine-6-phosphate acetylase, an enzyme of the *de novo* synthesis pathway, display a reduced UDP-GlcNAc pool and reduced cell proliferation. However, external supplementation of GlcNAc, which crosses the plasma membrane and is then phosphorylated by GlcNAc kinase, completely rescues the phenotype.[10] Finally, the subcellular localization of GlcNAc kinase in fibroblasts was found to be different from the localization of enzymes in the *de novo* UDP-GlcNAc biosynthetic pathway.[11] The spatial separation of the two pathways is presumably associated with different regulatory mechanisms of enzyme activity.

Mammalian GlcNAc kinase has been purified from several sources and cloned from man and mouse.[12–16] The 37 kDa human enzyme forms a homodimer in solution,[14] and in addition to its preferred substrate GlcNAc, it is able to phosphorylate *N*-acetylmannosamine (ManNAc) and glucose when the latter two are present at high (millimolar) concentrations.[17–19] As a member of the sugar-kinase/Hsp70/actin superfamily that is characterized by a common ATPase domain, GlcNAc kinase shares less than 15% amino acid sequence identity with any other member of this family.[20] It was recently shown that human GlcNAc kinase is phosphorylated on Tyr205 in platelets following thrombin activation[21] but due to a lack of structural information the implications of phosphorylation on enzyme activity and/or specificity are not clear.

Here we have determined the crystal structures of homodimeric human GlcNAc kinase in complex with ADP/glucose and in complex with GlcNAc at 2.72 and 1.9 Å resolution, respectively. Though key segments involved in sugar and ATP binding are conserved in sequence and structure compared to other members of the sugar-kinase/Hsp70/actin superfamily, GlcNAc kinase shows a unique mechanism to specifically bind GlcNAc, as both monomers of the homodimer form contacts to GlcNAc. The crystal structures suggest a mechanism for substrate specificity that involves rotation of the small and large domains of the monomer to form a "closed" configuration that tightly binds GlcNAc, whereas bound glucose and ADP fail to promote this movement and keep GlcNAc kinase in an "open" state.

Results and Discussion

Structure determination

The structure of GlcNAc kinase in complex with ADP and glucose was determined by multiwavelength anomalous dispersion from selenomethionine (SeMet) labelled crystals of space group $P2_12_12$. This structure was used as a search model during molecular replacement for elucidating the structure of GlcNAc kinase in complex with its preferred physiological substrate GlcNAc, which crystallized in space group $P1$. Both crystal structures contain two homodimers in the asymmetric unit, but these are packed differently. See Table 1 for data collection and refinement statistics.

Secondary and tertiary structure of the GlcNAc kinase protomer

We explain details of the fold and domain organisation of GlcNAc kinase in complex with GlcNAc, as this structure was refined to higher resolution. Continuous electron density was observed for all residues of the four molecules in the crystal asymmetric unit except for a loop defined by residues 292 to 298.

The secondary structure of GlcNAc kinase is composed of 11 β-strands and ten α-helices that are folded into a small N-terminal and a large C-terminal domain (Figure 1(a)) and labelled consecutively from the N to the C terminus (Figure 1(c)). The two domains feature a similar fold and probably arose from gene duplication.[22] The N-terminal, small domain (yellow, residues 1 to 117; Figure 1(a) and (c)) features a ββββαβαβα topography that is known as the core motif of the ribonuclease H fold[23] and is augmented by helix α10 (residues 308 to 334) that protrudes from the large domain. The five-stranded mixed β-sheet (β3, β2, β1, β4, β5) with strand β2 antiparallel to the four other strands of the sheet is covered on the outer face by helices α1 and α2 and on the inner face pointing towards the active site by helices α3 and α10 from the large domain. The large domain is composed of residues 118 to 307 and 335 to 344 (grey, Figure 1(a) and (c)). The ββββαβαβα topography of this domain is also observed in homologous structures and is extended by an insertion of four additional helices (α4, α5, α6, α7) between strand β8 and helix α8, and the C-terminal β-strand 11 extends a conserved five-stranded sheet to (β8, β7, β6, β9, β10, β11), where strands β7 and β11 are antiparallel to the remaining strands (Figure 1(c)). The side of the β-sheet facing the active site (and forming part of the