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The N-terminal extension is essential for the formation of the active dimeric structure of liver peroxisomal alanine:glyoxylate aminotransferase

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ABSTRACT

Alanine:glyoxylate aminotransferase (AGT) is a pyridoxal-phosphate (PLP)-dependent enzyme. Its deficiency causes the hereditary kidney stone disease primary hyperoxaluria type 1. AGT is a highly stable compact dimer and the first 21 residues of each subunit form an extension which wraps over the surface of the neighboring subunit. Naturally occurring and artificial amino acid replacements in this extension create changes in the functional properties of AGT in mammalian cells, including relocation of the enzyme from peroxisomes to mitochondria. In order to elucidate the structural and functional role of this N-terminal extension, we have analyzed the consequences of its removal using a variety of biochemical and cell biological methods. When expressed in *Escherichia coli*, the N-terminal deleted form of AGT showed the presence of the protein but in an insoluble form resulting in only a 10% soluble yield as compared to the full-length version. The purified soluble fraction showed reduced affinity for PLP and greatly reduced catalytic activity. Although maintaining a dimer form, it was highly prone to self-aggregation. When expressed in a mammalian cell line, the truncated construct was normally targeted to peroxisomes, where it formed large stable but catalytically inactive aggregates. These results suggest that the N-terminal extension plays an essential role in allowing AGT to attain its correct conformation and functional activity. The precise mechanism of this effect is still under investigation.

1. Introduction

Alanine:glyoxylate aminotransferase (AGT) is a pyridoxal 5′-phosphate (PLP)-dependent enzyme which catalyzes the transamination of alanine and glyoxylate to pyruvate and glycine in liver peroxisomes and/or mitochondria, depending on the species (Danpure et al., 1994; Danpure, 1997; Ichiyama et al., 2000; Birdsey et al., 2005). In humans, AGT is peroxisomal and its deficiency leads to the rare autosomal recessive disorder of metabolism primary hyperoxaluria type 1 (PH1, MIM 259900) (Danpure and Jennings, 1986). In the absence of AGT, glyoxylate can be oxidized to the metabolic end-product oxalate. The resulting increase in oxalate synthesis leads to the deposition of insoluble calcium oxalate in the kidneys and urinary tract. Following renal failure, calcium oxalate can deposit almost anywhere in the body (Danpure and Jennings, 1986; Danpure, 2001; Danpure and Rumsby, 2004).

The crystal structure of normal human AGT reveals that the enzyme belongs to the Fold Type I family of PLP-enzymes and is a compact homodimer, each subunit of which contains one PLP-binding site (PDB 1H0C) (Zhang et al., 2003). Each subunit can be divided into a large N-terminal domain (residues 1–282), containing most of the active site and dimerization interface, and a smaller C-terminal domain (residues 283–392) (Fig. 1) which contains all of the peroxisomal targeting information (Motley et al., 1995; Huber et al., 2005) and a couple of residues that structurally contribute to the active site. The first twenty or so residues of the large domain make up an N-terminal arm that wraps over the surface of the large domain of the opposing subunit, a structural feature that AGT shares with some, but not all, PLP-enzymes of the same family. The exact role of the N-terminal extensions in AGT is not currently clear, partly because they are predicted to have a limited effect on dimer stability due to their small amount of intersubunit contact

Abbreviations: AGT, alanine:glyoxylate aminotransferase; PH1, primary hyperoxaluria type 1; PLP, pyridoxal 5′-phosphate; CHO, Chinese hamster ovary; GO, glycolate oxidase; DLS, dynamic light scattering; PBS, phosphate buffered saline; BSA, bovine serum albumin; ANS, 1-anilino naphthalene sulphonic acid; BS_3, bis(sulfosuccinimidyl)suberate; $BS(PEG)_5$, bis-N-succinimidyl-(pentaethylene glycol)ester.

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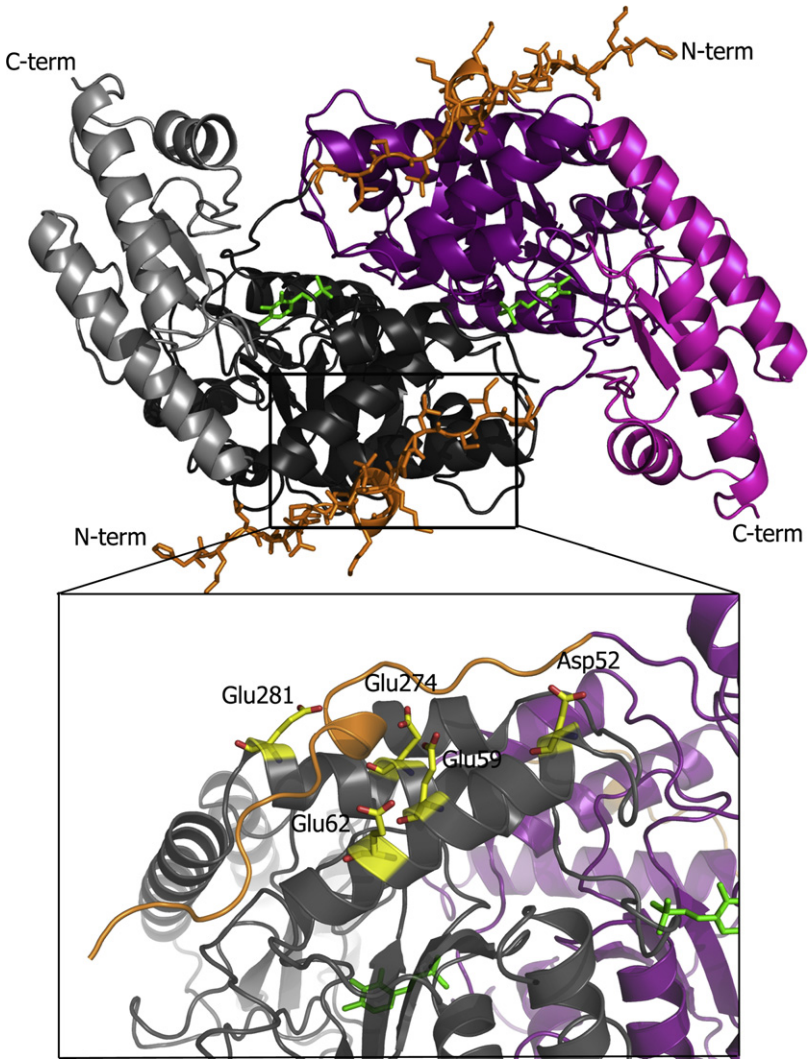

Fig. 1. 3D model of full-length AGT. One subunit is colored violet (large domain) and purple (small domain) while the other is colored black (large domain) and gray (small domain). *Inset*: detail of the interaction surface between the N-terminal arm of one subunit and the large domain of the neighboring subunit. Residues 1–21 of each subunit are represented as orange sticks, PLP molecules are represented as green sticks, negatively changed residues exposed upon the removal of the N-terminus are shown as yellow sticks. The figure was rendered using PyMOL. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

area, and partly because they do not contain any residue known to be involved in the catalytic site (Zhang et al., 2003). Nevertheless, mutations in this region, whether naturally found or artificially induced, can lead to significant alterations in AGT intracellular behavior, most notably peroxisome-to-mitochondrion mistargeting and delayed dimerization (Purdue et al., 1991a; Leiper et al., 1996; Lumb et al., 1999; Lumb and Danpure, 2000).

AGT is encoded by the *AGXT* gene, of which two common allelic variants have been described – the major and minor alleles (Purdue et al., 1990). The latter differs from the former mainly by the presence of two point mutations which lead to the Pro11Leu and Ile340Met amino acid substitutions, as well as several intronic and synonymous exonic changes that have no effect on the final gene product (Purdue et al., 1990, 1991b). The Pro11Leu polymorphism, which sits right in the middle of the N-terminal extension, has several measurable effects on the properties of AGT. Most relevantly, it interferes with dimerization, especially at high temperatures (Lumb and Danpure, 2000); it decreases the stability of the dimeric structure of recombinant purified AGT under chemical stress (Cellini et al., 2010a) as well as the specific catalytic activity of the protein (Cellini et al., 2009). Additionally, it generates a functionally weak mitochondrial targeting sequence (MTS) due to the generation of a Leu-X-X-Leu-Leu optimal consensus motif for binding to the TOM20 mitochondrial protein import receptor (Abe et al., 2000; Muto et al., 2001). Also, it sensitises AGT to the untoward effects of many of the missense mutations found in PH1, at least one of which (Gly170Arg) markedly increases the functional efficiency of the Pro11Leu-generated MTS (Purdue et al., 1991a; Leiper et al., 1996; Lumb et al., 1999). In addition, the replacement of both Pro10 and Pro11 by Leu generates a highly efficient MTS, even in the absence of the disease-specific Gly170Arg replacement (Lumb and Danpure, 2000). The effect of the Gly170Arg mutation seems to be intimately linked to its effect, together with the Pro11Leu polymorphism, on the attachment of the N-terminal extension to the surface of the neighboring subunit and the rate of dimerization. The fact that mutations which appeared to interfere with the intersubunit interaction of the polymorphic N-terminal extension also led to an interference of dimerization and subsequent increased peroxisome-to-mitochondrion mistargeting suggested that one of the main roles of this extension is to mediate the dimerization process (Leiper et al., 1996; Lumb and Danpure, 2000; Danpure, 2006; Djordjevic et al., 2010).

In the present work, we have attempted to shed light on the structural and functional role of the unusual, but not unique,