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Background

Primary hyperoxaluria type 1 (PH1) is caused by a functional deficiency of the intermediary metabolic enzyme alanine:glyoxylate aminotransferase (AGT). In order to understand the condition properly and to design rational treatment strategies, it is important to understand not only what mutations occur in AGT, but also how they achieve their disruptive effects. Although perturbations of AGT function are manifested variously as loss of catalytic activity, aggregation, accelerated degradation, and peroxisome-to-mitochondrion mistargeting, most are the consequences of decreased AGT stability. In order to understand the relationship between mutations and decreased stability, their locations in the context of the overall protein structure is required. Only then can pharmacological agents be designed to counteract their effects.

In our previous OHF-funded project, which ran from 1st July 2000 until 30th June 2002, we solved the crystal structure of normal human AGT. This work not only contributed significantly to our understanding of how AGT folds, dimerizes and acquires its catalytic activity, it also enabled us to localise all the non-synonymous polymorphisms and missense mutations and make predictions about how they might work. It also provided the essential framework upon which the present project was based.

Research Objectives

The main aim of the current project was to extend our previous studies on the detailed three-dimensional physico-chemical characteristics of the various polymorphic and mutant forms of AGT in order to design pharmacological agents that might counteract the untoward effects of the mutations on the cell-biological and enzymic properties of AGT.

Achievements

1. *Crystal structure of AGT(Gly170Arg)*

Gly170Arg is the most common mutation found in PH1. In European and North American patients, it has an allelic frequency of about 30%. It segregates and interacts synergistically with the Pro11Leu polymorphism. The two together are responsible for the peroxisome-to-mitochondrion AGT mistargeting phenotype. We have solved the x-ray crystal structure of mutant AGT containing the Gly170Arg replacement to a resolution of 2.6 Å. The coordinates have been deposited in the RCSB Protein Data Bank (PDB 1J04). The overall structure of AGT(Gly170Arg) is very similar to that of normal AGT (Zhang et al, *J. Mol. Biol*. 331, 643-652, 2003) (PDB 1H0C), except that the inter-

subunit interaction between the side chain of Arg197 and Lys5 in normal AGT is replaced by a network of hydrogen bonds bridged by a unique glycerol molecule (not found in normal AGT) between the side chain of Arg170 and Lys5 in the mutant form. Surprisingly, the glycerol appears to be able to fix the impaired intra-chain interactions caused by the Gly170Arg mutation. This might help to explain how this non-specific chemical chaperone can normalise the targeting of mistargeted mutant AGT(Pro11Leu, Gly170Arg) in transfected COS cells (see Lumb et al. *Biochem. J.* 374, 79-87, 2003). A variety of other mutant and polymorphic variants of AGT, including AGT(Ile244Thr), AGT(Gly82Glu) and ancestral AGT (containing the primate mitochondrial leader sequence), were crystallised and diffraction data obtained. Unfortunately, combinations of instability, aberrant and erratic folding, and crystallographic twinning resulted in uninterpretable diffraction patterns.

2. Localization of conserved glycerol binding sites in mutant and normal AGT.

Using the crystal structures of normal AGT and AGT(Gly170Arg), we have been able to analyse in detail the conserved glycerol binding sites, of which there are five per monomer. This analysis has allowed us to identify accessible surface cavities in the structure, particularly spanning the edge of the dimerization interface, which could be suitable docking sites for pharmacological agents. Modelling these sites has enabled us to carry out rapid computational screening of a large number of compounds in a variety of virtual databases without direct visual inspection. From these screens, we have generated a list of “hits”, which following refinement and iterative re-screening might lead to agents with many orders of magnitude greater affinity that the original compound (i.e. glycerol). It is these compounds that might be the forerunners of new pharmacological agents that could be used in the treatment of PH1.

3. Cell-based bioassay.

In parallel to the above, work supported by the Jules Thorn Charitable Trust (co-PI Gill Rumsby), we have been developing a cell-based bioassay suitable for screening potential candidate drugs generated in section 2 above. Preliminary testing of the “hits” above has been started. Although still very early days, it is clear that proof of principle of this approach has been established. One interesting side shoot of this is that we discovered that not all cells behave the same when it comes to phenotypic manifestations of AGT dysfunction. For example, although peroxisome-to-mitochondrion mistargeting of AGT(Pro11Leu, Gly170Arg) has been shown to occur in artificial in vitro systems, such as micro-injected human fibroblasts, transfected COS and CHO cells, as well as human liver in situ, it does not appear to occur in at least one cell line, namely the osteosarcoma-derived line U2OS. Our preliminary observations have shown that in these cells AGT(Pro11Leu, Gly170Arg) is not targeted to mitochondria. In stably-transformed U2OS cells it appears to be correctly targeted to peroxisomes, whereas in transiently transfected cells it aggregates into cytosolic lumps. The reason for this is unclear, but it might be related to a different folding (i.e. molecular chaperone) environment in U2OS cells or it might simply be a result of failure of the mitochondrial protein import process. Whatever the reason, this surprising observation shows that the cells used for a cell-based bioassay for drug screening need to be chosen with care.

Future

The work accomplished during this and our previous project has laid down a solid foundation for the development of new approaches to the pharmacological treatment of PH1. However, the route from the laboratory to the clinic is a long one and should not be embarked upon without adequate preparation. Much more work still needs to be done to bring together the structure-based virtual drug screening and the cell-based bioassay system, especially the development of the latter to accommodate high-throughput analysis.