

ENZYMATIC ACTIVITY OF THE FOUR HUMAN GLUTATHIONE S-TRANSFERASE PI VARIANTS TOWARDS NITROSOUREA ANTICANCER DRUGS

Submitted by

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to the University of Exeter as a thesis for the degree of Doctor of Philosophy in
Biological Sciences

(January 2013)

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Abstract

Glutathione S-transferases (GST) are a superfamily of detoxifying enzymes present in most life forms. They catalyse the conjugation of the tripeptide glutathione (GSH) to a wide variety of exogenous and endogenous compounds with electrophilic functional groups to form more soluble and non-toxic peptide derivatives. The over-expression of the human class Pi enzyme (hGSTP1) in tumours has been associated with multi-drug resistance. The work presented in this thesis is focused on the four polymorphisms of the hGSTP1 enzyme and their reactivity towards five anticancer nitrosourea drugs.

The coding sequences of hGSTP1-A, -C and -D enzymes were obtained from the hGSTP1-B cDNA through successive cloning into the pGEM-T and pET-28a vectors, and site directed mutagenesis. This was followed by protein over-expression and purification by nickel affinity chromatography and gel filtration.

Activity and inhibition assays, wavelength scans, mass spectrometry, denitrosation and thermofluor shift assays were performed to investigate the relationship between the four isozymes and the anticancer drugs (carmustine, lomustine, semustine, streptozocin and ethylnitrosourea). The enzymes were assayed for activity with 1-chloro-2,4-dinitrobenzene (CDNB) and hGSTP1-A and -D were found to be more active towards this substrate than the other hGSTP1 variants. These two variants share residue Ile104 which could influence catalysis towards different substrates. The mass spectrometry and denitrosation assays have shown that these drugs do not constitute substrates for the hGSTP1 enzymes. The inhibition assays suggested that the four drugs may be weak inhibitors of the hGSTP1 variants. However, the thermal shift assays did not show increased protein stability in the presence of the drugs.

The hGSTP1-D protein was successfully crystallised which allowed determination of the first crystallographic structure of this variant. This has allowed a detailed comparison of hGSTP1-D with other hGSTP1 protein structures available. The side chains of both amino acid residues Ile104 and Val113 of hGSTP1-D appear to occupy positions in the same orientation as the side chains of equivalent residues of other hGSTP1 enzymes. The use of the same crystallisation conditions for co-

crystallisation studies with hGSTP1-D and glutathione or the nitrosourea drugs did not result in protein crystals but further optimisation should be carried out.

Nonetheless, the findings of this project have helped to understand the relationship of the hGSTP1 natural variant enzymes with the five nitrosourea anticancer drugs and to realise that drug resistance in tumours over-expressing this class of hGSTs is not due to the fast metabolism of these drugs but most probably to the role that these enzymes play in cell apoptosis and survival.

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