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The role of the domain linker in the stability of Glutaredoxin-2

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Abstract

GST- when it first appears in the abstract changed from abbreviation (GST) to Glutathione *S*-transferase and the abbreviation is followed in brackets

Grx2 - when it first appears in the abstract changed from abbreviation (Grx2) to Glutaredoxin and the abbreviation is followed in brackets

Grx2 Y58W mutant: changed from being referred to as a wild type to Grx2 Y58W mutant

Titles

More descriptive information has been added to some of the titles of the subsection throughout the dissertation. The following have been changed to:

1.1 Protein domains

1.3.1 van der Waals interactions and hydrogen bonding in proteins

1.3.2 Hydrophobic effect in proteins

1.3.3 Electrostatic interactions in proteins

1.3.4. Entropic effect in proteins

2.2.1 Bioinformatics of the GST family

3.5 Identification of an unknown protein from a DEAE ion exchange matrix

3.6.1 Secondary structure characterisation f Grx2 mutants

3.6.2.1 Fluorescence Intrinsic spectroscopy of Grx2 mutants

3.6.2.2 Near-UV circular dichroism of Grx2 mutants

3.7 Conformational stability of Grx2 Y58W/L78A

3.7.2 Urea-induced equilibrium unfolding of Grx2 Y58W/L78A

3.7.3 Urea-induced equilibrium unfolding of Grx2 Y58W/L78A in the presence of ANS

4.3 Role of the linker region of Grx2 Y58W/L78A

4.4 Implication of this study in terms of function and structure of GSTs

Figure legends

The following information for figure legends has been added to describe them more clearly.

Figure 1. Ribbon representation of the GST in dimeric and monomeric form.

Figure 4. The sequencing results of plasmid Grx2 are presented above.

Figure 5. Over-expression studies of Grx2 Y58W/L78A using two different expression systems. 15 % polyacrylamide reducing SDS-PAGE gels showing Grx2 Y58W/L78A expressed in two different systems A) Escherichia coli BL21 (DE3)/pLysS cells and B) Escherichia coli T7 Express Iq cells.

Figure 6. Elution profile using DEAE ion exchange chromatography, purity and size determination of Grx2 Y58W. (The chromatographic method has been included in the title of the figure).

Figure 7. Elution profile (using DEAE ion exchange chromatography) and size determination of Grx2 Y58W/L78A labelled protein X. (The chromatographic method has been included in the title of the figure).

Figure 8. Sequence coverage and fragment ion spectra determined from mass spectroscopy identifying the unknown protein as Grx2 Y58W/L78A. Above is taken out on the figure legend.

Figure 9. Far-UV CD spectra for recombinant proteins Grx2 Y58W and Grx2 Y58W/L78A. Both spectra were obtained using protein concentration of 5 µM in 50 mM phosphate buffer, pH 7.0, 1 mM DTT and 0.02% NaN3. (Concentration written in full).

Figure 11. Spectra obtained using protein concentration of 20 µM in 50 mM phosphate buffer, pH 7.0, 1 mM DTT and 0.02% NaN3. (Protein concentration added).

Figure 12. Reversibility of unfolding of Grx2 Y58W/L78A monitored by fluorescence. (Comment to include Grx2 Y58W not appropriate as this figure refers only to Grx2 Y58W/L78A). Reversibility studies for Grx2 Y58W have been conducted by Gildenhuys, S. (2006). Folding mechanism of Glutaredoxin-2. PhD Thesis. University of the Witwatersrand. http://wiredspace.wits.ac.za/handle/10539/4845 (native and refolding conditions have also been added for the figure legend).

Figure 13. Urea-induced equilibrium unfolding of Y58W/L78A Grx2. (Written in the correct order)

Figure 14. Urea-induced equilibrium unfolding of Grx2 Y58W/L78A in the presence of ANS. (Grx2 mutant changed to Grx2 Y58W/L78A).

Figure 15. Representation of the residues surrounding Leu78 for Grx2 Y58W/L78A. (For Grx2 Y58W/L78A has been added to the legend).

Introduction

The introduction has been updated with more recent articles

(Pg. 5): 1.3.2 currently which refereed to 1985 and 1995 references is removed

(Pg. 7): POU domain written in full followed by the abbreviation in brackets

(Pg. 9): Dirr *et al*., 1994; *et al* included

Pg. 13: mainly taken out

Pg. 17 Xia *et al.,* 2001 (1999 replaced with 2001)

Pg. 20 (w/v) added for NaN_3

Pg. 22 Sulphate replaced with sulphate and throughout the dissertation

Pg.22 the concentration of components for separating and stacking gel have been written as final

Pg.22 dilution with sample buffer changed from 2-fold dilution to 1:1

Pg.23 not appropriate: information about sequencing already provided

Pg. 24 v/v included for MeOH and FA

Pg. 25 The following subheadings have been added into the table of contents

2.2.9.1; 2.2.10.1; 22.2.11.1; 2.2.11.2; 2.2.11.3

Pg.28 The sentence has been re constructed

2.2.11.2. The sentence has been changed to unfolded

Pg.30 Equation not written in bold anymore

Results

Pg. 32 two other amino acids have been included in the text (Met and Ile)

Pg. 33 a sentence is included in the figure legend explaining that all the sequences are being aligned to Grx2

Pg.34 an explanation showing where the data represented in figure 3a is from

Pg. 34 a copy of predicted amino acid sequence for Grx2 Y58W and Grx2 Y58W/L78A is included

Pg. 35 the nomenclature is included for both inserts

Pg. 35 citation showing that Grx2 Y58W is indeed soluble is included

Pg. 36 the pictures are enhanced and the correct information is shown

Pg. 38 Elution profile of the different samples that do not bind to the column are shown

Pg.39 Sentence explained mush better instead of 30 % increase in alpha helix content, it is the ellipticity signal for both proteins were found to be different because signal for Grx2 Y58W/L78A spectrum increased by 30 %. The numbers of residues shown for what the increase represents

Pg. 45 recovery is explained

Pg. 49 the rationale explained for conducting ANS binding studies. Only wild-type Grx2 ANAS and Grx2 y58w/l78a studies have been conducted

Enzyme activity – inappropriate- our lab is not equipped to handle such dangerous and flammable chemicals

Discussion

Pg.53 the correct conclusion is written for the Lin 2007 paper

Pg.54 delta G values are indicated as 5.7 because they are an average of the two techniques and it's explained in the text

Pg.54 more results are discussed on the purification protocol

Pg. 56 unfolding data for Grx2 Y58W is included for comparison

References

Spacing is fixed

The PNAS abbreviations is fixed

Font size is the same throughout