Targeted Ubiquitination of Glutathione Peroxidase 1

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Introduction

What is selenium?

- Essential micronutrient.
  - Recommended average daily intake for teens (14-18 yrs) and adults is 55 mcg.
- Cannot be synthesized by body.
- Found in foods such as seafood, meat, eggs, grains, and Brazil nuts.

What selenium is and why you need it (But not too much of it). (2023, May 31). Cleveland Clinic.

https://health.clevelandclinic.org/selenium-benefits

Why is selenium essential?

- Metabolized primarily into the amino acid selenocysteine (Sec) in liver.
- Sec is incorporated into selenoproteins that are essential for bodily functions.

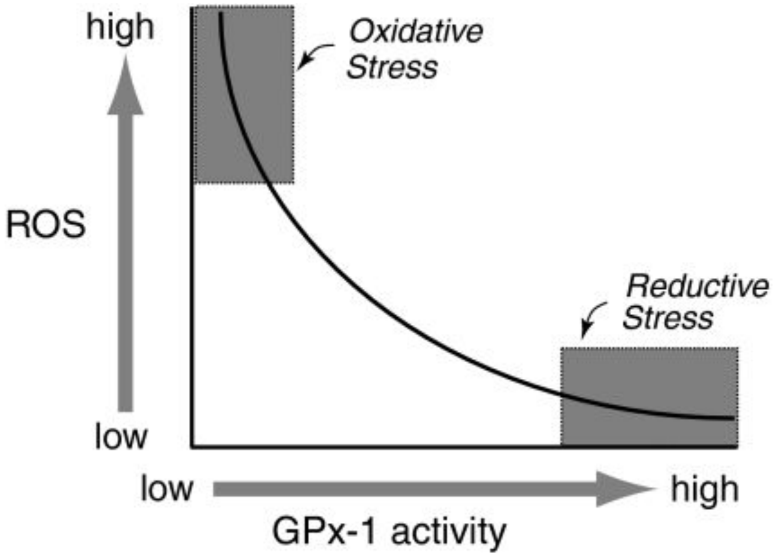
Selenium: foods, functions, how much do you need & more. (n.d.). Eufic.

https://www.eufic.org/en/vitamins-and-minerals/article/selenium-foods-functions-how-much-do-you-need-more

Introduction

Glutathione peroxidase 1 (GPX1)

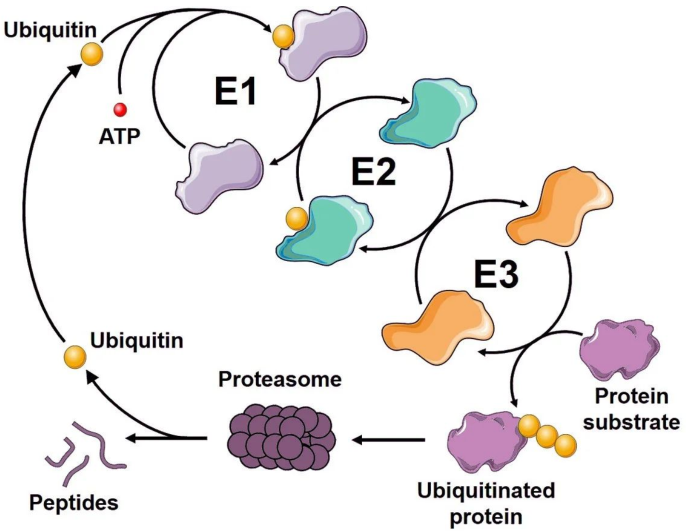
- A selenoprotein.
- Catalyzes reduction of hydrogen peroxide and soluble low molecular hydrogen peroxides into water and alcohols.



Lubos E, Loscalzo J, Handy DE. Glutathione peroxidase-1 in health and disease: from molecular mechanisms to therapeutic opportunities. *Antioxid Redox Signal*. 2011;15(7):1957-1997. doi:10.1089/ars.2010.3586

Ubiquitin-Proteasome System

- Protein degradation method.
- Ubiquitin binds to a protein's lysine residue, allowing the proteasome to recognize and degrade the protein.



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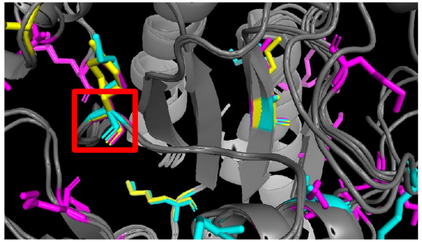
Introduction

AlphaFold 2.0 Structure Model of Human GPX1 and GPX4

A

B



GPX1: Yellow
GPX4: Magenta

GPX1 Amino Acid Sequence

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sp|P04041|GPX1_RAT     - - - - - M S A A R L S A V A Q S T V Y A F S A R P L A G G E P V S L G S L R G K V L L I E N V A S L U G T T T R D Y T   55
sp|P11352|GPX1_MOUSE   - - - - - M C A A R L S A A A Q S T V Y A F S A R P L T G G E P V S L G S L R G K V L L I E N V A S L U G T T I R D Y T   55
sp|P07203|GPX1_HUMAN   M C A A R L A A - - - A A A A A Q S V Y A F S A R P L A G G E P V S L G S L R G K V L L I E N V A S L U G T T V R D Y T   57
sp|Q8MJ14|GPX1_PIG     M C A A Q R S A A A L A A V A P R S V Y A F S A R P L A G G E P I S L G S L R G K V L L I E N V A S L U G T T V R D Y T   60
sp|P00435|GPX1_BOVIN   M C A A Q R S A A A L A A A A P R T V Y A F S A R P L A G G E P F N L S S L R G K V L L I E N V A S L U G T T V R D Y T   60

sp|P04041|GPX1_RAT     E M N D L Q K R L G P R G L V V L G F P C N Q F G H Q E N G K N E E I L N S L K Y V R P G G G F E P N F T L F E K C E V   115
sp|P11352|GPX1_MOUSE   E M N D L Q K R L G P R G L V V L G F P C N Q F G H Q E N G K N E E I L N S L K Y V R P G G G F E P N F T L F E K C E V   115
sp|P07203|GPX1_HUMAN   Q M N D L Q K R L G P R G L V V L G F P C N Q F G H Q E N A K N E E I L N S L K Y V R P G G G F E P N F M L F E K C E V   117
sp|Q8MJ14|GPX1_PIG     Q M N E L Q R R L G P R G L V V L G F P C N Q F G H Q E N A K N G E I L N C L K Y V R P G G G F E O N F M L F E K C E V   120
sp|P00435|GPX1_BOVIN   Q M N D L Q R R L G P R G L V V L G F P C N Q F G H Q E N A K N E E I L N V L K Y V R P G G G F E P N F M L F E K C E V   120

sp|P04041|GPX1_RAT     N G E K A H P L F T F L R N A L P A P S D D P T A L M T D P K Y I I W S P V C R N D I S W N F E K F L V G P D G V P V R   175
sp|P11352|GPX1_MOUSE   N G E K A H P L F T F L R N A L P T P S D D P T A L M T D P K Y I I W S P V C R N D I A W N F E K F L V G P D G V P V R   175
sp|P07203|GPX1_HUMAN   N G A G A H P L F A F L R E A L P A P S D D A T A L M T D P K L I T W S P V C R N D V A W N F E K F L V G P D G V P L R   177
sp|Q8MJ14|GPX1_PIG     N G A N A H P L F A F L R E A L P T P S D D A T A L M T D P K F I T W S P V C R N D I A W N F E K F L V G P D G V P L R   180
sp|P00435|GPX1_BOVIN   N G E K A H P L F A F L R E V L P T P S D D A T A L M T D P K F I T W S P V C R N D V S W N F E K F L V G P D G V P V R   180

sp|P04041|GPX1_RAT     R Y S R R F R T I D I E P D I E A L L S K Q P S N P   201
sp|P11352|GPX1_MOUSE   R Y S R R F R T I D I E P D I E T L L S Q Q S G N S   201
sp|P07203|GPX1_HUMAN   R Y S R R F Q T I D I E P D I E A L L S Q G P S C A   203
sp|Q8MJ14|GPX1_PIG     R Y S R R F L T I D I E P D I E A L L S Q E P S S A   206
sp|P00435|GPX1_BOVIN   R Y S R R F L T I D I E P D I E T L L S Q G A S A -   205
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K: Lysine

Hypothesis

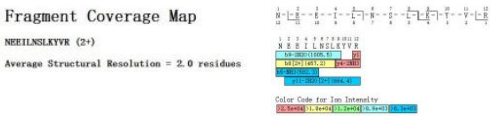
GPX1 is degraded through the ubiquitin-proteasome system.

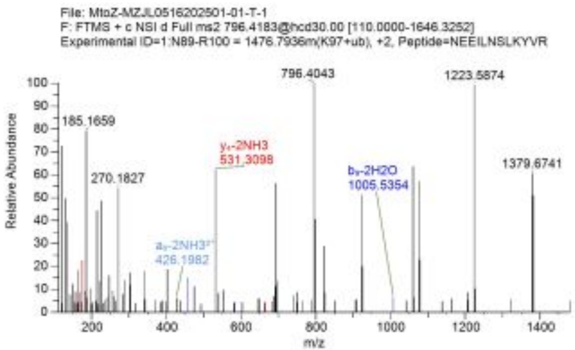
Methods

1. Mass Spectrometry

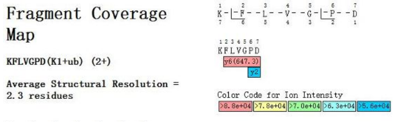
Predicted lysine sites K97 and K166 on GPX1 would be targeted for ubiquitination.

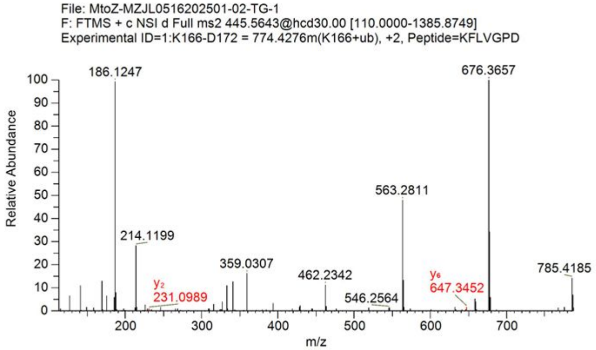
MS/MS Spectrum of the ubiquitination peptide at K97 in GPX1





MS/MS Spectrum of the ubiquitination peptide at K166 in GPX1

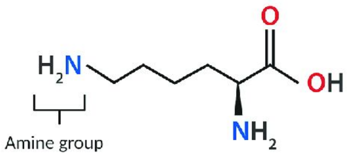




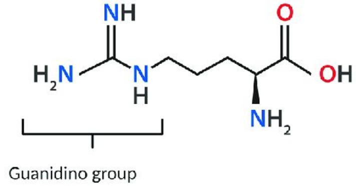
2. Mutagenesis

Human Embryonic Kidney 293 (HEK293) cells were transfected with plasmids expressing GPX1 with K97R or K166R mutations.

Lysine



Arginine



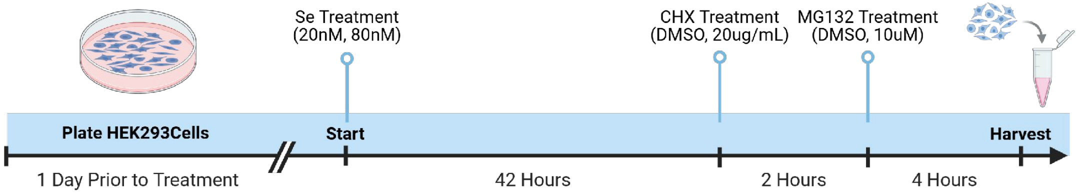
Arginine and Arginine-Rich peptides as modulators of protein aggregation and cytotoxicity associated with Alzheimer's disease. (n.d.). ResearchGate. https://www.researchgate.net/figure/Structure-of-the-arginine-monomer-in-comparison-to-lysine_fig1_355702450

Methods

3. Cell Treatment

The cells were treated with:

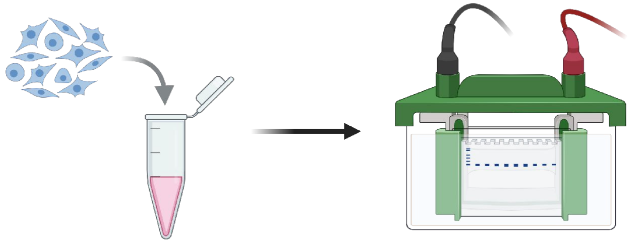
- Low or adequate selenium
- Cycloheximide (CHX)
  - Inhibits protein synthesis
- MG132
  - Inhibits proteasomal degradation



4. Analysis for GPX1

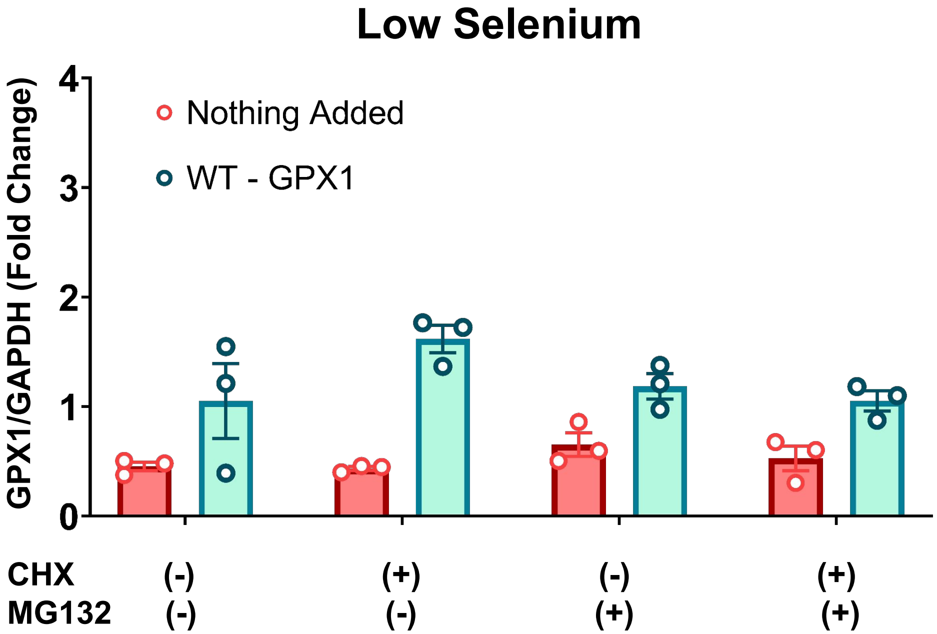
Total protein was extracted from the cells and measured with bicinchoninic acid assays.

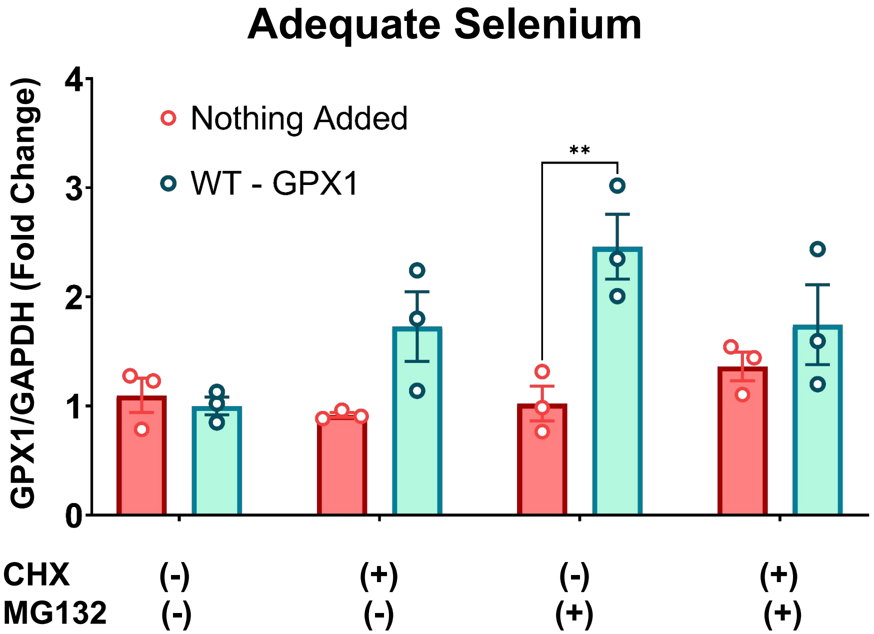
GPX1 levels were measured with Western blot analysis.



Results

Nothing Added vs Wild Type





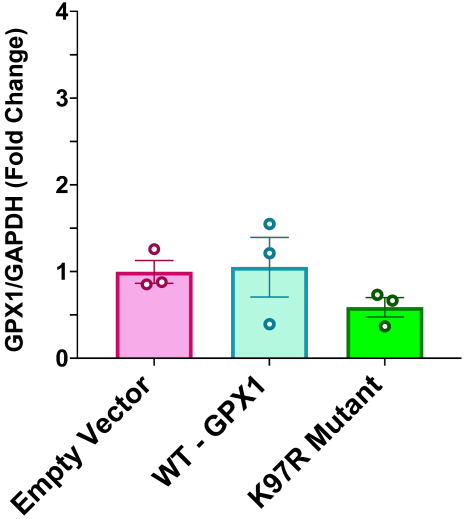
*p<0.05
**p<0.01
***p<0.001

Results

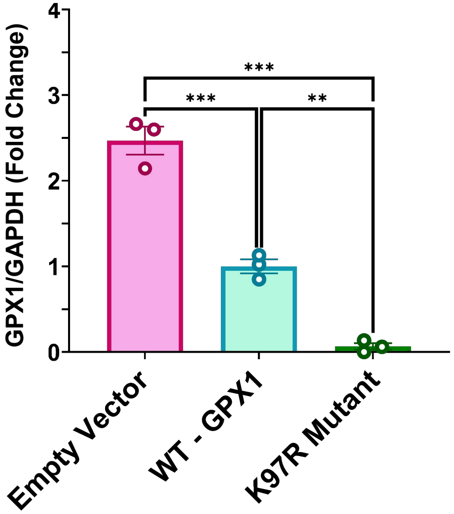
Empty Vector vs Wild Type vs Mutations

K97R Mutant

Low Selenium

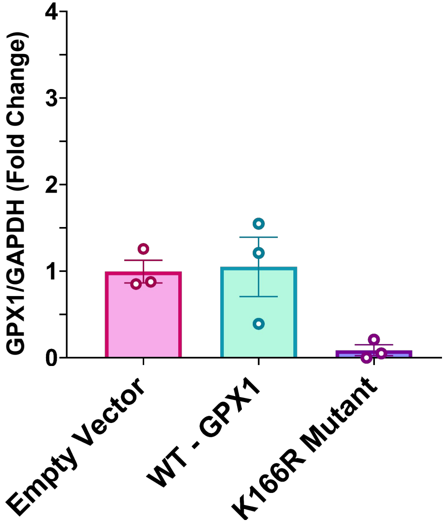


Adequate Selenium

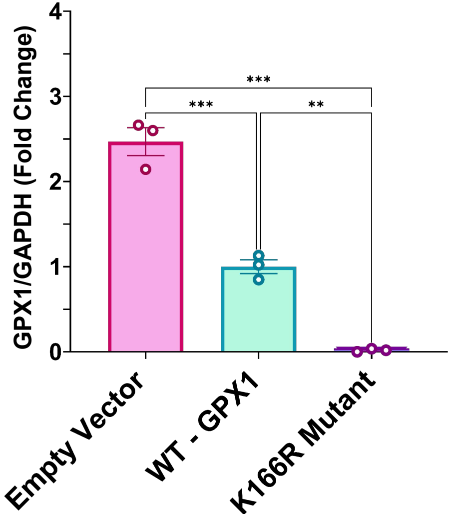


K166R Mutant

Low Selenium



Adequate Selenium



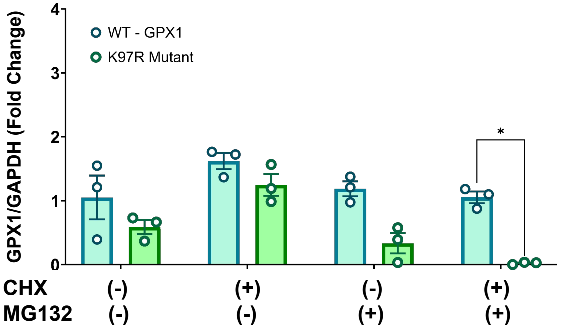
*p<0.05
**p<0.01
***p<0.001

Results

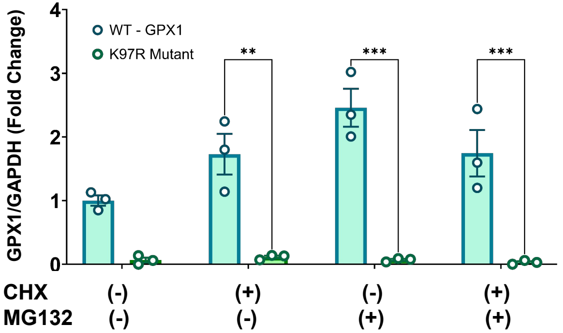
Wild Type vs Mutations

K97R Mutant

Low Selenium

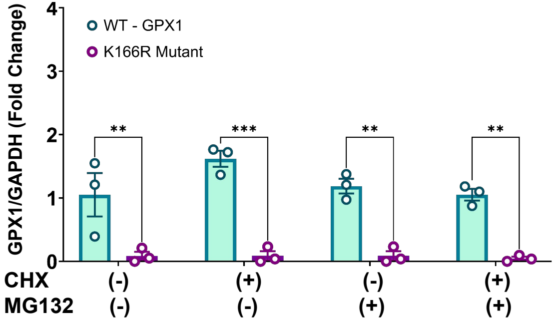


Adequate Selenium



K166R Mutant

Low Selenium



Adequate Selenium

*p<0.05
**p<0.01
***p<0.001

Conclusion

Is GPX1 degraded via the ubiquitin-proteasome system?

Maybe ⇒ needs further experimentation

- All controls worked.

K97

- May be ubiquitination site with low selenium.

K166

- Cannot yet be concluded as a ubiquitination site with low or adequate selenium.

Future Directions

Conduct further testing of the mutated sites of GPX1.

Increase Sample Size

Increase to n=6.

Central Dogma Analysis

Determine which stage is preventing GPX1 expression with mutations.

- Analyze mRNA expression.

References

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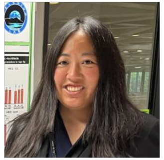

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