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Glutathione disulfide reductase : reducing oxidative stress

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Overview

1. Introduction
    - 1.1 About the enzyme
    - 1.2 Reaction catalyzed
2. Protein structure
3. Mechanism of reaction
4. Studying GR
5. Enzyme regulation and stability
6. Summary

About the enzyme

<u>Alternative names:</u> Glutathione reductase, NADPH-glutathione reductase, GSH reductase, GSSG reductase, usually abbreviated as GR.

<u>EC number*</u>: 1.6.4.2

- 1 → Oxidoreductase
- 6 → Acting on NADPH
- 4 → Have disulfide acceptor
- 2 → Specific for GSSG

<u>Isolated from:</u> Yeast (*Saccharomyces cerevisiae*), spinach, erythrocytes *etc.*

* www.ebi.ac.uk/thornton-srv/databases/cgi-bin/enzymes/GetPage.pl?ec_number=1.6.4.2 (Acessed 03-15-05)

Active site: Cys58-Cys63-His467

***Dimer molecular weight:** 105 kDa

***Isoelectric point:** 6.46

Catalytic efficiency: 2 $M^{-1}s^{-1}$

ε_{461}: 11.7 x 10^{-3} M^{-1} cm^{-1}

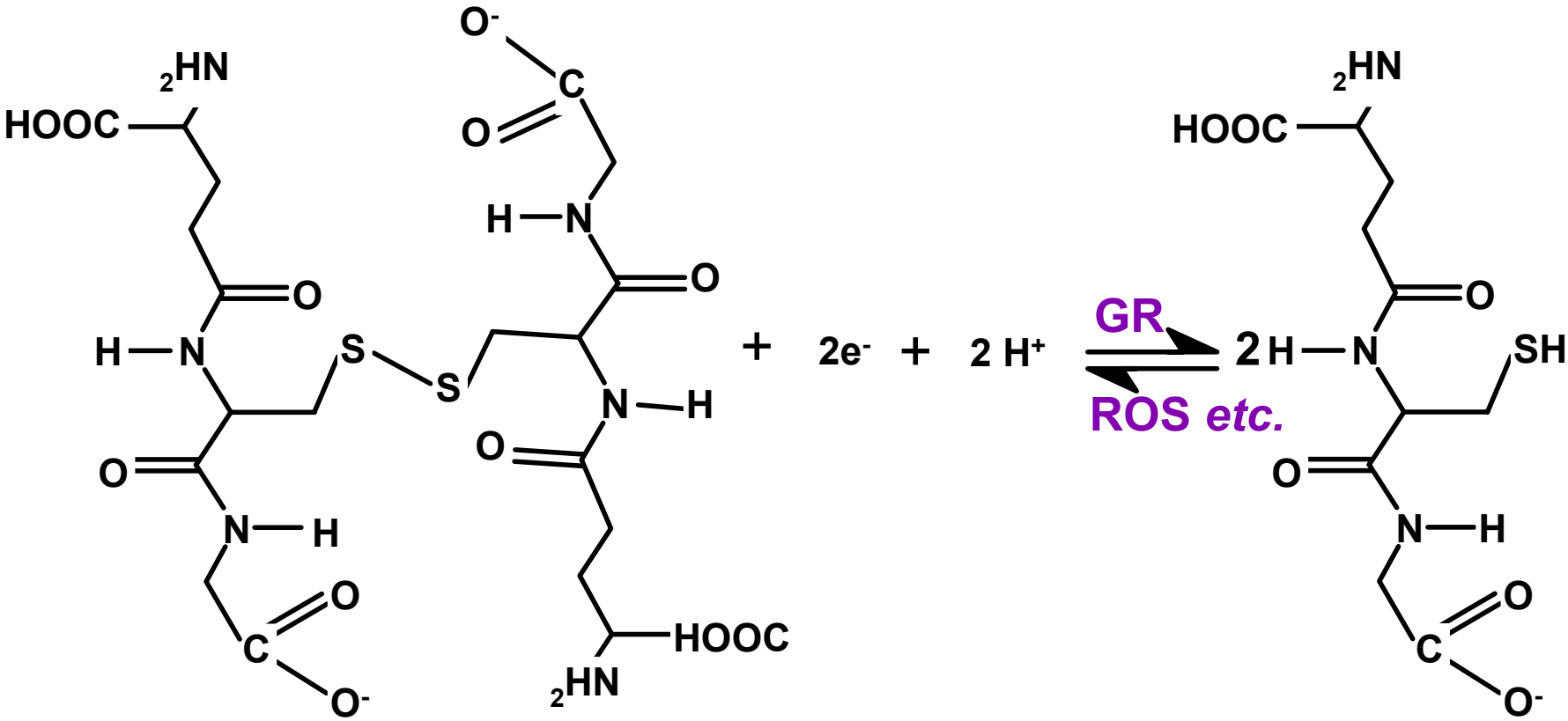
Turnover for GSSG: 9900 min^{-1}

Prosthetic group: FAD

Cofactor: NADPH

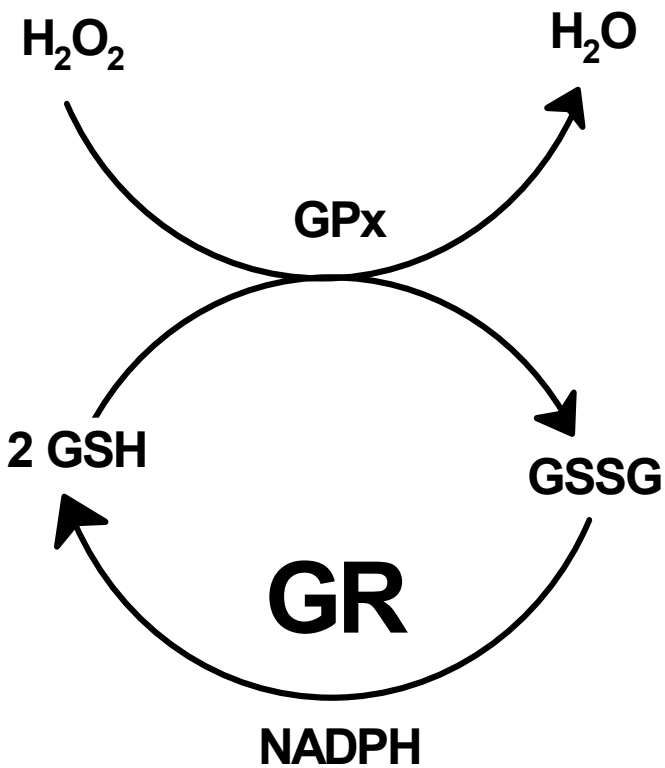
Mol Biochem Parasitol (2000) **107:**169–179: **Eur J Biochem*.(1979) **98:**487-99.

Reaction catalyzed



GSSG GSH

Reaction equation



Step 1:

$$NADPH + H^+ + E \leftrightarrow NADP^+ + EH_2$$

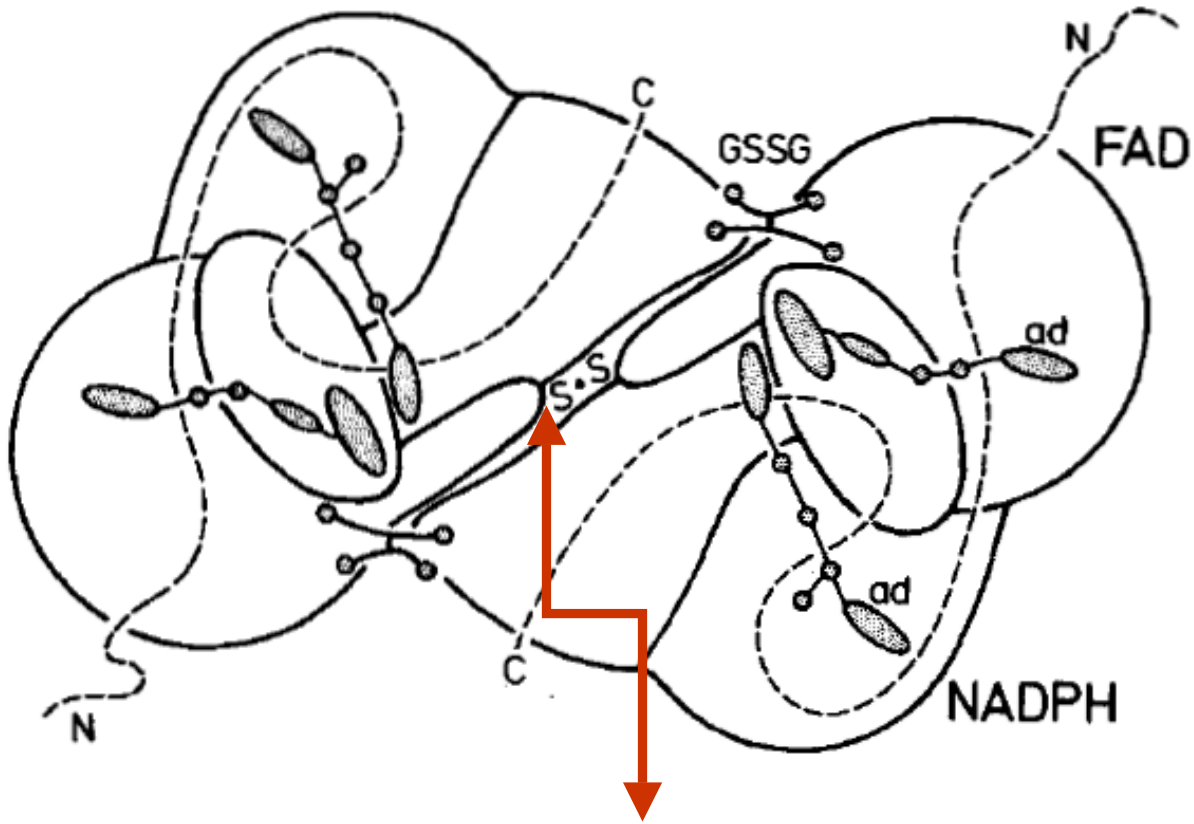
Step 2:

$$EH_2 + GSSG \leftrightarrow 2\ GSH$$

Where E = enzyme

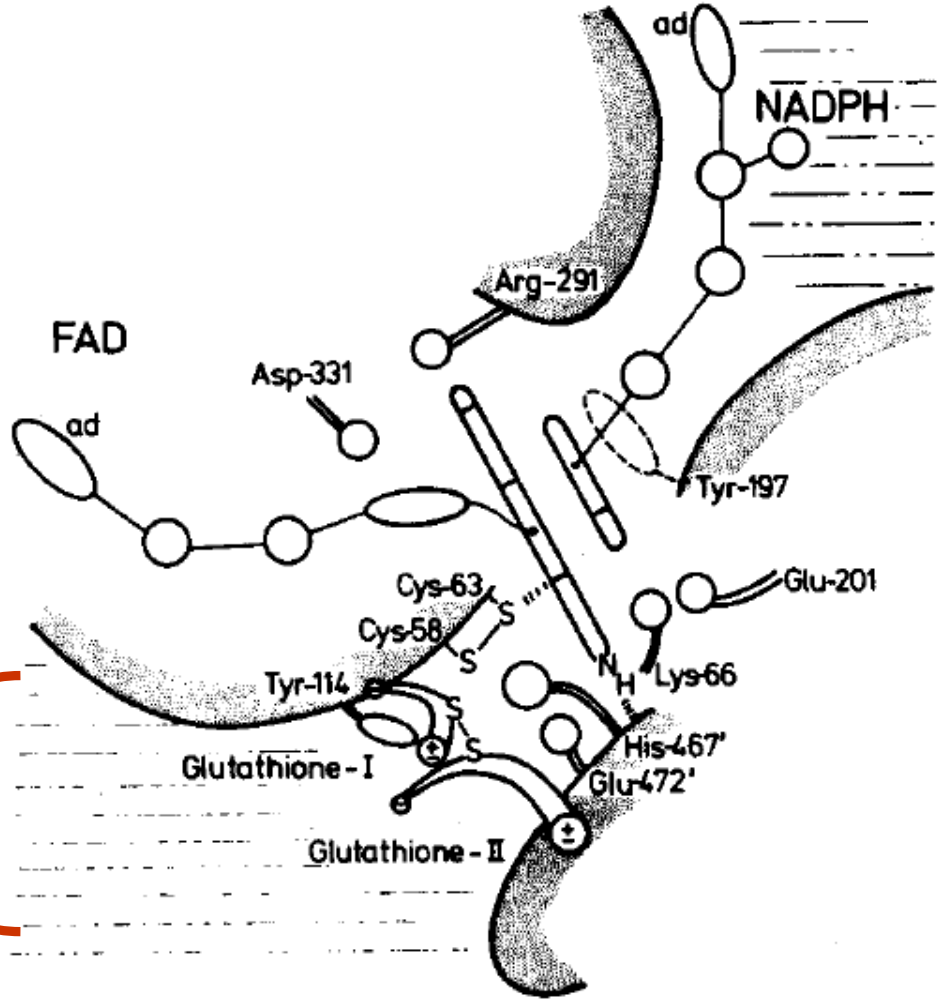
J Biol Chem. (1983) **258:**1752-1757.

GR has two equivalent subunits:
Catalytic center lies between these two subunits



J Biol Chem. (1983) **258:**1752-1757.

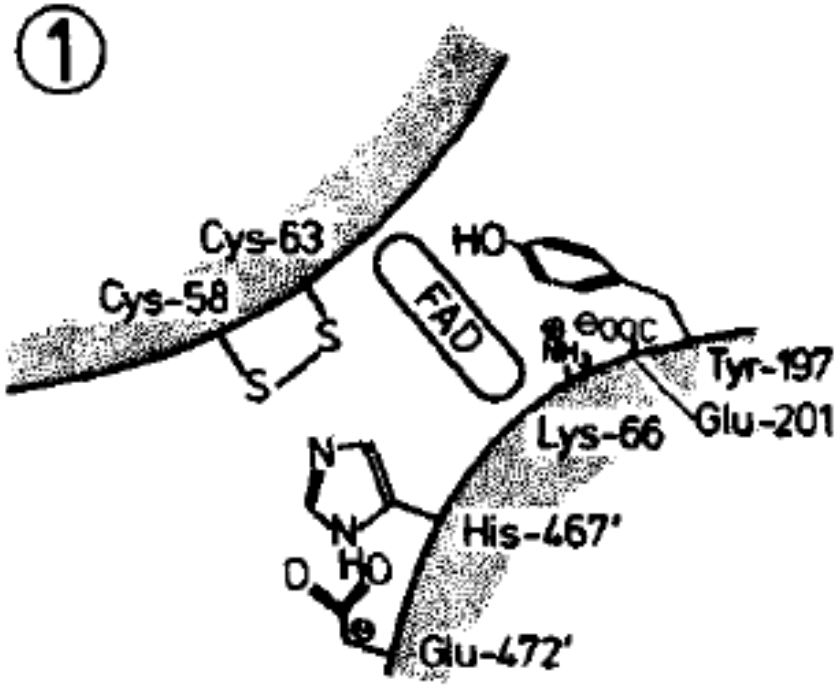
Catalytic center is divided into two parts: one binds NADPH and the other binds GSSG



Substrate GSSG represented as GSH I:GSH II

J Biol Chem. (1983) **258:**1752-1757.

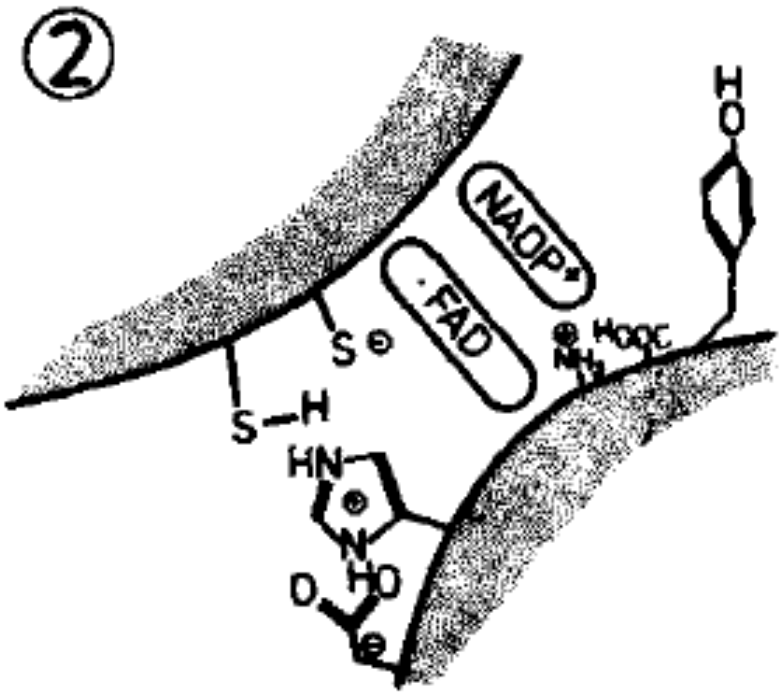
Mechanism step 1



- **Oxidized form of the enzyme.**
- **The -N of His 467 bonds with –OH of Glu 472.**

J Biol Chem. (1983) **258:**1752-1757.

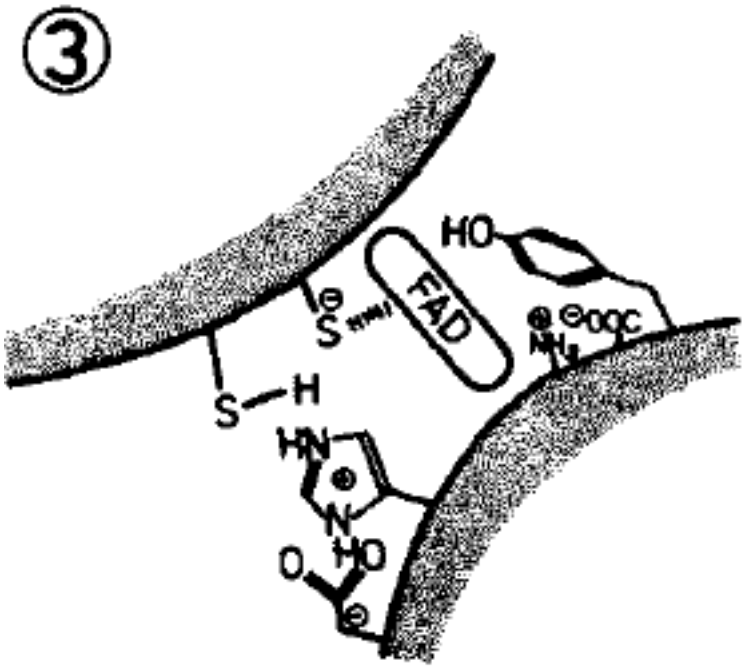
Mechanism step 2



- **NADPH reduces the enzyme to EH_2.**
- **On binding NADPH, Tyr 197 in the NADPH binding pocket moves away such that flavin of FAD can interact with nicotinamide of NADPH.**

J Biol Chem. (1983) **258:**1752-1757.

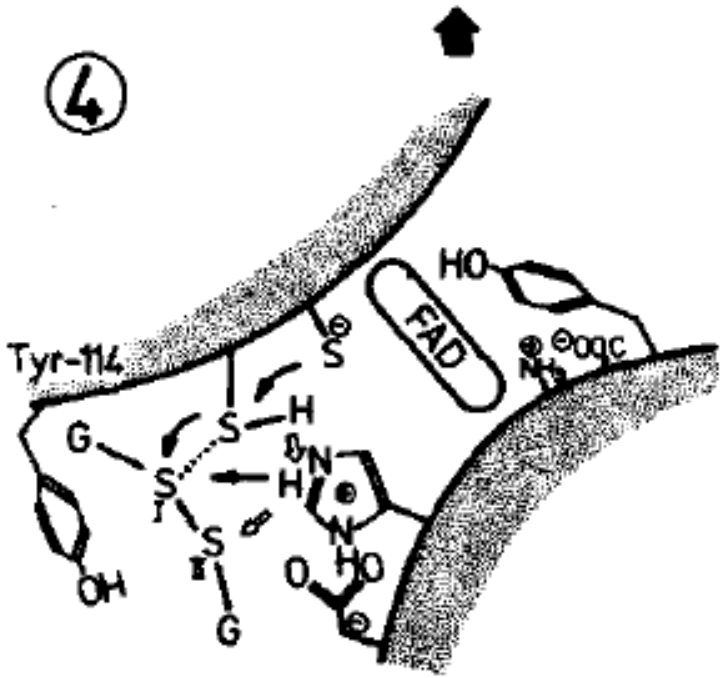
Mechanism step 3



- **Sulfur of Cys-63 moves closer to FAD forming the charge transfer complex.**

J Biol Chem. (1983) **258:**1752-1757.

Mechanism step 4

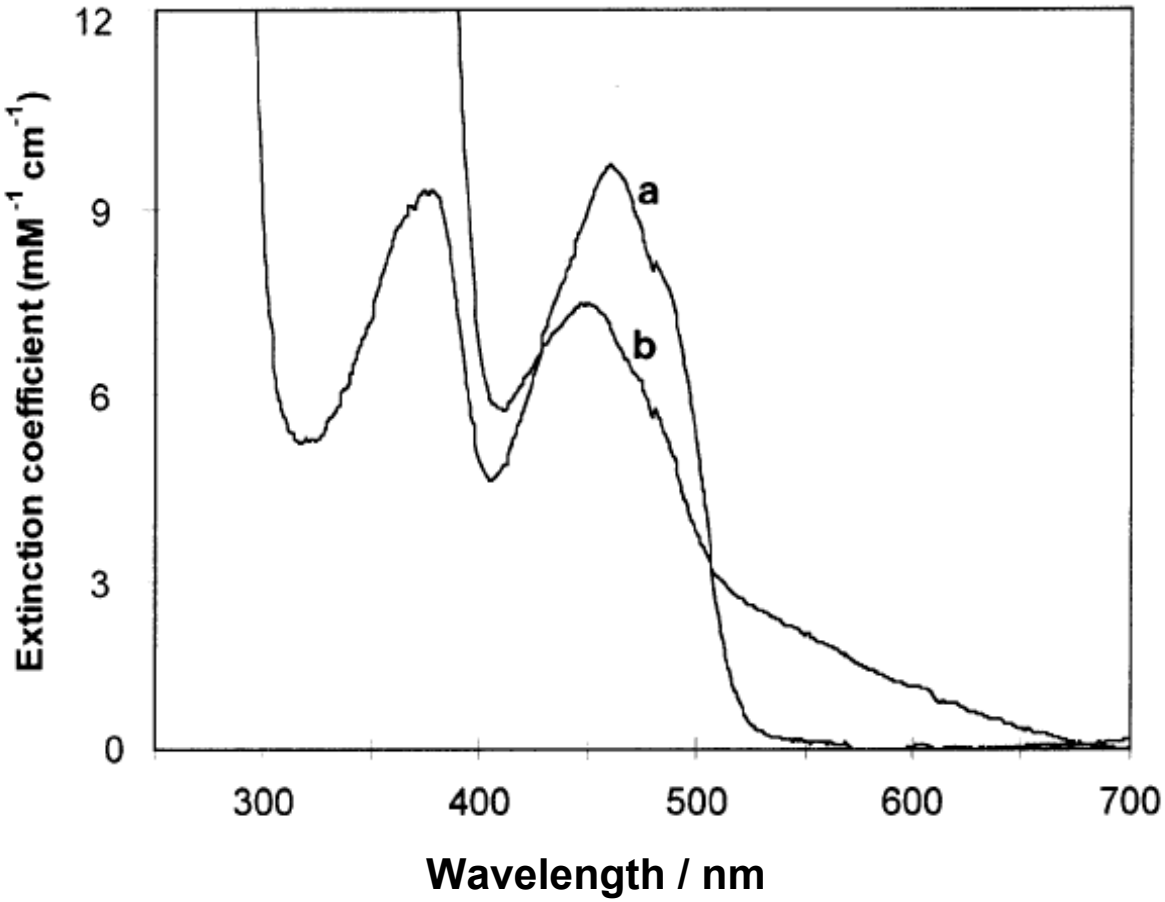


- **GSSG binds to reduced enzyme EH_2**
- **Glutathione I and Cys-58 form a mixed disulfide releasing glutathione II**
- **In the last step, glutathione I is released**

J Biol Chem. (1983) **258:**1752-1757.

Studying GR:

(1) Absorption spectra of GR



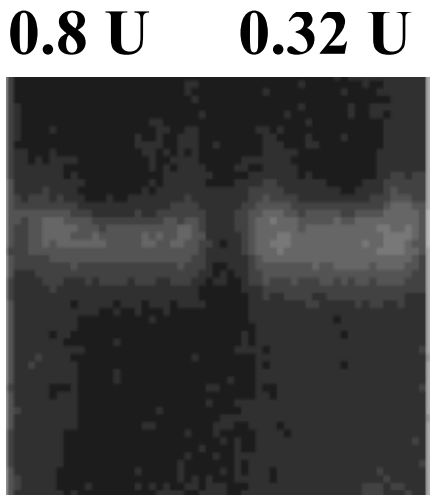
a: reduced form
b:oxidized form

Enzyme purity/activity can be tested by checking its ε_{461}

Molecular and Biochemical Parasitology (2000) **107:**169–179

(2) Activity assay

Activity can be followed by native PAGE using MTT/PMS* negative staining.

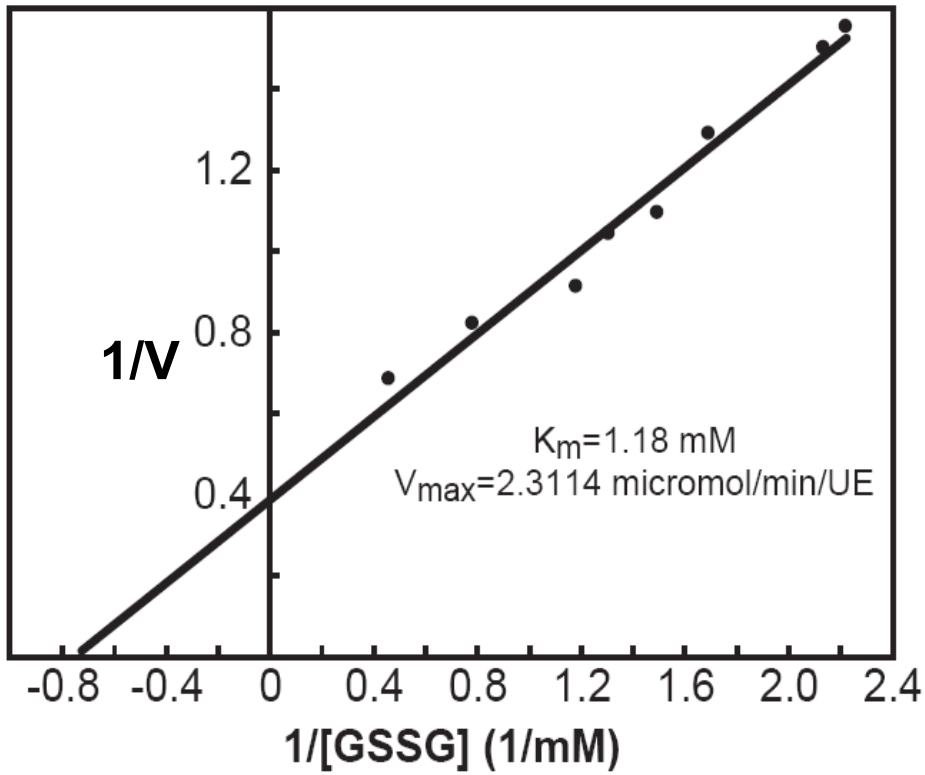


*__MTT__: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide;

PMS: phenazine methosulfate

Electrophoresis (2004) **25**: 2926–2931

(3) Lineweaver-Burk plots determined polarigraphically help in V_{max} and K_m determination



Average V_{max} = 2.30 ± 0.01 µmol/min/UE at

(25°C, pH = 8.0)

Average K_m= 1.17 ± 0.01 mM

Current Separations (2004) **20**: 117-120

Enzyme regulation

- **The gene encoding GR is mostly post-transcriptionally regulated.**
- **GR protein levels are dictated by the metabolic needs of the cell and oxidative stress.**
- **Activity of the enzyme can also vary with temperature and pH.**

Plant Physiol (2000) **122:**667-676

Enzyme stability

- **Prolonged storage (6 -12 months) of the enzyme at -20˚C or -80˚C reduces its activity by ~ 2%** *Clin chem.* (2000) **46:** 566-567.

- **On standing at room temperature, GR activity decreases. However, dilution of the enzymes seems to inhibit enzyme decay** *Clin chem.* (1976) **22:** 1005-1008.

Summary 1

- GR is indirectly involved in combating oxidative stress by replenishing the reducing equivalents of the cell, namely, GSH. Thus it is a secondary antioxidant enzyme.

- It is widely studied and most of its physical and chemical properties are well-known.

Summary 2

- **It holds an important place at all levels of life plants, yeast and humans. Thus, there is significant homology in the GR genetic code at all levels.**
- **Further studies need to be done to asses its regulation by competitive and non-competetive inhibition in the cell which might reduce its turnover.**
- **Loss of GR activity can prove to be detrimental to the cell.**
- **Thus GR inhibition forms a potential target for anti-cancer therapies.**