SFRR-E 2021 EUROPE

BELGRADE SERBIA 15-18 JUNE 2021

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The role of thioredoxin / thioredoxin reductase system in the redox homeostasis of the endoplasmic reticulum

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Abstract

Introduction: The endoplasmic reticulum (ER) is the major site of protein thiol oxidation during post-translational modification. Due to the dominance of oxidized proteins, the lumen of the ER is usually considered as an oxidative environment; although some processes which require reducing agents, such as NADPH, are also found here. The parallel occurrence of oxidized thiol-disulfides and reduced pyridine nucleotides may indicate that the ER lumen lacks components which connect the two systems. Our aim was to investigate the luminal presence of the thioredoxin (Trx) / thioredoxin reductase (TrxR) proteins, capable to link the protein thiol and pyridine nucleotide systems.

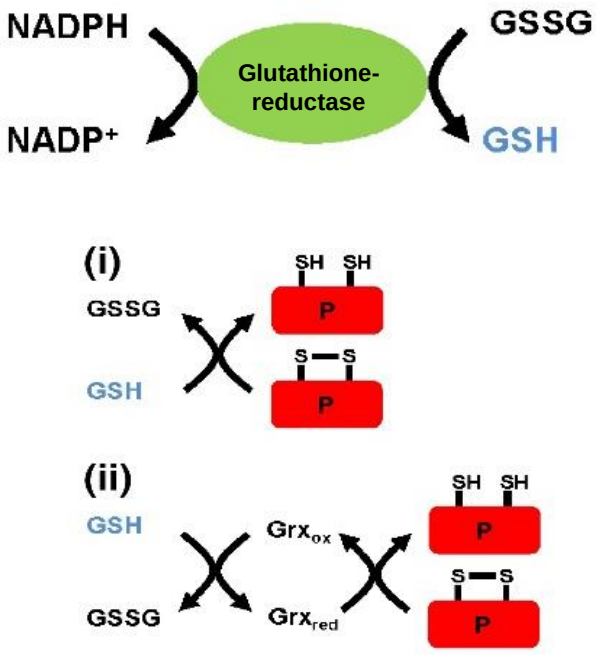
Methods: Protein expression of Trx/TrxR isoforms was examined on subcellular fractions by Western blot analysis. TrxR activity in each organelle was measured using a colorimetric kit. The intracellular distribution of Trx/TrxR isoforms was also examined by immunofluorescent microscopy. An *in silico* analysis was performed to analyze the predicted localization of each isoform.

Results: We showed that the specific activity of TrxR in the ER is around zero (0,02 U/mg ± 0,01), while we measured higher activities in the cytoplasm (1,26 U/mg ± 0,11) and mitochondria (1,57 U/mg ± 0,19). Analysis of rat liver subcellular fractions revealed that the two isoforms of Trx, and the three isoforms of TrxR are not expressed in the ER. Immunofluorescent analysis confirmed that Trx and TrxR isoforms did not show colocalization with ER-specific marker Grp94. *In silico* prediction analysis also predicted a very low probability of luminal localization for each isoform (0–5%).

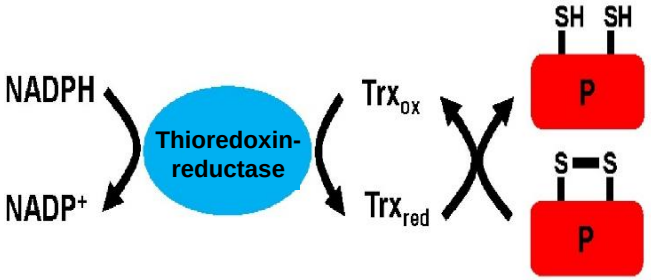
Conclusions: Our results show that none of the components of the Trx/TrxR system is expressed in the ER lumen. The absence of this electron transfer chain may explain the uncoupling of redox systems in the lumen, allowing the parallel presence of a reduced pyridine nucleotide pool and oxidized proteins.

- Introduction

1.a Glutathione-reductase



1.b Thioredoxin/thioredoxin-reductase system



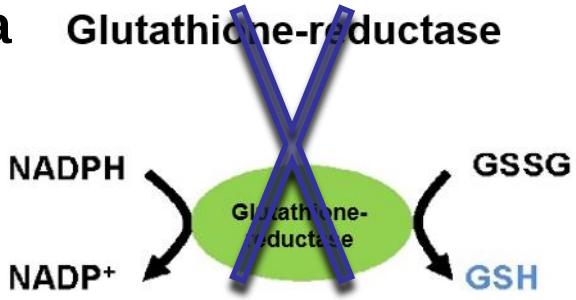
The redox conditions of the endoplasmic reticulum (ER) are different from the other subcellular compartments. The lumen of the ER is considered as an oxidative environment, however, reducing agent-requiring processes are also found here.

The colocalized thiol/disulfide and reduced/oxidized pyridine nucleotide systems are enzymatically linked in most compartments to form complex redox systems.
(*1.a*) One of these linking enzymes is glutathione reductase (GR).
(*1.b*) The other enzyme which is capable to connect the two redox systems in other compartments is thioredoxin reductase (TrxR).

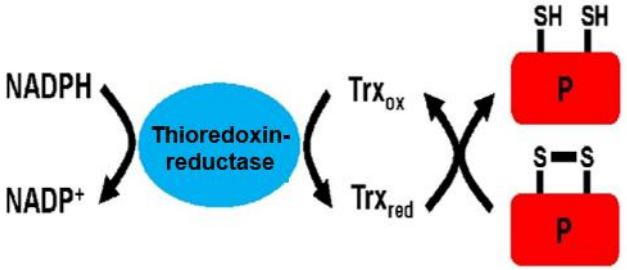
P: Protein
Grx: Glutaredoxin
Trx: Thioredoxin

- Aim

1.a Glutathione-reductase



1.b Thioredoxin/thioredoxin-reductase system



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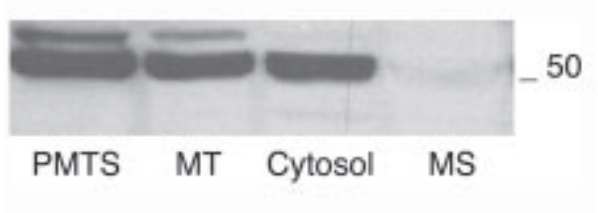
2.a[1]



The presence of these enzymes in the ER lumen would compromise both the function of luminal NADPH-dependent reductases and the oxidative protein folding. However, in the absence of linking enzymes, these redox systems can function separately.

GR is practically absent from the ER according to the previous results of our laboratory. *Figure 2.a* shows that GR protein is undetectable in the ER-containing microsomal (MS) fraction, examined by Western blot technique.

In contrast to GR, the Trx/TrxR redox system is complex and consists of several components, all of them having numerous isoforms. The exact localization of some of these components have not been studied thoroughly yet.

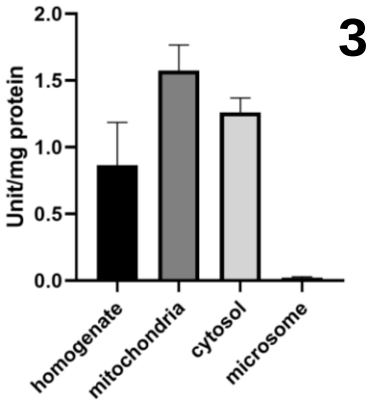
We aimed to investigate the presence of Trx/TrxR system in the ER. According to our hypothesis, this system is also missing from the lumen.

[1]Piccirella S et al (2006), *The Journal of Biological Chemistry*, 281:4671-4677

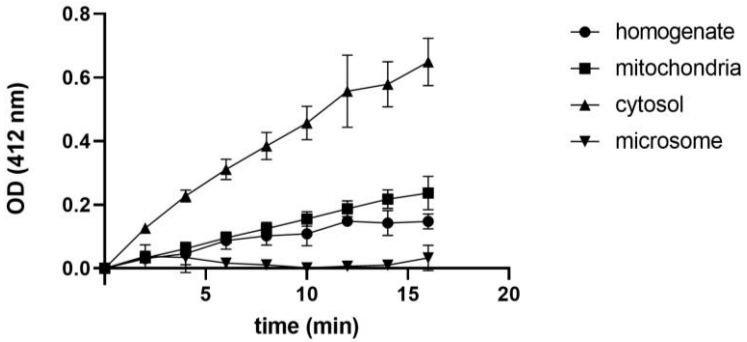
- Methods
  - Models:
    - rat liver subcellular fractions
    - human fibroblast cells
  - Measuring the activity of TrxR on subcellular fractions using a colorimetric kit
  - Examination of the subcellular localization of Trx/TrxR system by Western blot analysis on rat liver fractions
  - Examination of the subcellular localization of TrxR3 on human fibroblast cells by immunofluorescent analysis
  - *In silico* analysis to predict the subcellular localization of TrxR3

- Results

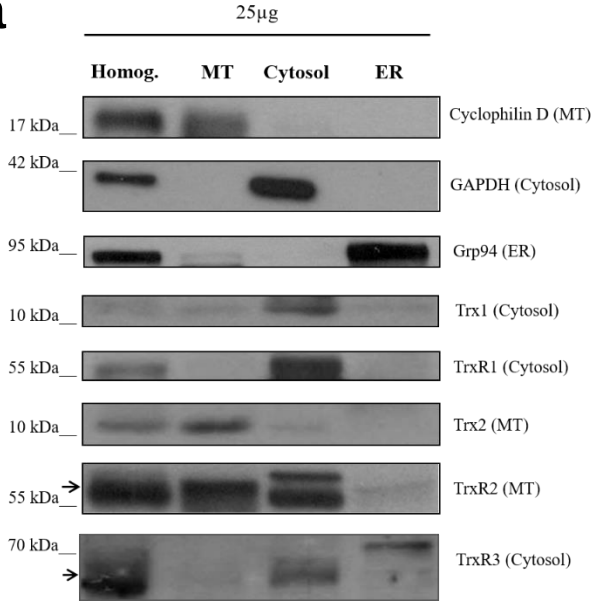
3.a



3.b



4.a

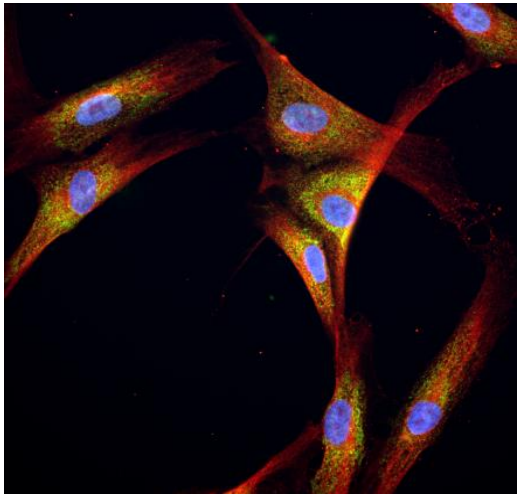


(*3.a,b*) TrxR activity was measured in rat liver fractions. TrxRs showed high specific activities in the mitochondrial fraction (1,57 U/mg ± 0,19) and in the cytoplasm (1,26 U/mg ± 0,11). The specific activity in the microsomal fraction was almost zero (0,02 U/mg ± 0,01) and the optical density (OD) showed no change over time, suggesting an absence of TrxR activity in the endoplasmic reticulum of the liver.

(*4.a*) Protein expression of each isoform of the Trx/TrxR system was examined in rat liver fractions by Western blot. The purity of the fractions was confirmed with organelle-specific marker proteins. Trx1 and TrxR1 showed a cytosolic localization. Trx2 and TrxR2 were found in the mitochondrial fraction, while TrxR3 showed cytosolic localization.

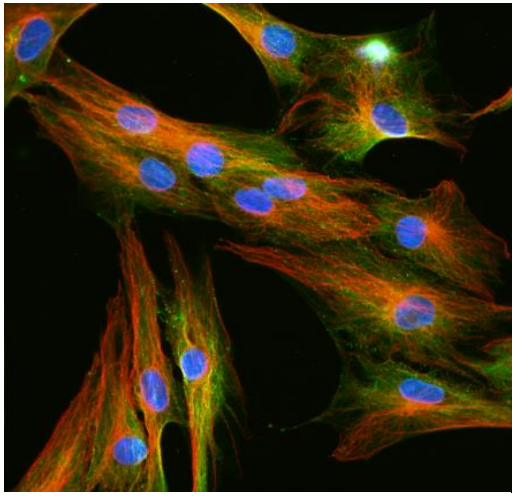
- Results

5.a



Immunofluorescent analysis of fibroblast cells (60x). Blue: nucleus, green: Grp94, red: TrxR3.

5.b



Immunofluorescent analysis of fibroblast cells (60x). Blue: nucleus, green: TrxR3, red: tubulin.

To further examine TrxR3, the component of the system having the most uncertain localization, we have performed colocalization experiments with immunofluorescence analysis.
(*5.a*) TrxR3 (red) didn't show colocalization with the ER-marker Grp94 (green), further indicating its absence from the lumen.
(*5.b*) For more precise determination of its localization, we also examined TrxR3 colocalization with tubulin cytoskeletal protein (red). The experiments indicated a cytosolic localization of the protein.

- Results

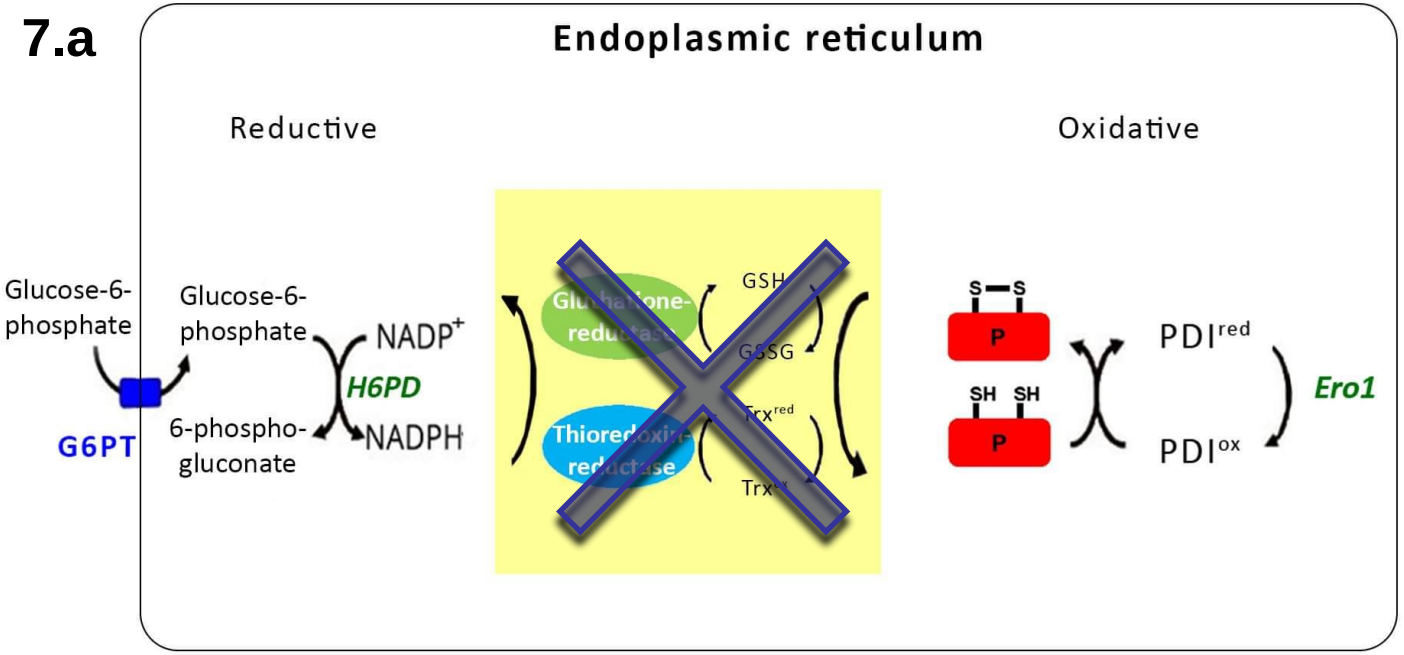
6.a

Localization	Target P	Predotar	PSORT II	yLoc	Euk-mPloc 2.0	Cello	Protein Prowler
Plasmamembrane	-	-	-	0%	0%	0,152	
Endoplasmic reticulum	0,047	0,01	4,30%	0%	0%	0,036	0,01
Extracellular	-	-	-	0%	0%	0,377	
Lysosome	-	-	-	0%	0%	0,033	
Golgi	-	-	-	0%	0%	0,031	
Peroxysome	-	-	4,30%	2,60%	0%	0,296	
Mitochondria	0,289	0,01	17,40%	0,80%	0%	1,147	0,03
Cytosol	0,748	-	43,50%	96,60%	100%	1,646	0,96
Nucleus	-	-	21,70%	0%	0%	0,502	

In silico analysis with several subcellular localization prediction programs were performed to further confirm the luminal absence of TrxR3.
The predictions showed uniformly the highest propability of cytoplasmic localization of the protein, while ER-localization of TrxR3 was unlikely in case of all prediction tools.

- Discussion, conclusion

7.a



According to our WB results, none of the components of the Trx/TrxR system is expressed in the ER lumen. In case of TrxR3, the luminal absence was further examined by ICC/IF and *in silico* analysis. TrxR also showed almost zero specific activity in the ER fraction.

In conclusion, our results suggests that the Trx/TrxR system is missing from the ER lumen. The absence of this electron transfer chain may explain the uncoupling of luminal redox systems, allowing the parallel presence of a reduced pyridine nucleotide pool and oxidized proteins.

- Acknowledgments

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