Redox Regulation of Dihydrofolate Reductase
Friend or Troublemaker?

Thomas Münzel, Andreas Daiber

Oxidative stress is a hallmark of cardiovascular diseases and a major contributor to vascular dysfunction. On the basis of recent concepts, vascular oxidative stress is caused mainly by infiltrating inflammatory cells such as monocytes/macrophages and leukocytes, producing so-called kindling radicals that lead to the activation of secondary, vascular enzymatic sources of reactive oxygen species (mainly superoxide). A prominent example is the uncoupled nitric oxide (NO) synthase, which means that an NO-producing antiatherosclerotic enzyme is getting switched to a superoxide-producing proatherosclerotic enzyme. Molecular mechanisms causing endothelial NO synthase (eNOS) uncoupling or dysfunction include phosphorylation at Thr495 and Tyr657, oxidative depletion of tetrahydrobiopterin (BH4), oxidative S-glutathionylation of cysteines in the reductase domain, S-glutathionylation of cysteines in the reductase domain, oxidative depletion of tetrahydrobiopterin (BH4), oxidative disruption of the zinc-sulfur dimer-binding site, and redox regulation of asymmetrical dimethylarginine formation and degradation. In theory, eNOS function is also regulated by S-nitrosylation in a negative feedback fashion. Another enzyme that contains several redox switches is the soluble guanylyl cyclase. It was reported that its activity is getting inhibited on oxidation by superoxide and peroxynitrite, by nitrosylation at different cysteine residues as well as oxidation of its heme moiety.

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Previous reports have shown that the enzyme responsible for de novo synthesis of the eNOS cofactor BH4, the GTP-cyclohydrolase-1, is redox-regulated at its expression level via increased proteasomal degradation under oxidative stress conditions. The 26S proteasome responsible for this degradation has been shown for getting activated by nitration of specific tyrosine residues in the setting of hyperglycemia and experimental hypertension, leaving only the salvage pathway for restoration of BH4 levels via reduction of tetrahydrobiopterin (BH2) by dihydrofolate reductase (DHFR). Taking into account that BH4 plays an essential role for eNOS function, its oxidative depletion or simultaneous inhibition of its de novo synthesis will ultimately lead to eNOS dysfunction/uncoupling, despite the futile counter-regulatory upregulation of DHFR expression and activity. Even more complicating the story is the observation that BH4 per se prevents oxidative inhibition of soluble guanylyl cyclase activity.

In their article in this ATVB issue, Cai et al report on a novel redox regulatory mechanism of DHFR. S-nitrosylation of DHFR by eNOS-derived NO prevents the degradation of the enzyme by the 26S proteasome. Knockdown of eNOS in primary endothelial cells and NO scavenging by PTO (2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide) decreased DHFR expression and this was prevented by MG132, a specific inhibitor of the 26S proteasome. Likewise, S-nitrosoglutathione, a known S-nitrosylating agent mimicked the protective effects by eNOS. The authors identified the S-nitrosylated cysteine residue by mutation experiments. DHFR stabilization is mediated by Cys7 nitrosylation. These results were confirmed in isolated aortic ring segments from wild-type and eNOS knockout mice. PTO promoted DHFR degradation in wild-type aorta, whereas MG132 prevented the proteasomal degradation.

Taken together, these results provide a new and important picture of redox regulation of the vascular tone by integrating the BH4 salvage pathway via DHFR in this process (for summary see Figure). The presented mechanism represents a negative feedback loop because low NO bioavailability under oxidative stress conditions (eg, either by direct scavenging by superoxide or by eNOS uncoupling) will lead to reduced DHFR expression, thereby causing further dysregulation of the eNOS activity. At a first view, this seems to represent a kind of suicide mechanism. Considering the fact, however, that BH4 also represents an essential cofactor for inducible NO synthase, this DHFR feedback loop may represent a rather important mechanism to control inducible NO synthase activity, especially under inflammatory conditions. It remains to be established in future whether control of DHFR function by S-nitrosylation represents a friend or foe.

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None.
Figure. Summary of redox regulatory pathways of vascular tone. Endothelial nitric oxide synthase (eNOS) and soluble guanylyl cyclase (sGC) are inactivated by several redox switches. Reactive oxygen and nitrogen species (ROS/RNS) also activate the 26S proteasome leading to degradation of the tetrahydrobiopterin (BH4) synthesis GTP-cyclodrolase (GCH-1) and the BH4→BH2 recycling enzyme dihydrofolate reductase (DHFR). BH4 is an essential cofactor of eNOS and also prevents oxidative inactivation of the sGC. NO is inactivated by superoxide and eNOS-derived NO, via S-nitrosylation, prevents proteasomal DHFR degradation upon tyrosine nitration of the 26S proteasome.

References


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