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Free Radicals in Biology and Medicine

Course Paper I

Chemistry of peroxynitrite in biological systems

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1. Abstract

Peroxynitrite ($ONOO^-$) is a reactive species, generated by reaction of superoxide ($\mathbf{O_2^{\bullet -}}$) with nitric oxide ($\mathbf{NO^{\bullet}}$). Peroxynitrite was shown to be involved in pathogenesis of many diseases. This paper is focused on chemistry of peroxynitrite formation and its interactions with different biomolecules. Reactions of peroxynitrite with sulfhydrils, transition metal centers, carbon dioxide, tyrosine residues represent major pathways accounting for biological effects of peroxynitrite. Kinetics of different reaction is summarized in a separate table. A separate chapters are dedicated to discussion of preparation of peroxynitrite as a reagent and peroxynitrite detection.

2. Introduction

Peroxynitrite ($ONOO^-$) is a reactive species, generated by reaction of superoxide ($\mathbf{O_2^{\bullet -}}$) with nitric oxide ($\mathbf{^{\bullet}NO}$). In addition to the generation of a pro-oxidant species, the formation of peroxynitrite results in decreased bioavailability of NO. Peroxynitrite was shown to be involved into pathogenesis of many diseases, including the following: acute and chronic inflammatory processes, atherosclerosis, rheumatoid arthritis, inflammatory bowel disease, adult respiratory distress syndrome, sepsis, ischemia-reperfusion, and neurodegenerative disorders.

3. Formation of peroxynitrite

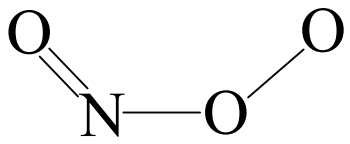
$$NO^{\bullet} + O_2^{\bullet -} \longrightarrow ONOO^- \qquad (1)$$

Production of peroxynitrite depends on $NO^{\bullet}$ and $O_2^{\bullet -}$ concentrations, which are regulated mainly by NOS and SOD

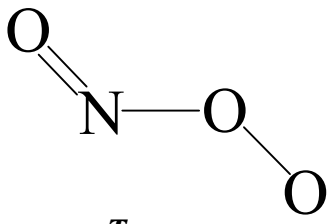
$$d[ONOO^-]/dt = k[^{\bullet}NO]*[O_2^{\bullet -}] \qquad K = 7*10^9\ M^{-1}\ s^{-1} \qquad (2)$$

4. Chemistry of Peroxynitrite

1. Peroxynitrite exists as two isomers, cis and trans:



Cis



Trans

(3)

2. Peroxynitrite anion exists in protonation equilibrium with peroxynitrous acid (ONOOH, pKa = 6.8)

$ONOO^- + H^+ \leftrightarrow ONOOH$ (4)

3. $ONOO^-$ is unstable and has a half-life of 1.9 sec at pH 7.4. It has two main mechanisms for decomposition:

$ONOOH \rightarrow NO_3^- + H^+$ 70% (5)

$ONOOH \rightarrow {}^{\bullet}HO + {}^{\bullet}NO_2$ 30% (6)

Production of $^{\bullet}HO$ is one of the major mechanism of peroxinitrite toxicity

4. Peroxynitrite reacts with carbon dioxide

$ONOO^- + \rightarrow ONO_2CO_2^-$ $K=4*10^4\ M^{-1}\ s^{-1}$ (7)

5. Nitrosoperoxycarbonate ($ONO_2CO_2^-$) falls apart to produce the following products:

$ONO_2CO_2^- \rightarrow CO_2 + NO_3^-$ 65% (8)

$ONO_2CO_2^- \rightarrow CO_3^{\bullet -} + {}^{\bullet}NO_2$ 35% (9)

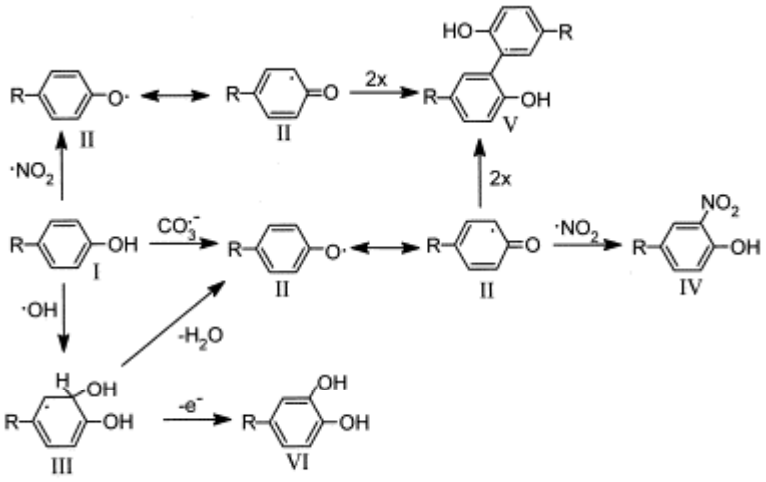
CO_2 was shown to increase peroxynitrate mediated one-electron oxidation and nitration of biomolecules.

6. Peroxynitrite can directly oxidize transition metals (Fe, Mn, Cu) in active centers of the enzymes.

$ONOO^- + Me^n \rightarrow Me^{n+1}=O + {}^{\bullet}NO_2$ (10)

7. Tyrosine oxidation and nitration by peroxinitrite.

Nitration of the tyrosine residues by peroxynitrite is one of the most important mechanisms of biological effects of peroxyitrite.

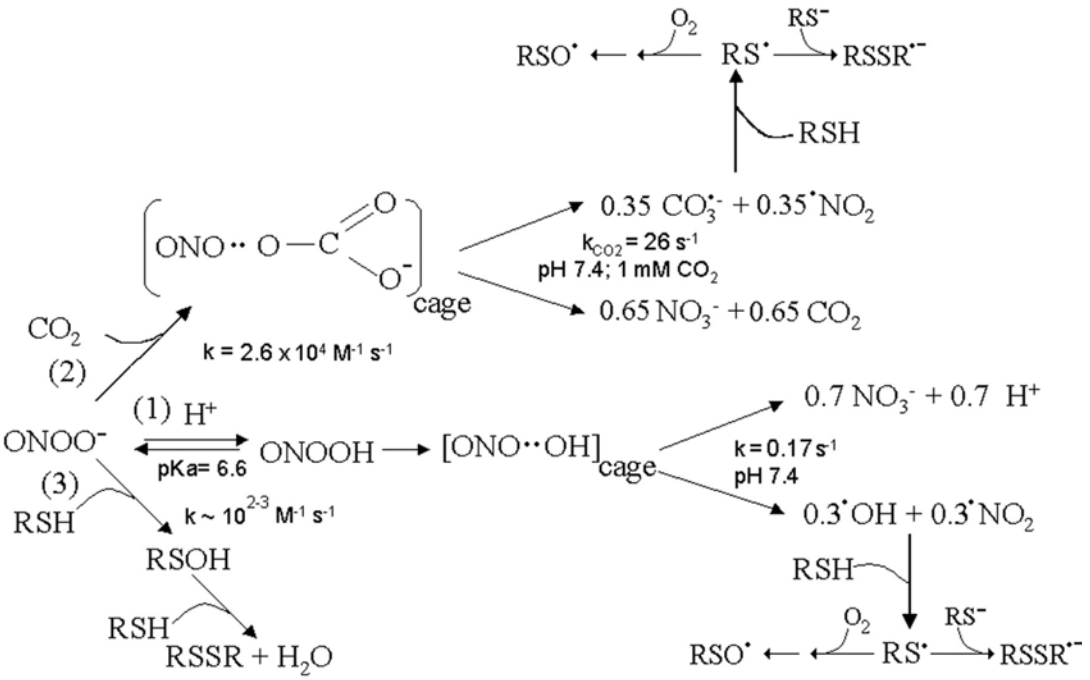


(11)

Fig. 1. I – tyrosine; II – tyrosyl radical; III – tyrosine-hydroxyl radical adduct; IV – 3-nitrosotyrosine; V – 3-3'-dityrosine; VI – 3-hydroxytyrosine

8. Peroxynitrite is involved in oxidation of thiols in vivo and in vitro. One of the most important targets is glutathione. J. Biol. Chem., Vol. 276, Issue 13, 9749-9754, March 30, 2001

CO_2 was shown to stimulate this reaction.



(12)

5. Kinetics of peroxynitrite reactions

Rate constants of Peroxynitrite reactions wit biomolecules and some other relevant compounds at physiological pH

	Reaction	K_s (M^{-1} s^{-1})		Reaction	K_s (M^{-1} s^{-1})
1	Fe(III)TMPyP	$2.2*10^6$	10	Cu-Zn SOD	10^3-10^5
2	Mn(II)TMPyP	$1.8*10^6$	11	CO_2	$4*10^4$
3	Ebselen	$1.6*10^6$	12	Bovine serum albumine	$6*10^3$
4	myeloperoxidase	$>10^6$	13	Cysteine	$5*10^3$
5	Horseradish peroxidase	$7*10^5$	14	Glutathione	$1.35*10^3$
6	Alcohol dehydrogenase	$3*10^5$	15	Methionine	$1.8*10^2$
7	Aconitase	$1.4*10^5$	16	Tryptophan	$1*10^2$
8	Cytochrome C	$1.3*10^4$	17	Ascorbate	$1*10^2$
9	Oxyhemoglobin	$1*10^4$	18		

6. Making peroxynitrite as a reagent.

A commonly used method of synthesis of peroxynitrite is to use reaction between nitrous acid and hydrogen peroxide:

$$HONO + H_2O_2 \rightarrow HOONO + H_2O \qquad (13)$$

NaOH should be added immediately to this reaction, because HOONO is very unstable.

Another approach is simultaneous generation of NO and O_2^- (peroxynitrite generating systems).

7. Detection of peroxynitrite in biological systems

The detection of peroxynitrite in biological systems has been a challenge over the past decade because of the (i) elusive nature of peroxynitrite which precludes its direct isolation and detection, (ii) necessity to find detector molecules that can efficiently outcompete the multiple reactions that peroxynitrite can undergo, (iii) nonexistence of footprints totally specific of peroxynitrite reactions, and (iv) the difficulty to discriminate between the biological effects of peroxynitrite versus that of its precursors, NO and O_2^-, and other NO-derived oxidants.

A. Probe oxidation/nitration

1. Oxidation of fluorescent probes

Dichlorofluorescin (DCFH) and dihydrorhodamine (DHR) are the most frequently used probes.

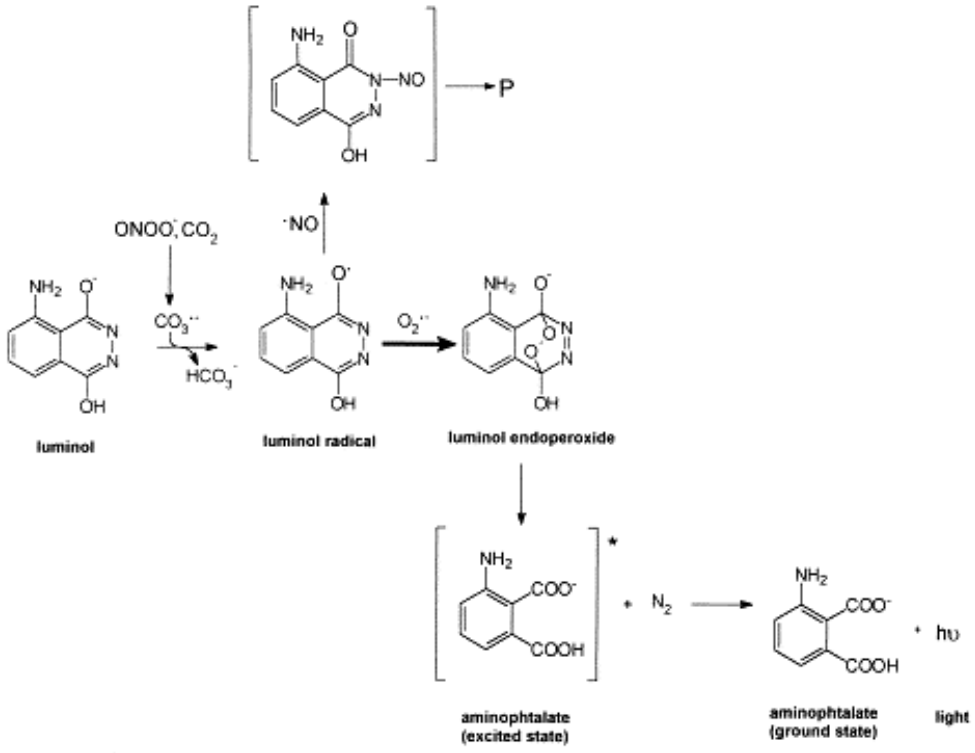
(14)

DCFH is oxidized by peroxynitrite to highly fluorescent DCF (14). DHR is oxidized by peroxynitrite to rhodamine (not shown)

2. Chemiluminescence probes

Luminol is one of the most commonly used chemiluminescence probes.

Mechanism of luminol chemiluminescence:



(15)

3. Nitration of phenolic compounds

Nitration of tyrosine or p-hydroxyphenylacetic acid (p-HPA) is assessed spectrophotometrically.

B. Footprinting

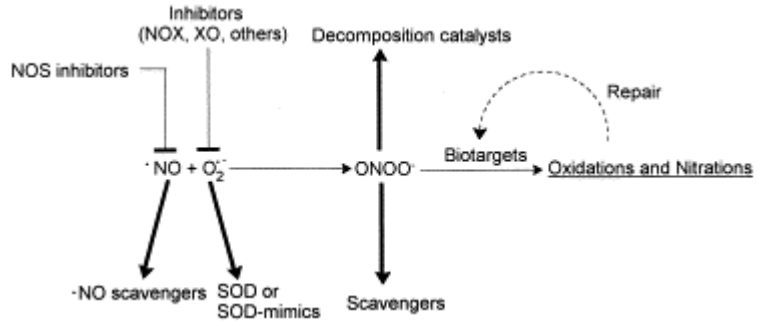
Another way to estimate peroxynitrite formation is to detect oxidative modifications that peroxinitrite promotes in biological molecules.

Such modifications have to be relatively stable and specific for peroxynitrite.

So far two main strategies are used: immunochemical detection of nitrated proteins and quantitation of 3-nitrotyrosine after protein hydrolysis.

C. Peroxynitrite pharmacology

Different pharmacological manipulations help us to determine whether signals that we get using other techniques are specific to peroxynitrite.



(16)

This figure shows potential sites of pharmacological intervention to decrease peroxynitrite production. Presumably such intervention should cause decrease of peroxynitrite-specific signal obtained by the other techniques.

D. Other methods

Several other methods are used:

- EPR-spin trapping of peroxynitrite-derived oxidants
- Aromatic hydroxylation
- Oxidation or formation of chromophores
- Cytochrome C^{2+} oxidation

4. Conlusions

Nitric oxide-superoxide interactions were shown to occur in vivo and lead to the formation of peroxinitrite. Reactions of peroxynitrite with sulfhydrils, transition metal centers, carbon dioxide, tyrosine residues are the most important. Peroxinitrite is short-lived, therefore its detection relies on modification of exogenous detector (probe oxidation) or endogenous target (footprinting).

5. References

1. Pryor WA, Squadrito GL The chemistry of peroxynitrite: a product from the reaction of nitric oxide with superoxide. Am J Physiol 1995 May;268(5 Pt 1):L699-722

2. Beckman JS, Beckman TW, Chen J, Marshall PA and Freeman BA (1990) Apparent hydroxyl radical production by peroxynitrite: Implications for endothelial injury from nitric oxide and superoxide. *Proc Natl Acad Sci USA* 87: 1620-1624

3. Radi, R., Beckman, J. S., Bush, K. M., and Freeman, B. A. (1991) Peroxynitrite oxidation of sulfhydryls: the cytotoxic potential of superoxide and nitric oxide. *J. Biol. Chem.* 266, 4244-4250.

4. Salgo, M. G., Bermúdez, E., Squadrito, G. L., and Pryor, W. A. (1995) Peroxynitrite causes DNA damage and oxidation of thiols in rat thymocytes. *Arch. Biochem. Biophys.* 322, 500-505.

5. Radi, R., Beckman, J. S., Bush, K. M., and Freeman, B. A. (1991) Peroxynitrite-induced membrane lipid peroxidation: the cytotoxic potential of superoxide and nitric oxide. *Arch. Biochem. Biophys.* 288, 481-487

6. Van Der Vliet, A., O'Neill, C. A., Halliwell, B., Cross, C. E., and Kaur, H. (1994) Aromatic hydroxylation and nitration of phenylalanine and tyrosine by peroxynitrite. evidence for hydroxyl radical production from peroxynitrite. *FEBS Lett.* 339, 89-92

7. Lymar, S. V., and Hurst, J. K. (1995) Rapid reaction between peroxonitrite ion and carbon dioxide: implications for biological activity. *J. Am. Chem. Soc.* 117, 8867-8868

8. Denicola, A., Freeman, B. A., Trujillo, M., and Radi, R. (1996) Peroxynitrite reaction with carbon dioxide/bicarbonate: kinetics and influence on peroxynitrite mediated oxidations. *Arch. Biochem. Biophys.* 333, 49-58

9. Squadrito, G. L., and Pryor, W. A. (1998) Oxidative chemistry of nitric oxide: The roles of superoxide, peroxynitrite, and carbon dioxide. *Free Radical Biol. Med.* 25, 392-403.

10. Squadrito, G. L., and Pryor, W. A. (1998) The nature of reactive species in systems that produce peroxynitrite. *Chem. Res. Toxicol.* 11, 718-719.

11. Pryor, W. A., Cueto, R., Jin, X., Koppenol, W. H., Ngu-Schwemlein, M., Squadrito, G. L., Uppu, P. L., and Uppu, R. M. (1995) A practical method for preparing peroxynitrite solutions of low ionic strength and free of hydrogen peroxide. *Free Radical Biol. Med.* 18, 75-83.

12. Jin, X. (1994) Nitric Oxide Toxicity Mediated by Peroxynitrite. Ph.D. Dissertation, Louisiana State University, Baton Rouge, LA.

13. Zhang, H., Squadrito, G. L., and Pryor, W. A. (1997) The mechanism of the peroxynitrite-carbon dioxide reaction probed using tyrosine. *Nitric Oxide* 1, 301-307.

14. Pryor, W. A., Lemercier, J.-N., Zhang, H., Uppu, R. M., and Squadrito, G. L. (1997) The catalytic role of carbon dioxide in the decomposition of peroxynitrite. *Free Radical Biol. Med.* 23, 331-338.

15. Goldstein, S., Squadrito, G. L., Pryor, W. A., and Czapski, G. (1996) Direct and indirect oxidations by peroxynitrite, neither involving the hydroxyl radical. *Free Radical Biol. Med.* 21, 965-974.

16. Squadrito Giuseppe L., Pryor William A. 1998. The Nature of reactive species in Systems that produce peroxynitrine. Chem Res. Toxicol. 1998. 11, 718 – 719

17. Lymar Sergei V., Hust James K. 1998. Radical nature of peroxynitrite reactivity. Chem Res. Toxicol. 1998, 11 714 – 715

18. Bartberger Michael D., Leif Olson P., Houk K. N. 1998. Mechanisms of peroxynitrite oxidations and rearrangements: the theoretical perspective. Chem. Res. Toxicol. 1998, 11 710 – 711

19. Radi, Raphael. 1998. Peroxynitrite reactions and diffusion in Biology. Chem. Res. Toxicol. 1998, 11 720 – 721

20. Koppenol Willem H., Kissner Reinard. Can O=NOOH undergo homolysis? Chem. Res. Toxicol. 1998, 11 87 – 90

21. Alvarez Beatriz, Rubbo Homero, Kirk Marion, Barnes Stephen, Freeman Bruce A, Radi Raphael. Peroxynitrite-dependent tyrptophan nitration. Chem. Res. Toxicol. 1996, 9 390 – 396

22. Zhang Houwen, Squadrito Giuseppe L., Uppu Roa, Pryor William A. Reaction of Peroxynitirte with Melatonin: a mechanistic study. Chem. Res. Toxicol., 1999, 12, 526 – 534

23. Lalu Manoj M., Wenjie Wang, Schulz Richard. Peroxynitrite in Myocardial Ischemia-Representation injury. Heart Failure reviews, 7, 359 – 369, 2002

24. Butler Anthony R., Williams Lyn. The physical role of Nitric oxide. ????????

25. Koppenil Willem H. Peroxynitrite uncloaked? Chem Res. Toxicol. 1998, 11, 716 – 717

26. Balazy Michael, Kaminski Pawel M., Mao Kaiun, Tan Jianzen, Wolin Michael S. S-nitroglutathione, a product of the reaction between Peroxynitrite and Glutathione that generates nitric oxide. The journal of biological chemistry. Col. 273, N 48 Nov.27, pp 32009 – 32015, 1998

27. Altug Sedat, Demiryurek Tuncay A., Kane Kathleen A., Kanzik Ilker. Evidence for the involvement of Peroxynitrite in ischaemic preconditioning in rat isolated hearts. British journal of pharmacology. (2000) 130, 125 – 131

28. Koppenol W. H. The chemistry of Peroxynitrite, a biological toxin. ???????

29. Beckman joseph S., Ischiropulos Harry, Zhu Ling, von der Woerd, Mark, Smith Graig, Chen Jun, Harrison Joseph, Martin James C., Tsai Michael. Kinetics of superoxide dismutase- and iron-catalized nitration of Phenolics by Peroxynitrite. Archives of biochemistry and biophisics. Vol. 298, N2, Nov 1, pp. 438 – 445, 1992

30. Landino, Lisa M., Crews Brenda C., Timmons, Michael D., Morrow Jason D., Marnett Lawrence J. Peroxynitrite, the coupling product of nitric oxide and superoxide, activates prostaglandin biosynthesis. Proc Natl Acad Sci USA vol 93, pp 15069 – 15-74, Dec 1996

31. Radi Rafael, Cosgrove Thomas P., Beckman Joseph S., Freeman Bruce A. Peroxynitrite-induced luminoi chemiluminiscence. Biochem. J. (1993) 290, 51 – 57.

32. Szabo Csaba. The pathophisiological role of Peroxynitrite in shock, inflammation, and ischemia-reperfusion injury. Shock,Vol.6, No.2, pp. 79 – 88, 1996

33. Radi Raphael, Beckman Joseph S., Bush Kenneth M., Freeman Bruce A. Peroxynitrite oxidation of sulfhydryls. The journal of biological chemistry. Vol. 266, no. 7, Mar 5, pp. 4244 – 4250, 1991

34. Goldstein Sara, Czapski Gidon, Lind Johan, Merenyi gabor. Effect of *NO on the decomposition of peroxynitrite: reaction of N_2O_3 with $ONOO^-$. Chem Res. Toxicol. 1999, 12, 132 – 136

35. Coffey Michael J., Phare Susan M., Peters-Golden Mark. Interaction between nitric oxide,reactive oxygen intermediates, and peroxynitrite in the regulation of 5-lipoxygenase metabolism. Biochimica et Biophysica acta 1584 (2002) 81 – 90

36. Koppenol W. H. The basic chemistry of nitrogen monoxide and peroxynitrite. Free radical biology and medicine. Vol. 25, Nos. 4/5, pp 385 – 391, 1998