Denitrosilation of S-nitrosothiols is catalyzed by methemoglobin in the presence of organic peroxides

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Abstract

S-nitrosoproteins and low-molecular-weight S-nitrosothiols are the main transport and storage forms of nitric oxide in human blood. S-nitrosoglutathione is the most abundant low-molecular-weight S-nitrosothiol in blood cells. Hemoglobin (Fig.1) in erythrocytes and serum albumin in blood plasma constitute the major part of nitrosylated proteins with average level of several micromoles. Presence of peroxides may be an important factor which influences nitric oxide metabolism, in particular the ratio of free and storage forms of NO may be altered. We showed that in the presence of organic peroxides trace amounts of methemoglobin (metHb) catalyzed denitrosylation of S-nitrosoglutathione and S-nitrosyl groups of cysteine residues in proteins.

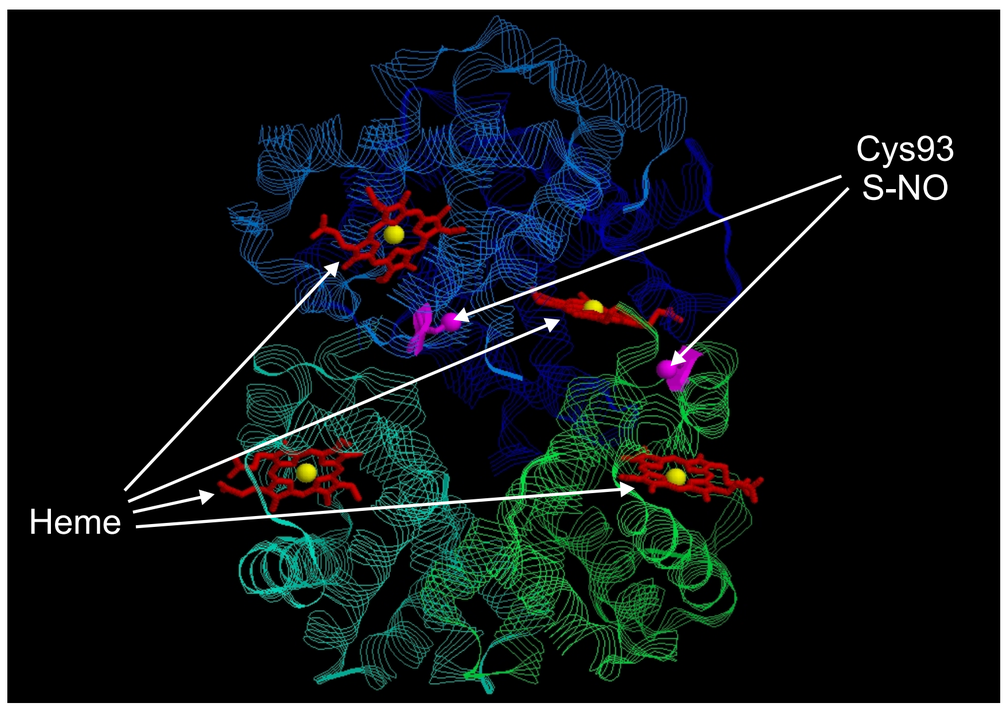

Fig. 1. Spatial structure of methemoglobin (1JY7.pdb). Heme cycles and nitrosylated Cys-93 are indicated.

Introduction

It is known that highly oxidized forms of hemoglobin (HbOFs) and other hemoproteins play important role in destructive processes during oxidative stress. Methemoglobin interaction with H_2O_2 results in HbOFs production [1]: less stable oxoferryl form with radical localized on the protein globule (Compound 1) and the oxoferryl cation of hemoglobin (Compound 2).

$$\mathbf{Hb(III) + H_2O_2 \longrightarrow {}^{\bullet}Hb(Fe\ IV{=}O)} \quad \mathbf{(1)}$$

$$\mathbf{\searrow\ Hb(Fe\ IV{=}O)} \quad \mathbf{(2)}$$

Organic peroxides are also involved in HbOFs production. Tert-Butylhydroperoxide (TBHP) was used as analogue of peroxides formed during lipid peroxidation processes in cell membranes. In contrast to H_2O_2, metHb interaction with TBHP gives rise mainly to formation of Compound 2 and organic free radicals [2]. In this work it was shown that these free radicals are involved in lowering of deposited NO level, i.e. denitrosilation of S-nitrosothiols.

Materials and Methods

Oxyhemoglobin (oxyHb) was prepared as described earlier [3]. Nitrosilation of hemoglobin SH-groups was carried out by prolonged incubation of oxyHb solution in the presence 20-fold excess of nitrite at pH 3.5. After several minutes oxyHb was oxidized to metHb and the solution was incubated with nitrite during 3 hours. Then pH was raised to neutral values and nitrite was removed by Sephadex G50 gel-filtration in phosphate buffer. Concentration of S-NO-groups was determined by the method of Saville [4].

HbOFs were obtained by addition to metHb of 100-1000 multiple excess of hydrogen peroxide or organic peroxide. Lipid peroxidation products of erythrocyte membranes were obtained by erythrocytes “ghosts” treatment with ultrasound (880 kHz, I= 2W/cm^2).

Thiochrome was identified by paper chromatography and its concentration was determined using fluorescent method [5].

Results

Interaction of organic peroxides (such as TBHP or lipid peroxidation products of erythrocyte membranes) with S-nitrosometHb resulted in cleavage of S-NO bond (Fig.2) of Cys-93 residue located near the heme cavity (Fig.1). We suggest that denitrosilation takes place due to reaction of alkoxyl and alkylperoxide radicals with the S-nitrosyl group. It is supported by the fact that upon reaction of TBHP with metHb the produced radicals may be intercepted by radical scavengers (thiochrome was used as a radical scavenger).

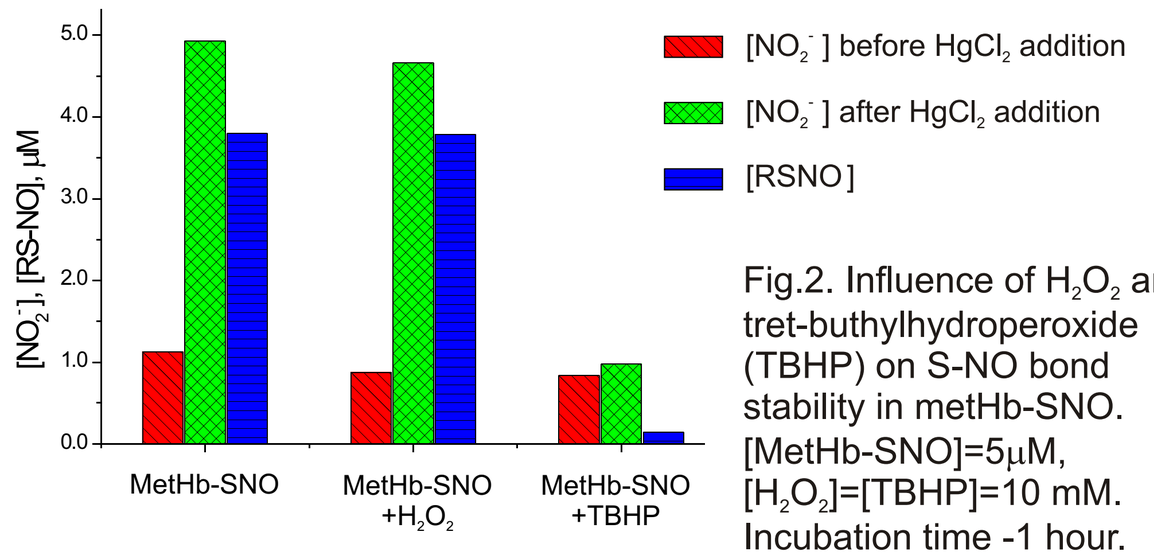

Fig.2. Influence of H_2O_2 and tret-buthylhydroperoxide (TBHP) on S-NO bond stability in metHb-SNO. [MetHb-SNO]=5μM, [H_2O_2]=[TBHP]=10 mM. Incubation time -1 hour.

Alkoxyl and peroxyl radicals are formed in reaction between metHb and organic peroxides (Fig.3).

$$Hb(III) + ROOH \longrightarrow Hb(IV{=}O) + RO^{\bullet} + H^{+}$$

$$RO^{\bullet} + ROOH \longrightarrow ROO^{\bullet} + ROH$$

Produced peroxyl radicals react with S-nitrosyl groups to form nitrate NO_3^-

$$ROO^{\bullet} \rightarrow ROONO \rightarrow NO_2 \rightarrow NO_3^-$$

$$R_1SNO \quad R_1S^{\bullet} \quad RO^{\bullet} \quad Hb(IV{=}O) \quad Hb(III)$$

Noteworthy, in contrast to organic peroxides interaction of hydrogen peroxide with S-nitrosometHb was not accompanied by denitrosilation of Cys-93 residue. It is known that reaction between metHb and hydrogen peroxide includes two-stage oxidation (two electrons are needed to reduce hydrogen peroxide) and produces the hemoglobin oxoferryl form with a radical located on the protein globule and a molecule of water. In this case, as opposed to tret-butyl-peroxide, the radical products do not leave the heme cavity.

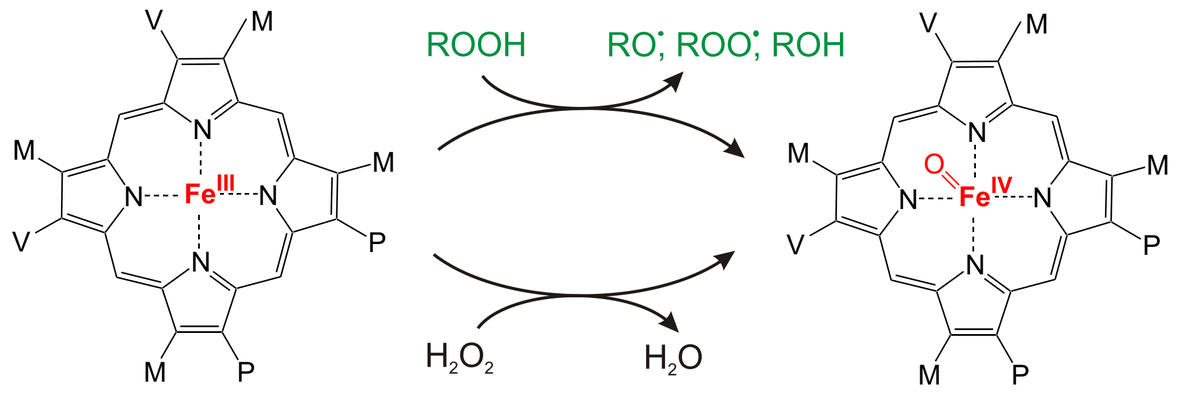

Fig. 3. Scheme of heme Fe ion oxidation under action of H_2O_2 or organic peroxides.

The results obtained testify that organic peroxides are among the main reasons of selective elimination of S-nitrosothiols, especially ferro- and ferri- forms of S-nitrosohemoglobin.

References

[1] Svistunenko, D.A., e.a. (1997) *J. Biol. Chem.*, **272,** 7117.; [2] Van der Zee, J., e.a. (1996) *Free Rad.Biol.Med.*, **20**, 199; [3] Benesch, R., e.a. (1972) *Biochemistry*, **11**, 3576.; [4] Saville, B. (1958) *Analyst,* **83**, 670.; [5] Stepuro, I.I. (2005) *PLEFA*, **72**, 115.

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