2. Experimental

2.1. Chemical and reagents

Fe^{III}-TAML activator (Fe^{III}-TAML) was obtained from GreenOx Catalysts, Inc. (USA). The concentration of Fe^{III}-TAML solutions was estimated by measuring absorbance using ε_{366} = 6600 $M^{-1}cm^{-1}$.

Luminol, hemin, and Tris were purchased from Aldrich (USA). Horseradish peroxidase (HRP, type VI-A, RZ 3.0) and tert-butyl hydroperoxide – from Sigma. Benzoyl peroxide (BP) was a gift of Dr N.Yudin. Hydrogen peroxide (30%), isopropanol, acetonitrile and ethanol were from ChemMed (Russia). All organic solvents were distilled prior to use. The concentration of H_2O_2 was estimated by measuring absorbance using ε_{240} = 43.6.

2.2. *Chemiluminescent determination of TAML activity in luminol oxidation with H_2O_2 in aqueous-organic solutions*

Determination of the catalytic activity of Fe^{III}-TAML was carried out as follows: 30 µl of Fe^{III}-TAML solution were mixed with 270 µl of 22.2 mM carbonate buffer (pH 9.9 or 10.5) containing 55.5 mM Tris, 30 µM luminol, 0.11 mM H_2O_2 and organic solvent in wells of black polystyrene plates for CL enzyme immunoassay (MaxiSorp, NUNC, Denmark). The final concentration of Fe^{III}-TAML in plate wells was 10^{-8} M. CL intensity was measured at room temperature on a luminometer SpectraMax L (USA). The light intensity was expressed in relative luminescence units (RLU).

2.3. *pH-dependence of Fe^{III}-TAML-catalyzed oxidation of luminol with H_2O_2 in aqueous-organic solutions*

pH-dependence of Fe^{III}-TAML-catalyzed oxidation of luminol was assayed as follows: 275 µl of 21.8 mM carbonate buffer, pH 9.0–10.5 containing 55.5 mM Tris, 0.11 mM hydrogen peroxide, 30 µM luminol and organic solvent were mixed with 25 µl of Fe^{III}-TAML solution (1.2 x 10^{-7} M) in wells of black polystyrene plates. CL kinetics was measured at room temperature on

a luminometer SpectraMax L (USA). The chemiluminescent signal in the absence of Fe^{III}-TAML was taken as background value.

2.3. *Determination of kinetic constants of Fe^{III}-TAML-catalyzed oxidation of luminol in 20% acetonitrile*

All kinetic measurements were performed using a Shimadzu UV-2401PC spectrophotometer equipped with a thermostatted cell holder at 25^0 C. Stock solutions of Fe^{III}-TAML (1.5 mM) and luminol (100 mM) were prepared in acetonitrile and 0.4 M NaOH, respectively. The catalytic luminol oxidation reactions were carried out in 20 mM carbonate, pH 10.5 with 50 mM Tris. Initial rates of luminol oxidation were calculated from the linear absorbance versus time plots using the differential coefficient of extinction (349 nm) for luminol of 5900 M^{-1} cm^{-1}, when the conversion of the luminol did not exceed 10%. A typical kinetic run was performed as follows: appropriate amounts of the stock solutions of Fe^{III}-TAML and luminol were added in a cuvette with the carbonate buffer. To initiate the oxidation a required amount of the stock solution of H_2O_2 was added to a 1 ml of reaction mixture. Calculations of the rate constants were carried out using a SigmaPlot 8.0 using the following equation.

$$\frac{d[\text{luminol}]}{d\text{t}} = \frac{k_I k_{II}[\text{Fe(III)-TAML}][\text{peroxide}][\text{luminol}]}{k_I[\text{peroxide}] + k_{II}[\text{luminol}]}$$

2.4. Chemiluminescent determination of organic peroxides

The determination of organic peroxides was carried out as follows: 30 µl of Fe^{III}-TAML solution were mixed with 270 µl of 22.2 mM carbonate buffer, pH 10.5 containing 55.5 mM Tris, 30 µM luminol, 0-55.6 µM BP or 0-556 µM TBH and 11% or 22.2% of organic solvent (ethanol, isopraponol or acetonitrile) in wells of black polystyrene plates for CL enzyme immunoassay. The final concentration of Fe^{III}-TAML in plate wells was 10^{-8} M. Chemiluminescence intensity was measured at room temperature on a luminometer SpectraMax L (USA).