EFFECT OF SUPEROXIDE DISMUTASE ON THE ANTIOXIDANT ACTIVITY OF NITROXYL RADICALS DURING THE OXIDATION OF METHYL LINOLEATE IN MICELLES

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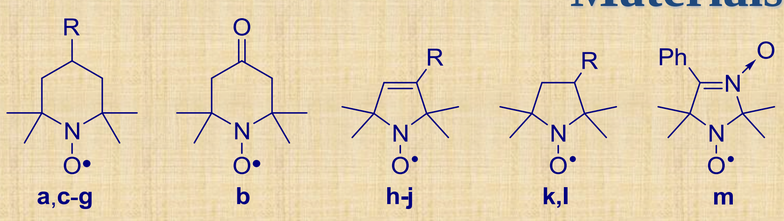
Objective:

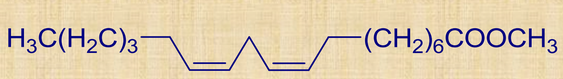
- Experimental research of the kinetics of methyl linoleate oxidation inhibited by nitroxyl radicals in micelles;
- Determination of the mechanism of this process and analysis of the role of hydroperoxyl radical.

Materials



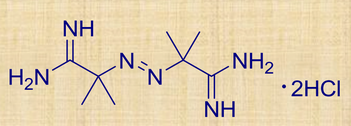
R = H (**a**), OH (**c**), OMe (**d**), OCOPh (**e**), Cl (**f**), N^+Me_3 $MeOSO_3^-$ (**g**), COOH (**h**), COOEt (**i**), $CONH_2$ (**j**, **k**), CH_2OH (**l**)

Nitroxyl radicals (>NO•)

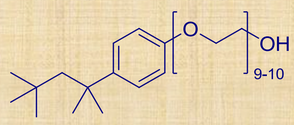


Methyl linoleate (LH), oxidation substrate

Superoxide dismutase (SOD):
lyophilized powder, 3,500 units/mg protein



AAPH, initiator



Triton X-100, detergent

Methods

- **Monitoring of oxygen consumption** (YSI 5300A Biological Oxygen Monitor)
- **ESR spectroscopy** (CMS 8400 ESR Spectrometer, Adani, Belarus)
- **Calculation of octanol-water log P values** (CLogP method)

Experimental conditions

LH (0.02 M) in Triton X-100 micelles (0.05 M),
air saturation, pH 7.4, 37 °C,
[AAPH] = 0.004 M, rate of initiation $W_i = 3.8 \cdot 10^{-9}$ M·s^{-1}
[SOD] = 0 or 100 U/mL

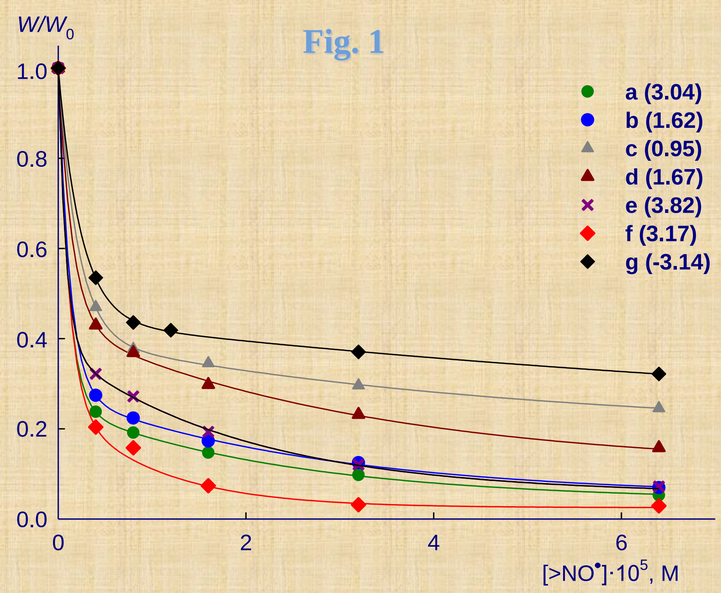
Calculation of inhibition coefficients (*f*)

$$f = \tau_{ind} W_i / [>NO^\bullet] \qquad \tau_{ind} = \int_0^\infty \left(1 - \left(\frac{W}{W_0}\right)^2\right) dt$$

Effect of >NO• a-g on the rate of oxidation of LH

(log *P* values for >NO• are given in parentheses)

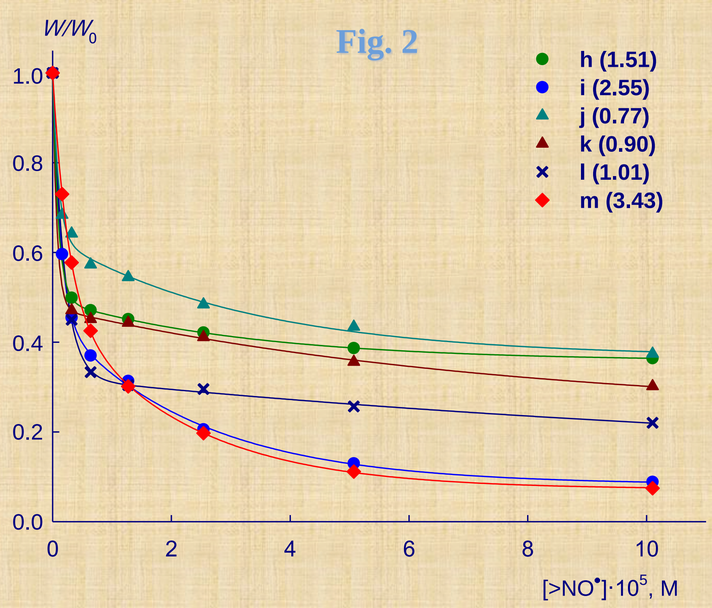
Fig. 1



Effect of >NO• h-m on the rate of oxidation of LH

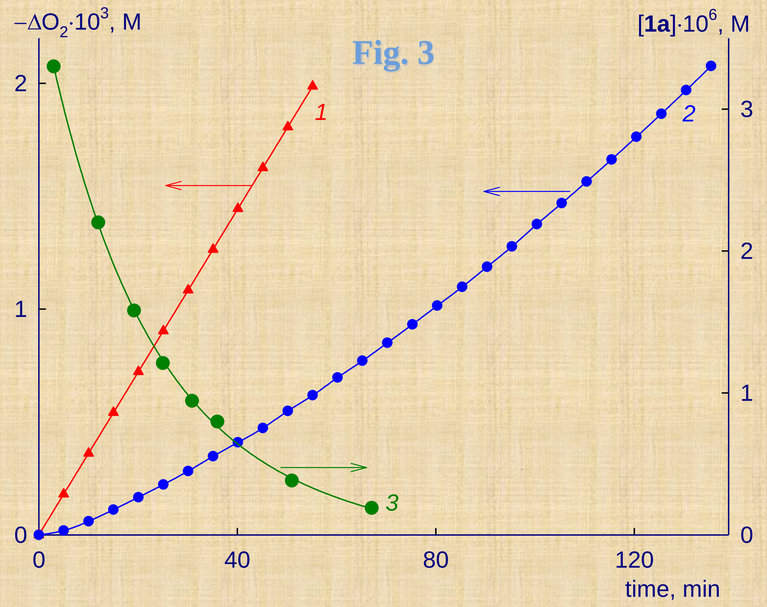
(log *P* values for >NO• are given in parentheses)

Fig. 2



Kinetics of O_2 and >NO• consumption during the oxidation of LH

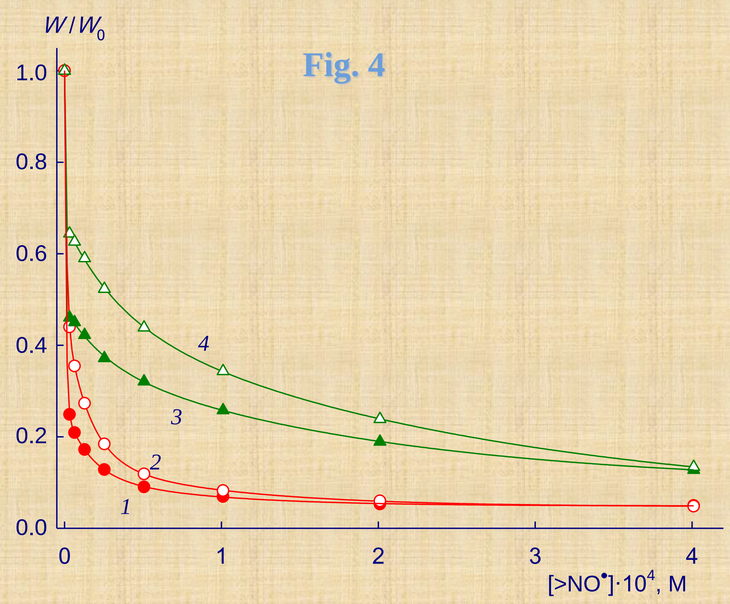
Fig. 3



1 – uninhibited oxidation; 2, 3 – $[\mathbf{a}]_0 = 4.0 \cdot 10^{-6}$ M

Effect of SOD on the rate of inhibited oxidation of LH

Fig. 4



1, 2 – >NO• **a**; 3, 4 – >NO• **c**
1, 3 – without SOD; 2, 4 – in the presence of 100 U/ml SOD

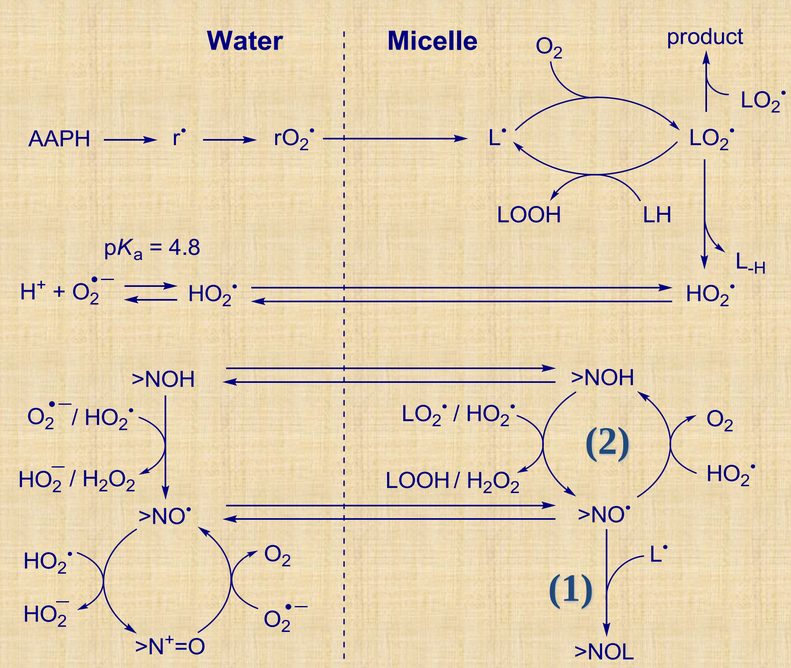
Basic regularities

1. Antioxidant activity of >NO• depends on log *P* values. More lipophylic antioxidants inhibit oxidation in micelles more effectively

2. One >NO• radical breaks 2-3 kinetics chains, i.e. >NO• is regenerating during the oxidation. Probable reason is $HO_2^\bullet$ formation during the oxidation of LH in micelles
(V. Roginsky, T. Barsukova, Chem. Phys. Lipids, 2001, **111**, 87).

3. In the presence of SOD >NO• reduces the rate of oxidation in the lower extent. The process is characterized by lower values of inhibition coefficients. These results demonstrate the important role of hydroperoxide radical in the mechanism of the process

Proposed mechanism of >NO• regeneration



Inhibition coefficients for >NO•

>NO•	*f* (without SOD)	*f* (in the presence of SOD)
a, **c-e**	**2.5 – 3.0**	**1.0 – 1.2**
i, **m**	**2.0 – 2.5**	**0.9 – 1.1**

Average Triton X-100 aggregation number: **130**

Average concentration of micelles: [Triton X-100]/130 = 0.05/130 = $\mathbf{3.9 \cdot 10^{-4}}$ **M**

Effect of SOD on inhibited LH oxidation at different [>NO•]

Low [>NO•] values (< 10^{-4} M)
Less than one >NO• radical per micelle. The main channel of chain termination is path (2). Addition of SOD eliminates path (2), therefore >NO• reduces the rate of oxidation in the lower extent

High [>NO•] values (~ $4 \cdot 10^{-4}$ M)
One or more >NO• radical per micelle. The main channel of chain termination is path (1). Addition of SOD doesn't effect on the rate of oxidation. End sections of the curves *1* and *2* in the figure 4 coincides with each other

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