

EPR STUDY OF REACTION PRODUCTS OF OXIDES OF NITROGEN WITH SOME ORGANIC COMPOUNDS

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The possibility of formation of paramagnetic centers in reactions between oxides of nitrogen and saturated and unsaturated fatty acids and aromatic amino acids was studied by an EPR method. During the reaction between NO_2 and oleic acid at 20°C several paramagnetic centers were found to be formed. Saturated fatty acids do not form paramagnetic centers on reacting with NO_2 . NO did not form paramagnetic centers, whether with butyric, palmitic, and stearic acids, or with oleic acid. NO_2 was shown to form paramagnetic centers with a saturated solution of tyrosine. The formation of paramagnetic centers was not observed as a result of the reaction of NO and NO_2 with saturated solutions of phenylalanine and tryptophan. It is postulated that unsaturated fatty acids and tyrosine residues of membrane lipoprotein complexes may act as targets for reaction with NO_2 , and that the formation of paramagnetic centers in the reaction between NO_2 and the above-mentioned substances is the primary act of injury to the membranes.

KEY WORDS: oxides of nitrogen; saturated and unsaturated fatty acids; aromatic amino acids; paramagnetic centers.

The free-radical nature of oxides of nitrogen enables them to react with various substances, including fatty acids and phenols of animal origin present as components of lipoprotein structures of membranes. This has been demonstrated by investigation [1, 2] which showed that an important role in the mechanism of the harmful action of oxides of nitrogen is played by free-radical oxidation of lipids. Consequently, oxides of nitrogen, on reacting with saturated and unsaturated fatty acids and also with aromatic amino acids, may give rise to the formation of paramagnetic centers.

In the present investigation the electron paramagnetic resonance (EPR) method was used to study reaction products of nitric oxide and nitrogen peroxide with fatty acids (oleic, butyric, palmitic, and stearic) and with aromatic amino acids (tyrosine, tryptophan, phenylalanine — obtained from the Koch-Light firm).

EXPERIMENTAL METHOD

EPR spectra were measured on a 3-cm radiospectrometer of reflecting type under conditions preventing signal saturation. Solutions of aromatic amino acids were freed from low-molecular-weight ions by passage through a 1×8 cm Dowex column. Purity checks were carried out on the Perkin-Elmer M-303 atomic absorption spectrophotometer (sensitivity 0.2 mg/liter). After NO and NO_2 had been passed at 20°C through the test substance, the latter was introduced into quartz capillary tubes with a bore of 3 mm, and transferred to a Dewar flask with liquid nitrogen, mounted in the resonator of the EPR spectrometer.

EXPERIMENTAL RESULTS

After the reaction between NO_2 and oleic acid several paramagnetic products were found. During the first minutes of the reaction between NO_2 and oleic acid the EPR spectrum consisted of a wide unresolved signal with a G-factor of 2003 and a wide line between the points of maximal slope $\Delta H = 8.4$ Oe (Fig. 1a). The

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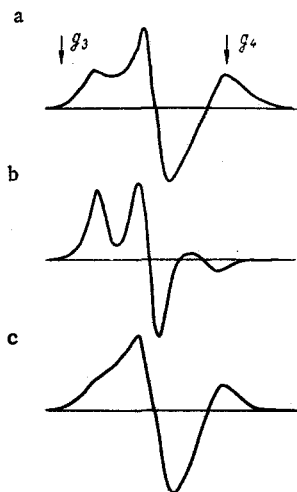


Fig. 1

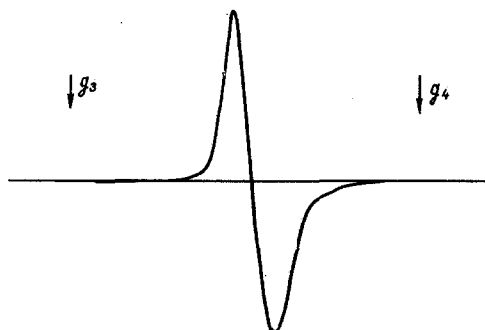


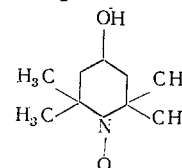
Fig. 2

Fig. 1. EPR spectra of oleic acid after reacting with NO_2 at 20°C . Duration of reaction: a) 1-2 min, b) 30 min, c) 24 h. Spectra measured at 77°K . Arrows indicate centers of third and fourth lines of Mn^{++} standard.

Fig. 2. EPR spectrum of a saturated solution of tyrosine following reaction with NO_2 at 20°C for 10 min. Spectrum measured at 77°K . Legend as in Fig. 1.

subsequent reaction between NO_2 and oleic acid led to a substantial change in the shape of the EPR spectrum

(Fig. 1b). Its parameters were the same as those of the EPR spectrum of the iminoxyl radical



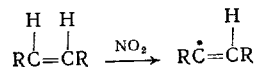
dissolved in oleic acid and measured at 77°K . These nitroxyl radicals remained stable for several hours, after which, as the concentration of these radicals diminished, changes were observed in the intensity of individual components of the EPR signal. The EPR signal of oleic acid 24 h after the reaction with NO_2 is shown in Fig. 1c.

No paramagnetic products were formed by the reaction between NO_2 and saturated fatty acids (butyric, palmitic, and stearic). No paramagnetic products likewise were found after the reaction between NO and both saturated and unsaturated fatty acids.

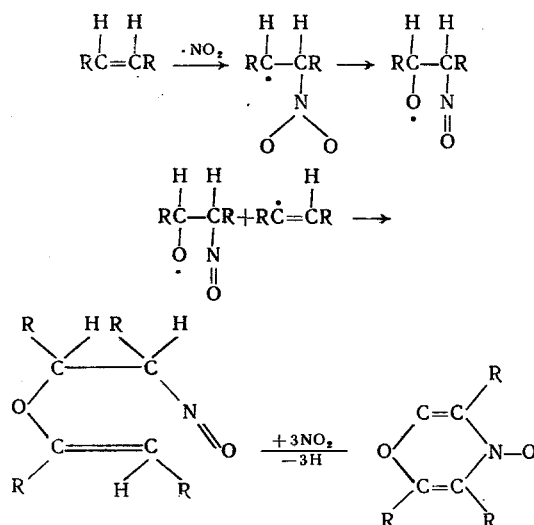
The formation of radicals of unsaturated fatty acids takes place through the removal of an electron or hydrogen atom from the reacting molecule. The least energy must be used to detach the hydrogen atom from the carbon atom in the α -position relative to the double bond [3]:



The wide EPR signal found in the initial stage of the reaction between NO_2 and oleic acid may be confirmation of the free-radical mechanism of oxidation of oleic acid. Rather more energy must be used to rupture the C-H bond of the carbon atom with the double bond:



At this stage the reaction is evidently not complete, and it subsequently continues in accordance with the following scheme:



The wide EPR spectrum observed 24 h after the reaction between oleic acid and NO_2 was evidently due to breakdown products of the nitroxyl radicals.

Passage of NO_2 through a deionized saturated solution of tyrosine at pH 7.0 and 20°C led to the appearance of a paramagnetic center, giving an EPR signal at 77°K with $G = 2005$ and a line width $\Delta H = 9 \text{ Oe}$ (Fig. 2). As the result of the reaction between NO and a saturated solution of tyrosine, no paramagnetic centers were formed. Paramagnetic products also were absent after the reaction of NO and NO_2 with saturated solutions of phenylalanine and tryptophan.

Paramagnetic products of tyrosine were found previously after irradiation of aqueous solution of this substance with ultraviolet light at 77°K [4-6]. The EPR signal arising in this case was due to two types of products: a phenoxyl radical and electrons captured by the surrounding medium. The absence of paramagnetic products following the reaction of NO_2 with tryptophan and phenylalanine and their appearance as a result of the reaction between NO_2 and tyrosine suggests that the singlet signal with $G = 2005$ and $\Delta H \approx 90\text{e}$ was due to the phenoxyl radical of tyrosine.

The reason why no paramagnetic products were observed to be formed by the reaction between NO and unsaturated fatty acids and tyrosine was evidently the weaker electron-acceptor properties of NO than of NO_2 [7].

It can be concluded from these results that unsaturated fatty acids and tyrosine residues of membrane lipoprotein structures may act as targets for interaction with NO_2 and that the formation of paramagnetic centers with the above-mentioned substances is the primary act of injury to the membranes.

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LITERATURE CITED

1. H. Thomas, P. Muller, and R. Wright, *J. Air Pollut. Contr. Assoc.*, **17**, 33 (1967).
2. H. Thomas, P. Muller, and R. Lyman, *Science*, **159**, 58 (1968).
3. E. J. E. Havkins, *Organic Peroxides* [Russian translation], Moscow-Leningrad (1964).
4. O. A. Azizova, *Biofizika*, **9**, 247 (1964).
5. O. A. Azizova and Yu. S. Malinin, in: *Free-radical Processes in Biological Systems* [in Russian], Moscow (1967).
6. L. P. Kayushin, Z. P. Gribova, and O. A. Azizova, *Electron Paramagnetic Resonance of Photoprocesses of Biological Compounds* [in Russian], Moscow (1973).
7. V. I. Vedenev, L. V. Gurvich, et al., *The Energy of Rupture of Chemical Bonds. Ionization Potentials and Affinity for Electrons* [in Russian], Moscow (1974).