Kinetic Parameters of Oxidation of Individual Fatty Acids with Ozone

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Using an original automated analyzer of double bonds we determined the rate constants for oxidation of saturated and unsaturated mono- and dienoic fatty acids (*in vivo* substrates for β-oxidation in the mitochondria) by the ozone titration method. The rate constant for O_3 oxidation is maximum for oleic monoenoic acid, lower for dienoic linoleic, and very low for saturated palmitic acid. The rate constant for oxidation of oleic fatty acid, which by one order of magnitude surpasses that for oxidation of essential arachidonic acid, indicates that oleic acid is a leading *in vivo* acceptor of active O_2 species. By the rate of trapping active oxygen species and in the quantitative aspect, endogenously produced oleic acid can be regarded as the main biological antioxidant.

Key Words: *oleic and arachidonic fatty acids; ozone; antioxidants; peroxidation*

Fatty acids (FA) in organic chemistry are classified as saturated, monoenoic with one double bond (-C=C-; DB), and polyenoic with more than one DB. The differences in physicochemical parameters of FA determine the properties of biomembranes, primarily realizing the function of long-term adaptation of cells [8]. However, this classification can hardly explain mutual biochemical transformations of FA *in vivo*, specifically, some common features of saturated mono- and dienoic FA and their appreciable differences from polyenoic FA [10].

We believe that from a physiological viewpoint, it is rational (particularly speaking about primates and humans) to classify FA on the basis of their *in vivo* role as saturated (C16:0 palmitic) and unsaturated, monoenoic (C18:1 oleic) and dienoic (C16:2 linoleic) acids serving as energy (ATP) substrates for β-oxidation process in the mitochondria; trienoic (ω-3 C18:3 α-linoleic and ω-6 γ-linoleic) with individual biological activity [13]; polyenoic (C20:4 arachidonic, C20:5 eicosapentaenoic and C22:6 docosahexaenic) serving as substrates for the synthesis of bioactive humoral regulators prostacyclins (prostaglandins), thromboxanes, and leukotrienes.

Due to physicochemical differences between C16 and C18 FA, the presence of one and two DB, saturated mono- and dienoic FA can β-oxidize cell mitochondria with different rate. For verification of this assumption we *in vitro* determined the kinetic parameters of ozone oxidation of individual saturated FA.

MATERIALS AND METHODS

Rate constants of O_3 oxidation for C16:0 palmitic saturated FA, C18:1 oleic, C18:2 linoleic, and C20:4 arachidonic essential polyenoic FA were measured using an original Russian-made double bond analyzer (ADS) [6] (Fig. 1).

By UV spectrophotometry, double bond analyzer evaluates the kinetics of O_3 consumption by measuring its concentration at the exit from the reactor. The titering device measures DB content in FA and expresses the results in millimoles of ozone spent for titration. The sensitivity of the method reaches 10^{-9} M; coef-

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ficient of variations in a series of measurements is 4.5%. Stilbene solution in CCl_4 in concentrations of 0.4-1.6×10^{-5} M DB was used as the reference sample (Fig. 2). Gravimetrically measured FA quantity was dissolved in CCl_4 and an aliquot of the solution was introduced into the ADS reactor. The number of DB was calculated by the formula

$$DB = \frac{A\ 1000 \times V_0}{q \times V_1},$$

where A is the number of moles of DB determined by the reference sample; V_0 volume of the solvent for dissolving weighed quantity of FA; V_1 volume of the sample introduced into the reactor; and q is weighed quantity of FA. The purity of FA samples (ICN), used for titration of FA was verified by thin-layer chromatography on Silica Gel. The kinetics of ozone oxidation of FA with different number of DB was evaluated (Fig. 3).

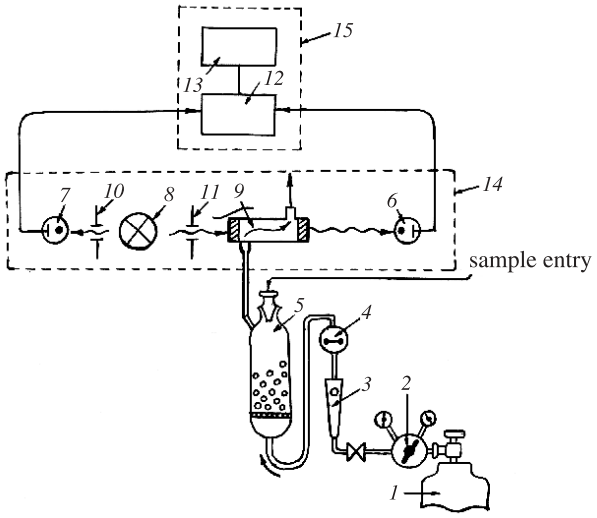

Fig. 1. Scheme of double bond analyzer. *1*) tank; *2*) reductor; *3*) O_2 flow valve; *4*) ozonizer; *5*) reactor; *6*, *7*) photoelements; *8*) mercuric lamp; *9*) optic cuvette; *10*, *11*) diaphragm; *12*) amplifier; *13*) potentiometer; *14*) current regulator; *15*) recorder.

RESULTS

Oxidation rate constants were different for individual FA: 6.0×10^{-2} liter/mol/sec for C16:0 palmitic FA, 1.0×10^6 liter/mol/sec for C18:1 oleic FA, and 6.1×10^4 liter/mol/sec for C18:2 linoleic FA.

This means that saturated and unsaturated FA, functionally serving as substrates for β-oxidation, can oxidize mitochondria with different rate. Oxidation by C18:1 oleic FA is much more rapid than by palmitic saturated FA. Oxidation rate constant for C18:2 Δ9,12 dienoic linoleic FA was lower than for monoenoic oleic FA. Linoleic FA has two DB in the carbon chain, which are oxidized by O_3 with different reaction rate constants. Considering *in vitro* oxidation of individual FA by O_3, we hypothesized the same relationship of their oxidation in the mitochondria; oxidation of FA by ozone and other active oxygen species was realized with similar reaction rate constants [7].

The last stage of our study was devoted to ozone titration of essential polyenoic arachidonic FA (ω-6 C20:4 arachidonic FA, 2.4×10^5 liter/mol/sec).

The constant of oxidation of arachidonic FA depended, among other factors, on the fact that O_3 oxidized not a solitary, but two conjugated DB in this molecule, forming diene conjugates and then disrupting the integrity its aliphatic chain [12]. Partly due to intramolecular structural changes, the rate of C20:4 arachidonic acid oxidation was not so high as that of C18:1 oleic FA.

Automated titration with O_3 most reliably showed the oxidation rate constant for the substances, including saturated, mono-, di-, and polyunsaturated FA, α-tocopherol, β-carotene, ascorbic acid, and flavonoids. Ozonolysis on this double bond analyzer allows measuring the kinetics of oxidizer consumption [1-3] and is the most reliable method for evaluation of the oxidation rate constant and FA structure (DB position in the aliphatic chain). In unsaturated FA O_3 disrupts the carbon chain at the site of DB [7]. Aldehydes and ketones (shortest carbohydrates) forming at the sites of DB rupture can be identified by gas chromatography and mass spectrometry. *In vivo* evaluation of ^{13}C FA fraction metabolism showed that oleic FA is oxidized to CO_2 by human and rat cell mitochondria 60% more actively than linoleic acid [11]. The rate of oxidation of 1-^{13}C oleic FA from human chylomicrons far surpasses that of palmitic unsaturated FA [14].

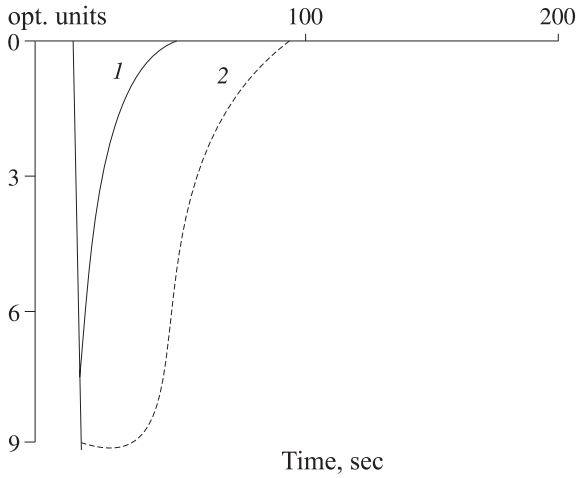

Fig. 2. Ozonograms of stilbene (*1*; reference sample) and fatty acid mixture (*2*).

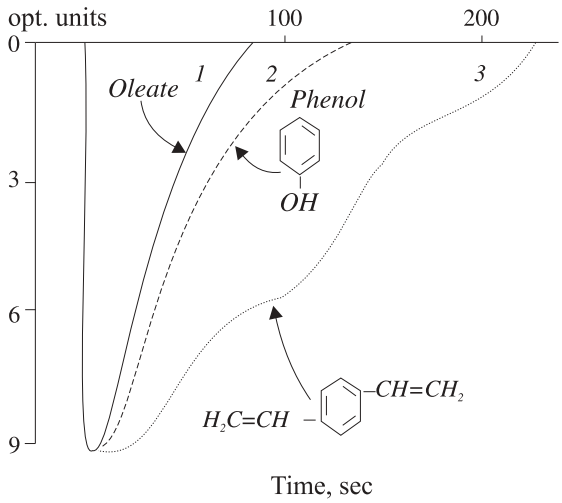

Fig. 3. Ozonation kinetics for substances with different number of double bonds (DB). *1*) fatty acid with one DB; *2*) phenol; *3*) DB ozonation for different positions in the molecule.

The difference in the oxidation rate constants for palmitic, oleic, and linoleic FA can be responsible for the pronounced increase in the concentrations of unsaturated FA and DB and the decrease in palmitic FA in the fraction of nonesterified FA in the blood during stress and epinephrine activation of hormone-dependent lipase in adipocytes. We also detected pronounced changes in DB content in serum lipids of patients with different pathologies during the acute stage and remission [9].

Ozone oxidation of *in vivo de novo* synthesized oleic FA by one order of magnitude surpasses that of essential arachidonic polyenoic FA; this suggests that oleic FA is one of the main acceptors ("traps") of active oxygen species [4] preventing peroxidation of polyenoic FA in plasma lipoproteins. This acid quantitatively and by the rate of *in vivo* trapping of active oxygen species seems to be the main endogenous biological "antioxidant" [5]. This will help to solve some problems of peroxidation and biological role of "antioxidants" [5]. The rate constants of oxidation reactions for oleic, monoenoic FA was for the first time determined by the method of automated ozone titration.

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