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## KINETICS AND MECHANISM OF CHEMICAL REACTIONS. CATALYSIS

# Kinetic Isotope Effect in the Oxidation of Unsaturated Fatty Acids

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**Abstract**—The kinetic parameters of the oxidation of a series of polyunsaturated fatty acids (PUFAs), linoleic and linolenic acids and ethyl esters thereof are determined by measuring the rate $R_{OX}$ of the process in chlorobenzene at 310 K. The selective replacement of H atoms by deuterium atoms results in a dramatic decrease in $R_{OX}$ (the H/D kinetic isotope effect, KIE). Furthermore, the addition of a deuterated PUFA to its nondeuterated analogue decreases $R_{OX}$ for the latter. It looks as if the deuterated PUFA partially protects nondeuterated one from oxidation. The information on the KIE obtained under conditions of initiated liquid-phase oxidation is reported in the current work for the first time; all the numerous previous reports have dealt with enzymatic oxidation.

*Keywords*: polyunsaturated fatty acids, linoleic acid, linolenic acid, H/D kinetic isotope effect, initiated oxidation, kinetics

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Polyunsaturated fatty acids (PUFAs) play a key role in the oxidation of many natural lipids [1–3]. The oxidation of PUFAs also contributes significantly to oxidative stress in biological systems [4–8]. A characteristic feature of PUFAs is the presence of bis-allylic $CH_2$ groups. Figure 1 shows the structure of the simplest PUFAs. It is bis-allylic fragments (positions 11, 14 in LnA and 11 in LA in Fig. 1) that provide a high oxidizability of PUFAs. A relatively high reactivity of these groups is associated with a relatively low dissociation energy of the C–H bonds, $D_{CH}$ = 318 kJ/mol as compared to 348 kJ/mol for simple allyl groups [9–11]. While the value of $D_{CH}$ can be measured only for the weakest C–H bonds, quantum-chemical calculations enable to evaluate these energies for all C–H bonds. Some values of $D_{CH}$ calculated by the DFT B3LYP/6-31G* method using a procedure similar to that used in [12] are given below in kJ/mol (numbers of the carbon atoms (in parentheses) correspond to Fig. 1): oleic acid, 356 (9, 12); LA, 360 (8), 302 (11), and 356 (14); LnA, 356 (8, 17), 297 (11), and 306 (14). Full names and acronyms of the PUFAs and esters thereof investigated in the present study are listed in Table 1. Comparison of the experimental and calculated values of $D_{CH}$ shows that they are in close agreement.

The number of active $CH_2$ groups in PUFAs of biological origin varies from one to five. The mechanism of the chain oxidation of LA and LnA and esters thereof is quite simple, being presently known in detail [13–19]. At the same time, the mechanism of oxidation of higher homologues of PUFAs, including biologically important arachidonic acid, is much more complicated and will not be considered in the present work.

Replacement of hydrogen atoms by deuterium in the active $CH_2$ groups leads to a significant reduction in the oxidizability of PUFAs. This phenomenon is known as the H/D kinetic isotope effect (KIE) [20, 21]. The oxidation of PUFAs can proceeds in the mode of a radical-initiated reaction (including chain-initiation reaction) or as a process initiated by enzymes. It is surprising that, while the enzymatic oxidation has been considered in dozens of publications (see, e.g., [22–30]), information on the KIE in the

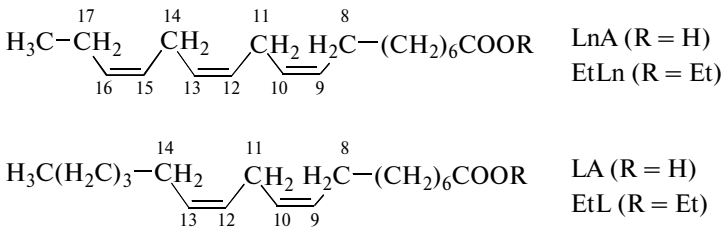


**Fig. 1.** Structure of the simplest PUFAs.

**Table 1.** Polyunsaturated fatty acids and esters thereof studied in the present work

| PUFAs and esters thereof | Acronym |
|---|---|
| Linoleic acid[a] | LA |
| Ethyl linoleate[a] | EtL |
| Linolenic acid[a] | LnA |
| Ethyl linolenate[a] | EtLn |
| 11,11-Dideuterolinoleic acid[b] | 11,11-$D_2$-LA |
| Ethyl 11,11-Dideuterolinoleate[b] | 11,11-$D_2$-EtL |
| 11,11,14,14-Tetradeuterolinolenic acid[b] | 11,11,14,14-$D_4$-LnA |
| Ethyl 11,11,14,14-tetradeuterolinolenate[b] | 11,11,14,14-$D_4$-EtLn |

[a] Purchased from "Sigma".
[b] Synthesized by V. Shmanai's group (Minsk State University); the extent of deuteration not less than 98% [31].

radical-initiated oxidation of PUFAs is virtually nonexistent. The present work, devoted to studying the KIE in the oxidation of PUFAs in the initiated-oxidation mode, is essentially a pioneering one.

## EXPERIMENTAL

Measurements were carried out in chlorobenzene in air at 310 K. In addition to the oxidation substrates, the reaction mixture contained 2,2-azobis(2,4-dimethylvaleronitrile) (AMVN, Polyscience Inc.), as the source (initiator) of active radicals, and 6-hydroxy-2,2,5,7,8-pentamethylchroman (HPMC, Sigma), as a reference antioxidant in the procedure of measuring the rate of generation of radicals $R_{in}$ (initiation). AMVN and HPMC were introduced into the reaction mixture as reference solutions in chlorobenzene. The kinetics of $O_2$ uptake during the oxidation was studied using a glass capillary microvolumeter. The oxidation rate, $R_{OX}$, was determined from the slope of the $[O_2]$ versus time dependence. The initiation rate was measured by means of the inhibitor method using HPMC.

## RESULTS AND DISCUSSION

The kinetics of the oxidation of PUFAs was studied in the controlled chain reaction mode. This approach enables to maintain $R_{OX}$ at a constant level during a particular kinetic experiment. The process under consideration can be described by a simple kinetic scheme [9–11]:

$$\text{AMVN} \xrightarrow[R_{in}]{+O_2,\ +LH} L^{\bullet},$$

$$L^{\bullet} + O_2 \xrightarrow{k_1} LO_2^{\bullet},$$

$$LO_2^{\bullet} + LH \xrightarrow{k_2} L^{\bullet} + LOOH,$$

$$LO_2^{\bullet} + LO_2^{\bullet} \xrightarrow{2k_3} \text{Products}.$$

The rate of the chain oxidation of LH PUFA is given by

$$R_{OX} = \frac{k_2[LH]R_{in}^{1/2}}{(2k_3)^{1/2}}. \quad (1)$$

The initiation rate $R_{in}$ was calculated from the induction period of the inhibited chain oxidation ($t_{ind}$) at the known concentration of HPMC reference antioxidant:

$$R_{in} = \frac{2[HPMC]}{t_{ind}}. \quad (2)$$

The fit of the experimental data to Eq. (1) is confirmed by the results of Fig. 2. The values of the oxidizability parameter $k_2/(2k_3)^{1/2}$ calculated from Eq. (1) are listed in Table 2.

The primary product of the oxidation of PUFAs is the conjugated pentadienyl radical, ~HC···CH···CH···CH···CH~ ($LO_2^{\bullet}$). The structure of this radical was confirmed by its EPR spectrum [32].

**Table 2.** Oxidation of PUFA and esters thereof at 310 K

| PUFA | $k_2/(2k_3)^{1/2}$, $L^{0.5}\,mol^{-0.5}\,s^{-0.5}$ |
|---|---|
| Methyl oleate | 0.0021[a] |
| LA | 0.0207 ± 0.009 (6)[b] |
| EtL | 0.0230 ± 0.009 (3) |
| LnA | 0.051 ± 0.004 (5) |
| EtLn | 0.046 ± 0.003 (3) |

[a] From [11].
[b] Figures in parentheses indicate the number of parallel runs.

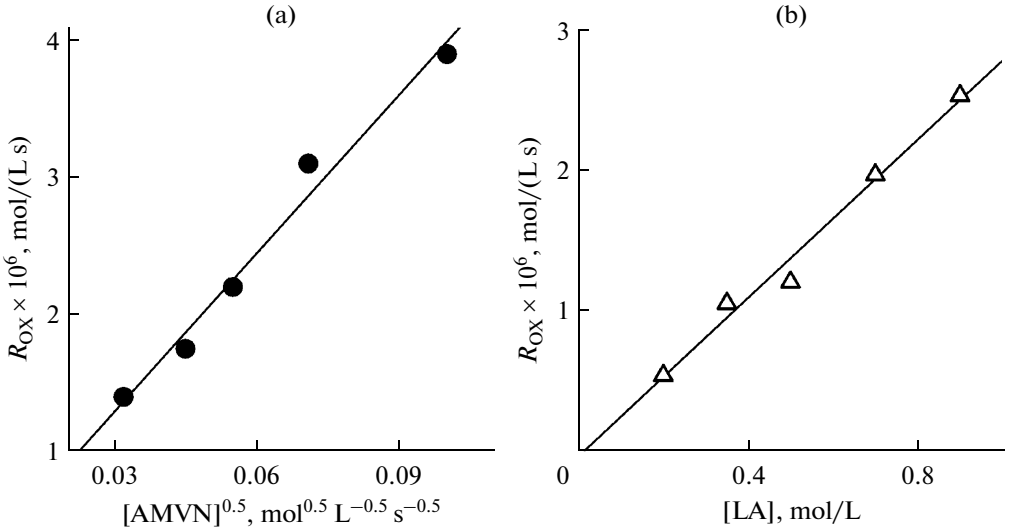


**Fig. 2.** Dependence of the oxidation rate of LA on (a) [AMVN] at [LA] = 1.0 mol/L and (b) [LA] at [AMVN] = 0.005 mol/L in chlorobenzene at 310 K.

The selective deuteration of the PUFAs (replacement of H atoms by deuterium atoms in the most active $CH_2$ groups at positions, 11, 11 in LA and 11,11,14,14 in LnA (Fig. 1) leads to a drastic reduction in $R_{OX}$. A typical kinetic experiment is shown in Fig. 3. It can be seen that the rate of the oxidation of uninhibited 11,11,14,14-$D_4$-LnA is virtually identical to the initiation rate. A similar effect is observed for the oxidation of 11,11-$D_2$-LA and the respective ethyl esters. Furthermore, the addition of HPMC, a typical antioxidant, which terminates oxidation chains in the reaction with $LO_2^{\bullet}$, does not change $R_{OX}$. These observations indicate that the oxidation of the above selectively deuterated PUFAs is a nonchain process.

### *Kinetic Isotope Effect*

The KIE can serve as a quantitative measure of the effect of deuteration on the chemical reaction kinetics. The most natural way to determine the KIE is to measure the ratio of the rates for the oxidation of nondeuterated $(R_{OX})_H$ and deuterated $(R_{OX})_D$ compounds.

**Table 3.** Kinetic isotope effect for some deuterated PUFAs in chlorobenzene at 310 K, [AMVN] = 0.01 mol/L

| PUFA | [PUFA], mol/L | KIE |
|---|---|---|
| 11,11-$D_2$-LA | 1.0 | ≥90 |
| 11,11-$D_2$-EtL | 1.0 | ≥120 |
| 11,11,14,14-$D_4$-LnA | 0.80 | ≥230 |
| 11,11,14,14-$D_4$-EtLn | 0.80 | ≥210 |

Unfortunately, this approach is not suitable for the above selectively deuterated PUFAs. The fact is that, as already mentioned, the rate of the oxidation of compounds of this kind does not differ from $R_{in}$. In this case, the $(R_{OX})_H/(R_{OX})_D$ ratio enables to estimate only the lower limit of the KIE (Table 3). This table shows that the estimated KIE values for the acids and the respective esters differ little from each other.

### *Oxidation of a Mixture of Deuterated and Nondeuterated PUFA*

Figure 4 displays the dependence of the rate of the oxidation of a mixture of deuterated and nondeuterated PUFAs (a mixture of 11,11-$D_2$-LA and LA) on its composition. The data presented in Fig. 4 show that the partially deuterated PUFA protects the nondeuterated analogue from chain oxidation. For example, the experimental value of the rate of oxidation of an equimolecular mixture of LA and 11,11-$D_2$-LA is $0.5 \times 10^{-6}$ mol/(L s), whereas the additive value of $R_{OX}$ is $2.4 \times 10^{-6}$ mol/(L s). Thus, the addition of 50% deuterated analogue of LA is responsible for approximately a fivefold reduction in $R_{OX}$.

Generally, the KIE for the oxidation of PUFAs is considerably higher than for other compounds. In particular, the KIE value is 10.6–15 for the reaction styrene peroxy radicals with substituted phenols [33, 34], 24 for the reaction of ascorbate with 2,2,6,6-tetramethylpiperidine-1-oxyl [35], 18 for the vitamin E radical with ubiquinol-10 [36], 3–8 for the reaction of ascorbate with the free radical formed from α-tocopherol in micellar solutions [37]. Note that the oxidation of PUFAs is so far the only example in which the KIE value changes nonadditively with the composi-

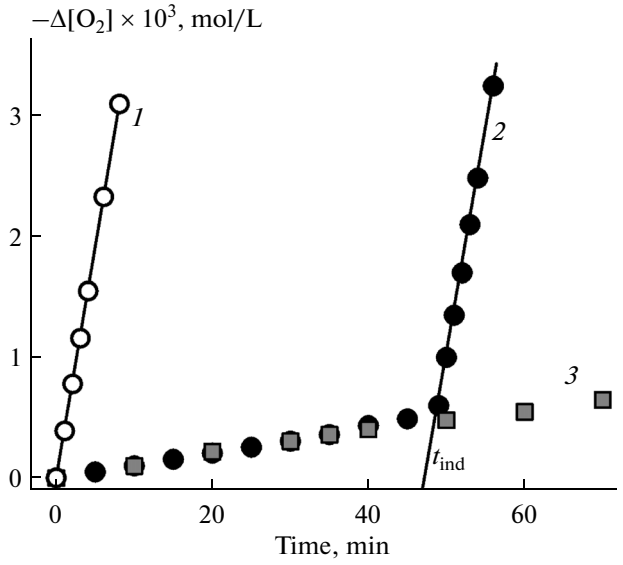


**Fig. 3.** Comparison of the kinetic characteristics of the oxidation of LnA and 11,11,14,14-$D_4$-LnA: (*1*) LnA oxidation (0.57 mol/L), (*2*) LnA oxidation (0.57 mol/L) in the presence of $1 \times 10^{-4}$ mol/L HPMC, and (*3*) 11,11,14,14-$D_4$-LnA oxidation (0.57 mol/L); chlorobenzene, [AMVN] = 0.025 mol/L, 310 K.

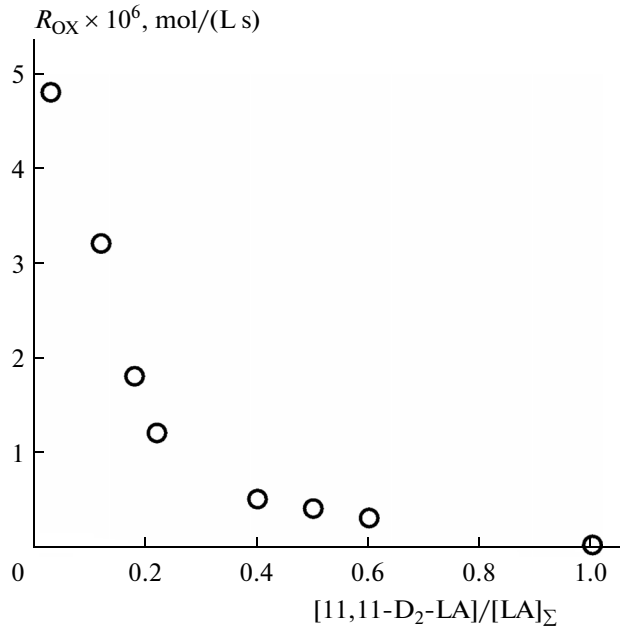


**Fig. 4.** Dependence of the rate of the oxidation of a mixture of LA and 11,11-$D_2$-LA on its composition. The total concentration of PUFA is 1.0 mol/L, [AMVN] = 0.025 mol/L, 310 K.

tion of the reaction mixture composed of nondeuterated and deuterated compounds.

Some kinetic features of the oxidation of PUFAs in which the bis-allylic $CH_2$ group are fully deuterated (11,11-$D_2$-LA, 11,11-$D_2$-EtL, 11,11,14,14-$D_4$-LnA, 11,11,14,14-$D_4$-EtLn) seem somewhat unexpected and have received so far no rational explanation. The matter is that, in addition to the above deuterated fragments, these compounds have potentially reactive allyl groups (~$CH_2$–CH=CH~). Given that the values of $R_{OX}$ for these compounds do not differ markedly from $R_{in}$, it can be argued that these allyl fragments do not take part in the oxidation. This means that the deuteration of a bis-allylic $CH_2$ group leads to a significant reduction in the reactivity simple allylic C–H bonds.

The literature has repeatedly pointed out that many diseases are caused by an increased rate of the oxidation of PUFAs [22–30]. It is expected that the decrease of $R_{OX}$ may lead to a reduction in the intensity of such diseases. One way to reduce $R_{OX}$ is the deuteration of PUFAs. It is such an effect, namely the nonadditive decrease of $R_{OX}$ with increasing concentrations of the deuterated component in the mixture (mixture of 11,11-$D_2$-LA and LA, Fig. 4) that is demonstrated by the results of the present study. This means that a mixture of deuterated and nondeuterated PUFAs can provide the same biological effect as the deuterated PUFA. In principle, this enables to achieve the same biological effect using a smaller concentration of the deuterated PUFA, a more valuable component. Note that, in contrast to the above results, the rate of the enzymatic oxidation of such mixtures obeys the additivity rule [22]. Therefore, it should be emphasized that the mechanism of the influence of PUFA deuteration on $R_{OX}$ is far from having been elucidated.

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