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CHEMISTRY

Polytopic Zinc Dipicolylamine Complex Containing a Ferrocene Moiety as a New Lipoxygenase LOX I-B Inhibitor

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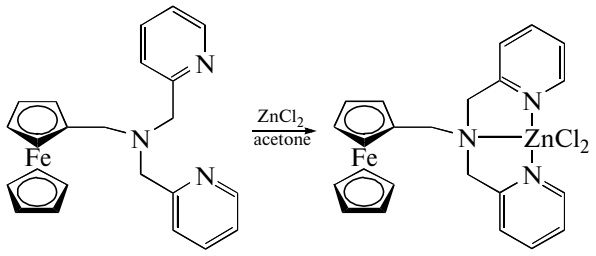
Lipoxygenases represent a class of non-heme iron-containing dioxygenase enzymes that catalyze regio- and stereoselective radical peroxidation of polyunsaturated fatty acids to hydroperoxides. This reaction is the first stage of the biosynthesis of leukotrienes, which initiate various pathological processes, in particular, they are involved in the pathogenesis of tumor diseases [1–3]. Therefore, the search for selective lipoxygenase inhibitors among organic and organoelement compounds is a topical task [4, 5].

Meanwhile, ferrocene derivatives, for example, ferrocifens, are known to exhibit antitumor activities [6] caused by the change in the oxidative state of the ferrocene moiety and its capability for the intramolecular electron transfer in the polytopic systems containing other redox active centers such as metal ions.

Dipicolylamine is an efficient tridentate ligand for the synthesis of metal complexes that forms a variety of coordination compounds. Molecular design of a polytopic compound combining ferrocene and dipicolylamine moieties in the same molecule suggests that this compound would possess simultaneously the complexing properties and the ability to undergo reversible redox transformations of the ferrocene moiety. By gradual complication of the molecule upon the introduction of various biometal atoms into the complex, it is possible to elucidate the role of each constituent of the metal complex in the mechanism of action on the lipoxygenase active site.

The purpose of this work is tailored synthesis of zinc dipicolylamine complex containing a ferrocene moiety and computer modeling and investigation of the reaction of the potential physiologically active and multitarget compound, having presumably an antiproliferative activity, with the lipoxygenase active site in the enzymatic peroxidation of the linoleic acid as the substrate.

Ferrocenylmethyl-bis(2-pyridylmethyl)amine (**1**) was used as the ligand [7] to prepare complex **2** with zinc chloride (Scheme 1). Compound **2** was characterized by IR, ^{1}H and ^{13}C NMR, and elemental analysis data.



1 **2**

Scheme 1.

Using the molecular docking method implemented in the AutoDock 4.2 program [8, 9], the possible ways of interaction of compound **2** with lipoxygenase (PDB ID 1IK3 [10]) were analyzed. A mode of binding for complex **2** was proposed in which the structure of complex **2** was sterically complementary to the predominantly hydrophobic cavity of the enzyme active site; hence, this compound can be regarded as a potential lipoxygenase inhibitor. In the absence of a metal ion, the conformation of compound **1** similar to that occurring in complex **2** is sterically unfavorable and, hence, its existence in a rather small active site cavity seems unlikely.

We studied the effect of ferrocene derivatives **1** and **2** on the activity of the lipoxygenase LOX I-B enzyme toward the oxidation of the linoleic acid substrate giving hydroperoxide. The figure presents the kinetic

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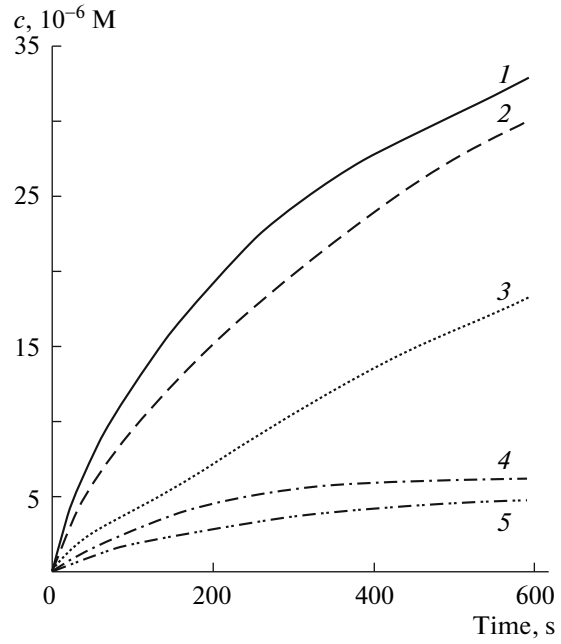

Kinetic curves for the linoleic acid oxidation under the action of lipoxygenase LOX I-B enzyme in the presence of compounds **1** and **2**. (*1*) Control; (*2–5*): concentration, μmol/L: (*2*, compound **1**) 75; (*3*, compound **2**) 15; (*4*, compound **2**) 20; (*5*, compound **2**) 25.

curves for the operation of this enzyme in the presence of compounds **1** and **2**.

The degree of lipoxygenase inhibition (A, %) was determined as

$$A, \% = \frac{100\nu_0(\text{in the presence of compound } \mathbf{1} \text{ or } \mathbf{2})}{\nu_0\,(\text{DMSO control})}.$$

The initial rate (ν_0) was calculated from the relation $\nu_0 = \frac{\Delta c}{\Delta t} = \frac{\Delta A}{\Delta t \varepsilon} = \frac{\tan\alpha}{\Delta t \varepsilon}$ where c is the product concentration, t is the reaction time, ε is the molar extinction coefficient ($\varepsilon = 2.5 \times 10^4$ L mol^{-1} cm^{-1}), tanα is the slope of the kinetic curve. The IC_{50} value was found by describing the obtained data by a logistical curve $A, \% = 100\frac{1}{(1 + [I]/IC_{50})}$, where $[I]$ is the inhibitor concentration. According to the data on the variation of the initial rates upon increase in the inhibitor (complex **2**) concentration, the IC_{50} value was found to be 15.2 μM.

It is noteworthy that the study of the effect of compound **1** on the lipoxygenase activity showed no noticeable inhibition. Even when the concentration of **1** was 100 μM, the initial reaction rate almost did not change. On the other hand, the concentration dependence for compound **2** is indicative of its high activity (figure). Presumably, the enzyme inhibition in the presence of **2** is caused by complexation of the metal atom with the dipicolylamine ligand **1**. This has a critical effect on the geometric parameters of the molecule. The inhibiting action of **2** is caused apparently by the direct binding of the metal complex to the enzyme; the compound in question can be classified as a redox inactive lipoxygenase inhibitor.

The IR spectra were recorded on a Thermo Nicolet IR200 FT IR spectrophotometer in KBr pellets. The NMR spectra were run on a Bruker AMX-400 spectrometer in $CDCl_3$ (^{1}H, 400 MHz; ^{13}C, 100 MHz). The absorption spectra were measured on a Thermo Evolution 300 BB spectrophotometer.

The following substances were used: *N,N*-dimethylaminoferrocene iodomethylate, di(2-picolyl)amine (Sigma–Aldrich, 97%), reagent grade $ZnCl_2 \cdot 2H_2O$, lipoxygenase (Sigma, Lipoxidase from Glycine max (soybean), Type I-B), linoleic acid (Sigma, 99%). Ferrocenylmethyl-bis(2-pyridylmethyl)amine (**1**) was prepared by a reported method [7].

Complex of ferrocenylmethyl-bis(2-pyridylmethyl)amine with $ZnCl_2$ (2). $ZnCl_2 \cdot 2H_2O$ (88 mg, 0.51 mmol) was added with stirring to a solution of **1** (200 mg, 0.50 mmol) in acetone (0.4 mL). The mixture was stirred with heating at 30–40°C for 30 min. The resulting needle crystals were washed with petroleum ether and dried in air for 24 h. Yield 213 mg (80%).

For $C_{23}H_{23}N_3FeZnCl_2$ anal. calcd. (%): C, 51.77; H, 4.34; N, 7.87. Found (%): C, 51.59; H, 4.36; N, 7.70.

IR, cm^{-1}: 1574, 1604, 3032, 3055, 3080, 3093, 2861, 2929, 2952.

^{1}H NMR (δ, ppm): 3.46 (s, 2H), 3.54 (s, 4H), 4.01 (s, 5H), 4.18 (s, 2H), 4.02 (s, 2H), 7.30 (t, 2H, $J_{H-H} = 8$ Hz), 7.52 (t, 2H, $J_{H-H} = 8$ Hz), 7.92 (t, 2H, $J_{H-H} = 8$ Hz), 9.26 (d, 2H, $J_{H-H} = 4$ Hz).

^{13}C NMR (δ, ppm): 51.00, 55.41, 68.88, 69.11, 123.35, 124.61, 139.87, 149.96, 153.54.

The activity of lipoxygenase LOX I-B was determined by spectrophotometry [11]. The content of the hydroperoxide resulting from oxidation of linoleic acid was measured at λ_{max} 234 nm. The test solution contained 2 mL of a linoleic acid solution (0.3×10^{-3} mol/L), borate buffer, pH 9.0 (0.89 mL), and a solution of the compound in DMSO (0.01 mL) in the concentration range of 0 to 100 μmol/L. The reaction was triggered by adding 0.1 mL of a solution of the enzyme (500 units), and the measurement was carried out for 10 min at 25°C. Every experiment was repeated three times.

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