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Effect of endocannabinoids on soybean lipoxygenase-1 activity

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ABSTRACT

Endocannabinoids appear to be involved in a variety of physiological processes. Lipoxygenase activity has been known to be affected by unsaturated fatty acids or phenolic compounds. In this study, we examined whether endocannabinoids containing both N-acyl group and phenolic group can affect the activity of soybean lipoxygenase (LOX)-1, similar to mammalian 15-lipoxygenase in physicochemical properties. First, N-arachidonoyl dopamine and N-oleoyl dopamine were found to inhibit soybean LOX-1-catalyzed oxygenation of linoleic acid in a non-competitive manner with a K_i value of 3.7 μM and 6.2 μM, respectively. Meanwhile, other endocannabinoids failed to show a remarkable inhibition of soybean LOX-1. Separately, N-arachidonoyl dopamine and N-arachidonoyl serotonin were observed to inactivate soybean LOX-1 with K_{in} value of 27 μM and 24 μM, respectively, and k_3 value of 0.12 min^{-1} and 0.35 min^{-1}, respectively. Furthermore, such an inactivation was enhanced by ascorbic acid, but suppressed by 13(S)-hydroperoxy-9,11-octadecadienoic acid. Taken together, it is proposed that endocannabinoids containing polyunsaturated acyl moiety and phenolic group may be efficient for the inhibition as well as inactivation of 15-lipoxygenase.

1. Introduction

Lipoxygenases (LOXs), a family of non-heme iron-containing dioxygenases, catalyzes the addition of molecular oxygen to polyunsaturated fatty acids containing at least one (*Z*, *Z*)-pentadiene system to produce corresponding hydroperoxides [1–5]. Recently, our recent studies demonstrated that polyunsaturated lysophosphatidylcholines and lysophosphatidylethanolamines containing linoleoyl, arachidonoyl or docosahexaenoyl groups were efficiently oxygenated by reticulocyte LOX, leukocyte LOX or soybean LOX-1 [6–8]. In general, LOXs contain an essential iron atom, which is present as Fe^{2+} in the ground-state inactive form; enzymatic activation occurs through lipid hydroperoxide-driven oxidation of Fe^{2+} to Fe^{3+} [1–3]. LOXs are widely distributed among plants and animals [1–3]. It is well-known that in plant system, LOXs catalyze oxygenation of linoleic acid and linolenic acid to generate cellular regulators, such as jasmonic acid, traumatic acid, and alkenals, responsible for the growth regulation and wound healing [9,10]. In animals, LOXs are known to convert arachidonoic acid to lipid mediators, such as leukotrienes or lipoxins [11,12], which are implicated in various diseases such as atherosclerosis, cancer, inflammation and asthma [13]. Since LOX metabolites of polyunsaturated fatty acids are involved in cellular response under normal or disease conditions [14–17], the regulation of LOX-catalyzed oxygenation of polyunsaturated fatty acids may result in the alteration of a pathophysiological response in animals. In humans, lipoxygenase plays a key role in the biosynthesis of leukotrienes, proinflammatory mediators mainly released from myeloid cells. Thus, inhibitors of lipoxygenases have attracted attention initially as potential agents for the treatment of inflammatory and allergic diseases, but their therapeutic potential has now been expanded to certain types of cancer and cardiovascular diseases [18–21]. Soybean lipoxygenase-1 (soybean LOX-1) is a plant-derived 15-LOX catalyzing efficiently the oxidation of linoleic acid to 13-hydroperoxy-9,11-octadecadienoic acid. Because of structural and functional similarities with mammalian 15-LOX, soybean LOX-1 is commonly used as a model for mammalian 15-LOX for both mechanistic and inhibition studies [1–3,14–17].

Earlier, soybean LOX-1 had been reported to undergo inactivation during the incubation with polyunsaturated fatty acids, and

Abbreviations: 13-HpODE, 13(S)-hydroperoxyoctadecadienoic acid; 15(S)-HpETE, 15(S)-hydroperoxyeicosatatraenoic acid; E_{ox}, active ferric enzyme form; E_{red}, inactive ferrous enzyme form; LOX, lipoxygenase; NA-DA, N-arachidonoyl dopamine; NA-Gly, N-arachidonoyl glycine; NA-Tau, N-arachidonoyl taurine; NA-Ser, N-arachidonoyl serine; NA-5HT, N-arachidonoyl serotonin; NO-DA, N-oleoyl dopamine; NP-DA, N-palmitoyl dopamine.

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the inactivation was suggested to be ascribed to the oxidants from oxygenation products [22]. In support of this, the activities of soybean LOX-1 or potato 5-LOX was observed to decline gradually during preincubation with 5(S)-hydroperoxyeicosatetraenoic, 9(S)-hydroperoxy-10(E), 12(Z), 15(Z)-octadecatrienoic acid or 15(S)-hydroxyperoxyeicosatetraenoic [23–25], supporting the assumption that the unstable intermediates from the respective hydroperoxy product may be responsible for the gradual loss of enzyme activity. Recently, it was observed that polyunsaturated lysophosphatidylcholines, which can be utilized as substrates for lipoxygenase, could inhibit lipoxygenase [26]. Separately, there are many reports about the inhibitory effect of some phenolic compounds on lypoxygenases activity [27–29]. Endocannabinoids are compounds containing fatty acids link to amino acid or neurotransmitter. Most endocannabinoids are differentially distributed in the body and the level of several ones undergoes hormonal regulation in the brain [30]. Endocannabinoids are of great interest as potential tools to probe new binding sites of G protein coupled receptors (GPCRs), transporters or ion channels [31]. Previously, N-arachidonoyl glycine and N-arachidonoyl dopamine had been reported to be substrates for lipoxygenase [32,33]. Nonetheless, the reports concerning the regulation of endocannabinoids on lipoxygenase or cyclooxygenase activity is limited [34,35].

The present study evaluated the inhibitory or inactivating effect of N-acylated endocannabinoids on soybean LOX-1 activity. Here, we demonstrate that N-acyl dopamine inhibited soybean LOX-1 potently and N-arachidonoyl dopamine or N-arachidonoyl serotonin is a strong inactivator of soybean LOX-1. The purpose of this study is to examine the structural requirement of endocannabinoids for the efficient inhibitor of soybean LOX-1, and to provide an idea for the design of a potential inhibitor of mammalian 15-LOX.

2. Material and methods

2.1. Materials

Soybean lipoxygenase-1 (type 1-B), linoleic acid, ascorbic acid, dopamine hydrochloride, serotonin and xylenol orange were purchased from Sigma–Aldrich Company (Alabaster, Alabama, USA). Iron (II) sulfate was from Kanto Chemical Co., Inc (Tokyo, Japan). Arachidonic acid, N-arachidonoyl dopamine (NA-DA), N-oleoyl dopamine (NO-DA), N-palmitoyl dopamine (NP-DA), N-arachidonoyl taurine (NA-Tau), N-arachidonoyl glycine (NA-Gly), N-arachidonoyl serine (NA-Ser), N-arachidonoyl serotonin (NA-5HT) were obtained from Cayman Chemical (Ann Arbor, MI, USA). 13(S)-hydroperoxy-9,11-octadecadienoic acid (13-HpODE, >98%) and 15(S)-hydroperoxy-5,8,11,13-eicosatetraenoic acid (15-HpETE, >98%) were prepared as described before [25]. Briefly, soybean LOX-1 (0.8 unit/mL) was incubated with linoleic acid or arachidonoic acid (50 μM) in borax buffer (50 mM, pH 9.0) at 25 °C for 30 min. Subsequently, the mixture was passed through a C_{18} column (2 cm × 1 cm), and the products were eluted with methanol and concentrated under N_2 gas.

2.2. Determination of kinetic values in soybean LOX-1 – catalyzed oxygenation of endocannabinoids

Oxygenation of NA-DA, NA-Tau, NA-Gly, NA-Ser, arachidonoic acid or linoleic acid by soy-LOX-1 was monitored by measuring the increase of the absorbance at 234 nm (A_{234}) reflecting to the formation of conjugated diene. In kinetic study, endocannabinoids (0–40 μM) were incubated soybean LOX-1 (0.05 units/mL) in 50 mM borax buffer (pH 9.0). One unit is defined as the activity of enzyme forming 1 μmole of oxygenation product per min. The kinetic parameters, km and kcat, were determined by non-linear regression analysis using enzyme kinetic analysis in GraphPad Prism 5 software.

2.3. Determination of LOX activities by UV spectrophotometer and Eadie–Hofstee Plot analysis for inhibition of LOX activities by N-acyl dopamine

Soybean LOX-1 (0.05 units/mL) was incubated with linoleic acid (0–80 μM) in the presence of N-arachidonoyl dopamine or N-oleoyl dopamine in borax buffer (50 mM, pH 9.0) at 25 °C for 1 min. The remaining LOX activity was determined by measuring

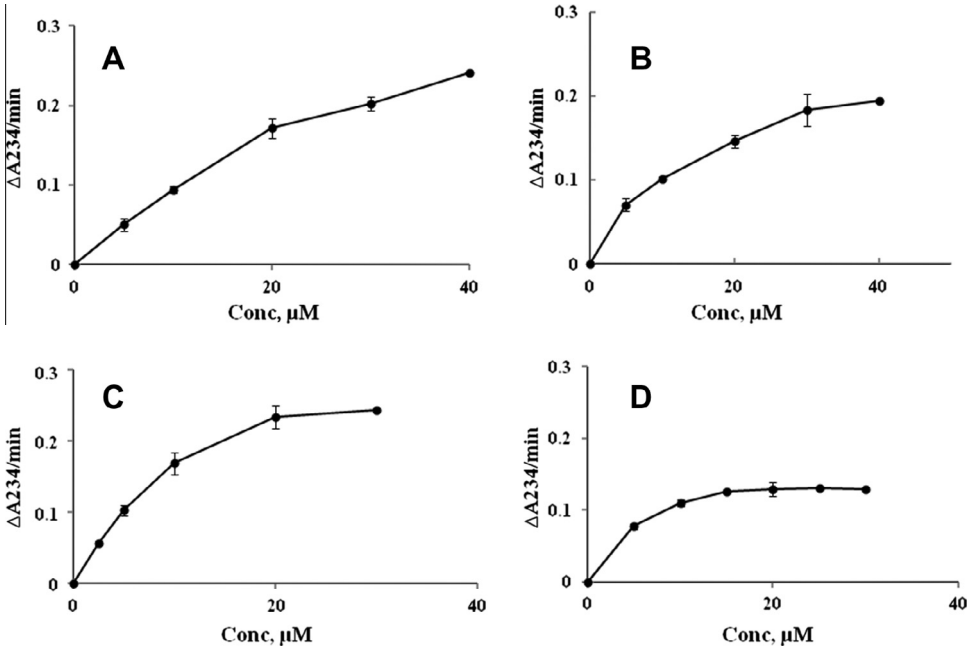

Fig. 1. Effect of substrate concentration on LOX-catalyzed oxygenation of various endocannabinoids. Soybean LOX-1 (0.05 units/mL) was incubated with some endocannabinoids of various concentration (0–40 μM) in 50 mM borax buffer (350 μl, pH 9.0). (A) N-arachidonoyl taurine, (B) N-arachidonoyl dopamine, (C) N-arachidonoyl glycine, (D) N-arachidonoyl serine. Data are expressed as a means ± SD value of triplicate assays.