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N-linoleoylamino acids as chiral probes of substrate binding by soybean lipoxygenase-1

Charles H. Clapp *, Justin Pachuski, Natasha F. Bassett, Kathleen A. Bishop, Gillian Carter, Megan Young, Thomas Young, Yuhan Fu

Department of Chemistry, Bucknell University, Lewisburg, PA 17837, United States

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ABSTRACT

Lipoxygenases catalyze the oxygenation of polyunsaturated fatty acids and their derivatives to produce conjugated diene hydroperoxides. Soybean lipoxygenase-1 (SBLO-1) has been the subject of intensive structural and mechanistic study, but the manner in which this enzyme binds substrates is uncertain. Previous studies suggest that the fatty acyl group of the substrate binds in an internal cavity near the catalytic iron with the polar end at the surface of the protein or perhaps external to the protein. To test this model, we have investigated two pairs of enantiomeric N-linoleoylamino acids as substrates for SBLO-1. If the amino acid moiety binds external to the protein, the kinetics and product distribution should show little or no sensitivity to the stereochemical configuration of the amino acid moiety. Consistent with this expectation, N-linoleoyl-L-valine (LLV) and N-linoleoyl-D-valine (LDV) are both good substrates with k_{cat}/K_m values that are equal within error and about 40% higher than k_{cat}/K_m for linoleic acid. Experiments with N-linoleoyl-L-tryptophan (LLT) and N-linoleoyl-D-tryptophan (LDT) were complicated by the low critical micelle concentrations (CMC = 6–8 μM) of these substances. Below the CMC, LDT is a better substrate by a factor of 2.7. The rates of oxygenation of LDT and LLT continue to rise above the CMC, with modest stereoselectivity in favor of the D enantiomer. With all of the substrates tested, the major product is the 13(*S*)-hydroperoxide, and the distribution of minor products is not appreciably affected by the configuration of the amino acid moiety. The absence of stereoselectivity with LLV and LDV, the modest magnitude of the stereoselectivity with LLT and LDT, and the ability micellar forms of LLT and LDT to increase the concentration of available substrate are all consistent with the hypothesis that the amino acid moiety binds largely external to SBLO-1 and interacts with it only weakly.

1. Introduction

Lipoxygenases catalyze the oxygenation of *Z,Z*-1,4-diene units in polyunsaturated fatty acids and their derivatives to produce conjugated diene hydroperoxides [1,2]. These enzymes are widespread in plants [3] and animals [4], and they also occur in prokaryotes [5]. Lipoxygenases contribute to the synthesis of important signaling molecules, including jasmonic acid [6] in plants and leukotrienes, lipoxins and resolvins in animals [7–9]. Some lipoxygenases act on phospholipids [10–13] and appear to be involved in membrane modification [1,14], storage-lipid mobilization [15], and ferroptosis [16]. Inhibitors of human lipoxygenases are of interest as potential drugs for the treatment of inflammation, asthma, cancer and other disorders [4,7,8,17,18].

Much of our mechanistic insight into lipoxygenases comes from studies on soybean lipoxygenase-1 (SBLO-1). This enzyme catalyzes the oxygenation of linoleate to primarily 13(*S*)-hydroperoxy-9(*Z*),11(*E*)-octadecadienoate (13-HPOD) [19]. SBLO-1 will also oxygenate other fatty acids, phospholipids and fatty acid derivatives that have a *Z,Z* 1,4-diene unit in the ω6 position, and the major product contains oxygen at that position [10–13,20]. Like most lipoxygenases, SBLO-1 is a nonheme iron protein [21]. The catalytic mechanism (Scheme 1) is thought to involve transfer

Abbreviations: BSTFA, N,O-bis(trimethylsilyl)trifluoroacetamide; CMC, critical micelle concentration; ENDOR, electron-nuclear double resonance; EPR, electron paramagnetic resonance; ESI, electrospray ionization; GC/MS, gas chromatography/mass spectrometry; HPLC, high pressure liquid chromatography; 9-HODE, 9-hydroxy-10(*E*),12(*Z*)-octadecadienoic acid; 13-HODE, 13-hydroxy-9(*Z*),11(*E*)-octadecadienoic acid; 13-HPOD, 13(*S*)-hydroperoxy-9(*Z*),11(*E*)-octadecadienoic acid; HRMS, high resolution mass spectrometry; LDT, N-linoleoyl-D-tryptophan; LLT, N-linoleoyl-L-tryptophan; LDV, N-linoleoyl-D-valine; LLV, N-linoleoyl-L-valine; MS/MS, tandem mass spectrometry; NMR, nuclear magnetic resonance; SBLO-1, soybean lipoxygenase-1.

* Corresponding author.
E-mail address: cclapp@bucknell.edu (C.H. Clapp).

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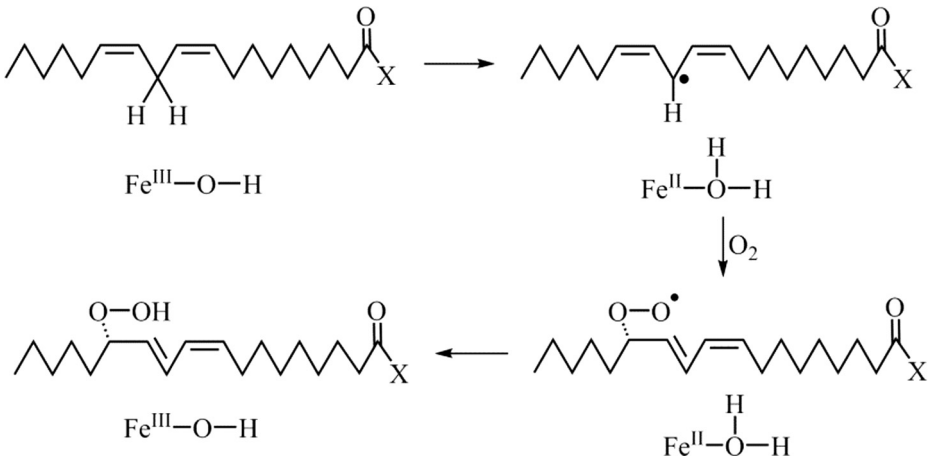
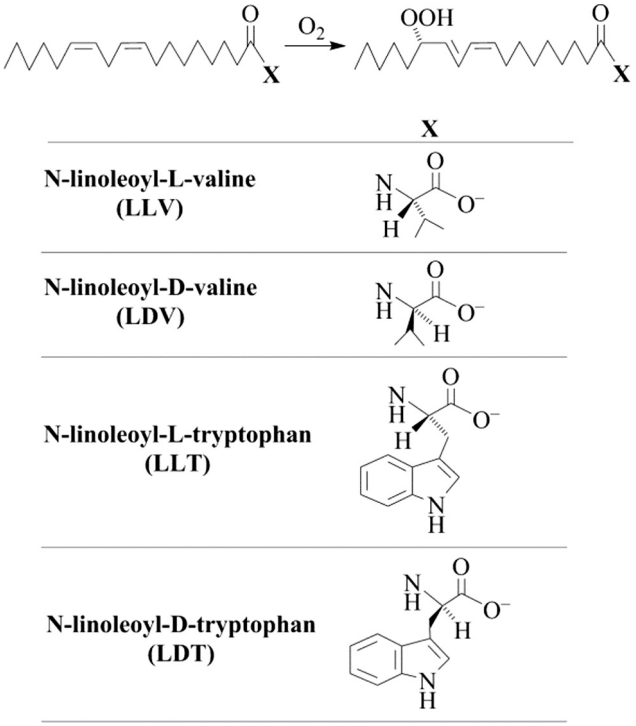


Scheme 1. Reaction mechanism of SBLO-1.

of a hydrogen atom from the bisallylic carbon of the substrate to a ferric hydroxide species in a process that involves hydrogen tunneling [21–24]. The resulting pentadienyl radical reacts with O_2, with the enzyme likely providing an oxygen channel to control the regio- and stereochemistry [25]. The mechanism postulates that the bisallylic carbon of the substrate binds near the Fe^{+3} ion, and this hypothesis is strongly supported by recent ENDOR studies using samples of linoleate labelled with ^{13}C and SBLO-1 in which the catalytic iron has been replaced by manganese [26].

The iron in SBLO-1 is located in the center of a large helical domain [27,28]. The manner in which substrates bind to SBLO-1 and other lipoxygenases in order to position the bisallylic carbon close to the iron has been difficult to establish, and progress towards this goal has been reviewed [29,30]. The ability of SBLO-1 to oxygenate phospholipids and other substrates with large substituents at the polar terminus is most readily accommodated by the hypothesis that substrates bind with the alkyl tail in an internal cavity close to the iron and the polar terminus near the surface [1,31]. Binding in this manner would allow large substituents at the polar terminus to remain largely outside the protein. This binding model is supported by recent studies with spin-labelled substrate analogues [29,32]. The model requires some reorganization of the surface of the protein in order to provide access to the internal cavity, and recent studies suggest that the mobility of helix-2 plays a role in providing this access [33].

Our group has reported that SBLO-1 will oxygenate linoleyltrimethylammonium ion, in which the negatively charged carboxylate group of linoleate is replaced by a positively charged quaternary ammonium group [34]. This finding is consistent with the hypothesis that the polar terminus of substrates has minimal interaction with the protein. As a further test of this model, we have investigated the action of SBLO-1 on the N-linoleoylamino acids shown in Scheme 2. In each case, the major product was determined to be the 13(*S*)-hydroperoxide (Scheme 2); as in the case of linoleate [19], small amounts of other isomeric products were detected. Our goal in these studies was to determine whether the stereochemical configuration of the amino acid moiety affects the kinetics and product distribution. If the amino acid moiety binds external to the protein, the activity of SBLO-1 on these substances is expected to show little or no sensitivity to the configuration of the chiral center. There has been a previous report of activity of SBLO-1 on N-arachidonoylamino acids [35], but the work reported here is the first to focus on the stereoselectivity of the action of SBLO-1 on substrates of this kind.



Scheme 2. Structures of Substrates.

2. Materials and methods

2.1. Materials

SBLO-1 was purified by the method of Axelrod [36], and its concentration was determined using $A^{1\%}_{280nm} = 1.6$ [37]. Linoleoyl chloride, methyl L-valine hydrochloride, methyl D-valine hydrochloride, methyl L-tryptophan hydrochloride, and methyl D-tryptophan hydrochloride were obtained from Sigma Aldrich. 13(*S*)-HPOD was produced enzymatically from linoleic acid using SBLO-1 and reduced with $NaBH_4$ to give 13(*S*)-hydroxy-9(Z),11(*E*)-octadecadienoic acid (13(*S*)-HODE). 9(*S*)-HODE, (±)13-HODE, and (±)9-HODE were