Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 31–34

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

NEW MINIMAL SUBSTRATE STRUCTURAL REQUIREMENTS IN THE ENZYMATIC PEROXIDATION OF ALKENES WITH SOYBEAN LIPOXYGENASE

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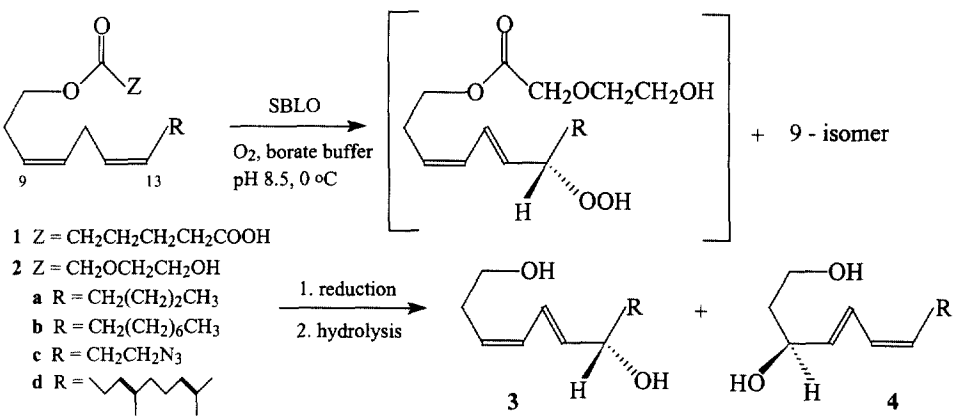
Received 5 August 1998; accepted 10 November 1998

Abstract: A carboxylic or a charged head group in fatty acid analogs is not an essential structural requirement for binding and catalysis in soybean lipoxygenase-1 catalyzed oxidations. Published by Elsevier Science Ltd.

In connection with past studies on the enzymatic oxidation of modified substrates with soybean lipoxygenase-1 (SBLO),[1] a question arose concerning the importance of the carboxyl head group to the design of substrate structure **1**. The carboxyl group in fatty acids, which are substrates for lipoxygenase-1, had long been considered important for enzymatic recognition and binding.[2] However, Harris et al.[3] reported that glycerol esters of linoleic acid bearing an ionic phosphatidylcholine group are also substrates for the enzyme. Furthermore, it was demonstrated that the regiospecificity of lipoxygenase-1 catalyzed oxidations is largely influenced by the hydrophobic interaction within the hydrocarbon units flanking the *cis,cis*-pentadienyl moiety.[4] However, if electrostatic and/or hydrogen-bonding interactions are, at least, partially involved in binding, these results do not exclude the relevance of a charged, or strongly polar, head group as part of the minimal structural requirements. We now report that a charged head group is not an essential structural requirement as illustrated by the oxidation of **2**, which has a nonionic hydroxy terminus. Treatment of **2** with SBLO leads to the expected chiral diols **3** and **4** in good yield with relative rates



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0960-894X/99/$ - see front matter. Published by Elsevier Science Ltd.
PII: S0960-894X(98)00680-5

comparable to that observed for the carboxylated prosthetic group in **1**.[1]

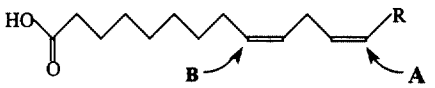
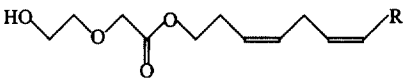
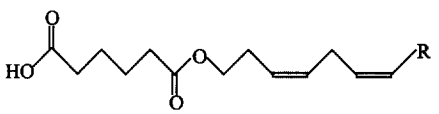
Initially, we attempted to replace the carboxyl head group in the substrate structure **1** by employing a hexanoyl ester group as the prosthetic modifier. However, treatment of such a substrate with lipoxygenase-1 in the presence of oxygen gave only a trace of oxidation product, presumably due to the insolubility of this material in the aqueous buffer. We anticipated that partial water solubility could be attained by introduction of nonionic heterogroups Into the substrate backbone. Therefore, we examined the reaction of the prosthetic modified substrates **2a-d** having the ether-hydroxyl proximal unit.

A 73% isolated yield of the chiral diols **3a** and **4a** in a 99:1 ratio was obtained by incubation of 0.60 g (2.1 mmol) of the partially soluble substrate **2a** in 60 mL of borate buffer pH 8.5 at 0 °C with 400 mg of SBLO[5] (activity = 127,000 units/mg of protein) in the presence of O_2 (0.2 L/min) with vigorous stirring, followed by reduction of the intermediate hydroperoxide and hydrolytic removal of the ester group under conditions described previously.[1,4] A control incubation in the absence of enzyme gave no measurable oxidation. The diol **3a** was obtained in optically active form with a 98% enantiomeric excess (ee) as determined by HPLC analysis of the corresponding (+)-MTPA esters.[6] In keeping with the previous findings for the stereospecificity of lipoxygenase-1,[1,4] this alcohol was found to have the *S*-stereochemistry by comparison of the optical rotation for **3a** with literature values. A similar incubation of substrate **2b** afforded a 10:90 ratio of the isomeric diols **3b** and **4b**. Diol **3b** was also obtained as the *S*-stereoisomer with an optical purity of 96% ee. The regioisomeric diol **4b** was obtained in optically active form having the *S*-configuration (96% ee). The absolute configuration was determined by ozonolytic cleavage in methanol at -78 °C and s equential reduction with $NaBH_4$ to afford the known *S*-1,2,4-butanetriol ($[\alpha]_D$ -12° *c* 0.5 MeOH).

The azido substrate **2c** was readily oxidized by SBLO to afford the azido diol **3c** (98% ee) as the dominant product (**3:4**, 89:11). This was the first example of SBLO oxidation of a compound possessing a nitrogen moiety in the fatty acid portion of the substrate. Furthermore, the azido-diol could be potentially useful for the preparation of chiral amino alcohols. Lastly, the oxidation of **2d** with SBLO afforded diol **4d** as the only detectable regioisomer with a 96% ee. The stereochemistry of **4d** was found to be the *S*-configuration by the ozonolytic method described above.

The oxidation of **2a-d** demonstrates that a carboxyl or a charged head group is not an essential requirement for lipoxygenase-1 binding or catalysis. In addition, the regiospecificity of oxidation for **2a** deserves comment. It was previously reported that the regiospecificity of oxidation is strongly influenced by the hydrophobic difference between the proximal and distal groups.[4] This trend was noted for the series of substrates **1a-f** having terminal groups ranging from C_5H_{11}-$C_{10}H_{21}$ as shown in Table 1. However, these data did not exclude the possibility that a proper "pocket fit" may act in concert with the hydrophobicity effects for controlling the specificity of oxidation. *A priori*, hydrophobic considerations alone predict that the regioisomers **4a**, **4b**, and **4d** would be the predominant products of SBLO oxidation of their corresponding substrates, since the hydrophobicity[7] of the C_5H_{11}(log P =

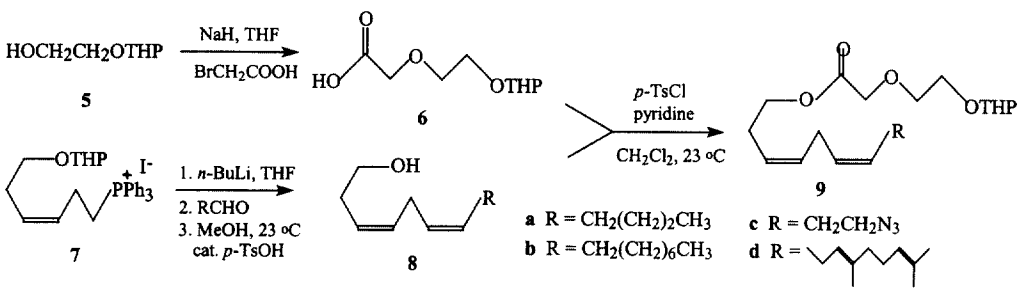
Table 1. Regiospecificity vs. Size of the Proximal and Distal Groups

		R	Proximal(Å)/ Distal (Å)	Regiospecificity (A vs B)
		C_5H_{11}	10.9 / 6.2	97 : 3
	**2a**	C_5H_{11}	10.6 / 6.2	99 : 1
	2b	C_8H_{17}	10.6 / 9.9	10 : 90
	2c	$C_2H_4N_3$	10.6 / 5.9	89 : 11
	2d		10.6 / 10.9	1 : 99
	**1a**	C_5H_{11}	11.9 / 6.2	95 : 5
	1b	C_6H_{13}	11.9 / 7.4	87 : 13
	1c	C_7H_{15}	11.9 / 8.7	76 : 24
	1d	C_8H_{17}	11.9 / 9.9	50 : 50
	1e	C_9H_{19}	11.9 / 11.1	35 : 65
	1f	$C_{10}H_{21}$	11.9 / 12.4	25 : 75
	1g		11.9 / 10.9	1 : 99

+2.82), C_8H_{17} (log P = +4.41), and homogeranyl (log P = +6.1) moieties are much greater than that of the hydroxylated terminus (log P = -3.70). As shown in Table 1, the specificity for **2a** (**3:4**, 99:1) suggests that hydrophobicity alone cannot be the sole determinant of regiospecificity. If the orientation of substrate is solely determined by size of the binding pocket whereby the enzyme preferentially binds the group that has a "proper pocket fit" then substrates **2a** and **2c** with the hydroxylated prosthetic group would be expected to display specificity similar to that of the natural substrate linoleic acid because the proximal groups are of similar lengths (~10.6 A) in all of these compounds. This is illustrated in Table 1. However, a substrate in which the sizes of the proximal and distal groups are nearly equal, as is the case for **2b** and **2d**, would be expected to provide an approximate 1:1 ratio of regioisomeric products. Clearly the regiospecific formation of **4c** and **4d** (**3:4**, 10:90 and 1:99, respectively) reflects a strong preference for binding the more hydrophobic appendage. Consequently, neither the hydrophobic effects nor a size-fit relationship alone determines the orientation of substrate during binding. These findings may have a significant bearing on further synthetic development of this biocatalytic method for the oxidation of alkenes and provide a broader scope for the synthesis of chiral alcohols and amino alcohols.

The substrates were synthesized[8] as shown below. The addition of the tetrahydropyranyl (THP)-protected alcohol

5[9] to NaH in THF at 0 °C followed by the addition of bromoacetic acid afforded a 54% yield of the labile acid **6** after refluxing 24 h. The dienoic alcohols **8a-d** were prepared according to literature procedures,[4] which involved condensation of the ylid derived from the phosphonium iodide **7**[10] with the appropriate aldehydic partner and subsequent removal of the THP protecting group with p-toluenesulfonic acid in methanol. Curiously, coupling of the acid to the dienols proved difficult using standard DCC or Mitsunobu procedures. By contrast, the addition of *p*-toluenesulfonyl chloride (1.4 equiv) and pyridine (2.5 equiv) to the acid **6** (1.5 equiv) in CH_2Cl_2 (23 °C, 30 min) followed by the addition of alcohol **8** afforded the protected substrates **9** in 81-92% isolated yields after workup and chromatography. Removal of the THP-protecting group was best accomplished by treatment of **9** in 20% aqueous dioxane with a catalytic amount of sulfuric acid for 6-8 h at 23 °C to afford the hydroxylated substrates **2a-d** all having >95% *Z,Z*-diene geometry as determined by ^1H NMR and ^{13}C NMR spectroscopy.



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5. Lipoxygenase 1 (Type I, from soybeans) was obtained from Sigma Co. and used without further purification. The enzyme activity was determined by the standard assay procedure: Axelrod, B.; Cheesbrough, T. M.; Laasko, S. *Methods Enzymol.* **1981**, *71*, 441.

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