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RESEARCH ARTICLE

Natural Lipoxygenesis Isolation Technology

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Abstract

This article discusses the isolation of natural lipoxygenase (LOX), an iron-containing enzyme that catalyzes the oxidation of polyunsaturated fatty acids, a multistep biochemical process. Information is presented on the construction of polyunsaturated hydrocarbon chains, known variants of their cross-linking reactions, the condensation of magnesium halide derivatives of terminal acetylenic acids with polyyne propargyl halides, the condensation of magnesium halide derivatives of terminal acetylenic acids with methyl groups of polyunsaturated compounds of the propargyl or allyl type, isolation from natural sources or production by chemical synthesis, and existing approaches to the development of preparative methods for the isolation of HETE and LX from natural sources.

KEYWORDS

Lipoxygenase, enzyme, catalyst, iron-containing, biochemical process, sources, unique climate, hydrocarbon chains.

INTRODUCTION

Lipoxygenases are enzymes that catalyze the oxygenation of polyunsaturated fatty acids, including linoleic, arachidonic, and α-linolenic acids. These enzymes play a key role in regulating physiological processes in plants, animals, and microorganisms and possess considerable biotechnological potential. The present study systematizes the known natural sources of lipoxygenases in Central Asia, including endemic plants, agricultural crops, microbial strains, and algae. It also discusses methods of enzyme isolation, comparative activity, and the prospects for the practical application of lipoxygenases [1].

The products of lipoxygenase activity—hydroperoxides of polyunsaturated fatty acids—play an essential role in numerous biological processes, including inflammation, the formation of plant aromas, fruit ripening, defense against pathogens, photosynthetic reactions, and the biosynthesis of oxylipin hormones.

Lipoxygenases are metalloenzymes containing an iron ion and participating in the oxidative modification of polyunsaturated fatty acids. Their mechanism of action involves the formation of hydroperoxides, which serve as precursors of biologically active molecules such as leukotrienes, jasmonates, volatile aromatic compounds, and oxylipins [2].

Particular interest in lipoxygenases is associated with their practical significance in the following fields:

- the food industry, including the improvement of bread quality and the enhancement of plant-derived aromas;
- pharmacology, where they serve as molecular targets for antitumor and anti-inflammatory drugs;
- the cosmetics industry;
- agricultural technologies and the study of plant stress physiology.

In recent years, lipoxygenases have attracted increasing attention in medicine, biotechnology, pharmacology, and the food industry. In Central Asia, the investigation of natural lipoxygenase sources is particularly relevant because of the region's rich biodiversity and abundance of endemic plant species.

The biological significance of lipoxygenases lies in their involvement in:

- the development of fruit aroma and color;
- plant defense responses;
- the biosynthesis of leukotrienes and prostaglandins in animals;
- seed maturation;
- food fermentation processes.

Central Asia is characterized by a high degree of plant endemism, extreme climatic conditions, and the extensive cultivation of oil-bearing crops. These features create substantial potential for identifying unique natural sources of lipoxygenases. Lipoxygenases exhibit pronounced species specificity, which makes the search for novel enzyme sources particularly valuable.

Major Groups of Natural Lipoxygenase Sources

Source	Examples	Significance
Plants	Soybean, wheat, safflower, cotton, sesame	The most widespread source of lipoxygenases
Microorganisms	Fungi (*Aspergillus*, *Penicillium*) and bacteria (*Pseudomonas*, *Bacillus*)	Suitable for biotechnological production
Animal tissues	Mammalian blood and marine organisms	Sources of 5-, 12-, and 15-lipoxygenases
Algae	Brown and green algae	Contain unique lipoxygenase isoforms

Low-molecular-weight lipid-derived bioregulators, collectively referred to as eicosanoids, occupy an important position among biologically active natural compounds. They are formed in the body through the enzymatic transformation of naturally occurring C20 polyunsaturated fatty acids, including 8Z,11Z,14Z-eicosatrienoic acid, also known as dihomo-γ-linolenic acid; 5Z,8Z,11Z,14Z-eicosatetraenoic acid, or arachidonic acid; and 5Z,8Z,11Z,14Z,17Z-eicosapentaenoic acid, also known as timnodonic acid. The eicosanoid class includes such well-known compounds as prostaglandins (PGs), thromboxanes (TXs), and other related mediators.

These compounds include cyclooxygenase metabolites, as well as mono- and polyhydroxy acids, including leukotrienes (LTs), hepoxilins (Hxs), lipoxins (LXs), and other lipoxygenase-derived metabolites. More recently, a third pathway of polyunsaturated fatty acid metabolism has been identified. This pathway is mediated by cytochrome P450 enzymes and results in the formation of a range of oxygenated metabolites, including epoxyeicosatrienoic acids (EETs), dihydroxyeicosatrienoic acids (diHETs), 19-hydroxyeicosatetraenoic acid (19-HETE), 20-hydroxyeicosatetraenoic acid (20-HETE), and other monooxygenase metabolites.

According to the literature, lipoxins, including 5(S),6(R),15(S)-trihydroxy-7E,9E,11Z,13E-eicosatetraenoic acid (lipoxin A_4, LXA_4) and 5(S),14(R),15(S)-trihydroxy-6E,8Z,10E,12E-eicosatetraenoic acid (lipoxin B_4, LXB_4), are formed through the interaction of 15(S)-hydroperoxyeicosatetraenoic acid (15-HPETE) or 15(S)-hydroxyeicosatetraenoic acid (15-HETE) with leukocytes. They may also be produced through the oxidation of arachidonic acid by soybean lipoxygenase [3].

The formation of eicosanoids in the body is associated with the activation of various cell types and the development of

diverse physiological and pathophysiological processes. These include inflammatory reactions, alterations in smooth muscle tone, immune responses, and various allergic conditions.

At present, mono- and polyoxygenated acyclic lipoxygenase metabolites of fatty acids are among the most intensively studied compounds. Of particular interest are 5-hydroxy-6E,8Z,11Z,14Z-eicosatetraenoic acid (5-HETE), 15-hydroxy-5Z,8Z,11Z,13E-eicosatetraenoic acid (15-HETE), and the products of the double oxygenation of arachidonic acid, namely lipoxins (LXs).

Monohydroxy fatty acids, particularly hydroxyeicosatetraenoic acids (HETEs), exert diverse effects at the cellular level and serve as intermediates in the biosynthesis of lipoxins.

Interest in lipoxins as immunoregulatory agents has increased considerably because of the growing prevalence of diseases associated with immune system dysfunction, including various forms of allergy and cancer, which have become major contemporary health challenges.

Studies of the biological roles of mono- and polyhydroxy fatty acids, including HETEs and lipoxins (LXs), and of their functions in the body require individual reference samples of these compounds. Their radiolabeled analogues are of particular importance. Therefore, the development of preparative methods for the synthesis of hydroxyeicosapolyenoic acids, such as 15-HETE and 5-HETE, polyoxygenated fatty acids, including LXs, and their isotope-labeled analogues represents a relevant research objective.

Three principal approaches may be used to develop preparative methods for obtaining HETEs and LXs: isolation from natural sources, chemical synthesis, and enzymatic synthesis. These biologically active lipid mediators are produced in cells in extremely small quantities; therefore, their isolation from natural sources is impractical. Total chemical synthesis involves numerous stages, is labor-intensive, and requires chiral synthons to obtain the naturally occurring S-configuration. Enzymatic synthesis is particularly promising in this respect, as it enables the production not only of eicosanoids themselves but also of their tritium-labeled analogues. Enzymatic synthesis essentially requires two key components: a substrate, such as arachidonic acid or other polyunsaturated fatty acids, and an enzyme, namely lipoxygenase.

At the beginning of the present study, preparative chemical methods for synthesizing acetylenic precursors of eicosapolyenoic acids had already been reported. However, the overall yields of the target compounds were insufficiently high, which substantially reduced the efficiency of the synthetic process. The key synthetic steps involve the condensation of synthons, particularly terminal acetylenic precursors with derivatives of the propargylic or allylic type [4].

Two variants of cross-coupling reactions for constructing a 1,4-polyunsaturated hydrocarbon chain have been reported:

1. condensation of magnesium halide derivatives of terminal acetylenic acids with polyynic propargyl halides;

2. condensation of magnesium halide derivatives of terminal acetylenic acids with methyl derivatives of polyunsaturated compounds of the propargylic or allylic type.

Polyunsaturated fatty acids, including 5,8,11-eicosatrienoic acid, also known as dihomo-γ-linolenic acid, may be obtained either by isolation from natural sources or by chemical synthesis. Their tritium-labeled analogues can likewise be synthesized using the previously developed acetylenic strategy involving polyynic acids. Selective hydrogenation at the final stage determines the stereochemical direction of the synthesis [5].

The possibility of synthesizing lipoxins from arachidonic acid using a single enzyme, soybean 15-lipoxygenase isolated from natural sources, can be explained by the broad substrate specificity of this enzyme and its ability to further oxidize 15-H(P)ETE through the formation of 5,15-dihydroxyeicosatetraenoic acid (5,15-diHETE). Lipoxins may also be formed through the interaction of 5,15-diHETE with 12-lipoxygenase isolated from porcine leukocytes.

CONCLUSION

We compared the products formed under different conditions of enzymatic lipoxin synthesis using lipoxygenase enzyme preparations, specifically 5-lipoxygenase (5-LO) and 15-lipoxygenase (15-LO).

As a result, samples of lipoxins A_4 and B_4 were obtained in yields of 1.5–2.0%. These relatively low yields indicated the need for a detailed investigation of the synthesis process at each intermediate stage, particularly during the formation of 5-HETE and 15-HETE.

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