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Inhibition of lipoxygenase by sesamol corroborates its potential anti-inflammatory activity

P.S. Yashaswini, A.G. Appu Rao, Sridevi A. Singh *

Department of Protein Chemistry & Technology, CSIR—Central Food Technological Research Institute, Mysuru, 570020, India

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ABSTRACT

Reactive oxygen species, the byproducts of oxygenases reaction, when in excess, promote degenerative diseases like cardiovascular, cancer and arthritis. Sesame lignans- sesamin, sesamolin and the phenolic degradation product of sesamolin, sesamol, are empirically known for their health promoting properties like antioxidant, antimutagenic, antiaging and antiinflammatory activities. In the current study, the effect of sesamol on the inflammatory oxygenase – lipoxygenase (LOX) was investigated. Enzyme kinetics and spectroscopic techniques were used to understand the inhibition mechanism. Sesamol was a potent inhibitor of soy LOX-1. It inhibited soy LOX-1 in a dose dependent manner with IC_{50} value of 51.84 μM and K_i of 4.9 μM. Binding studies using circular dichroism and corroborated by surface plasmon resonance, revealed that sesamol does not bind or change the conformation of LOX. Further, sesamol prevented the conversion of inactive LOX (Fe^{2+}) to active LOX (Fe^{3+}) by arresting the oxidation state of iron and prolonging the lag phase by virtue of its ability to scavenge hydroperoxides. Understanding the mechanism of action of such molecules will help in their application and promotion as nutraceuticals.

1. Introduction

Antioxidants, including bioactive molecules like flavonoids, lignans, phenolic acids, and tannins, are reported to have several biological effects [1]. Lignans, a chemically diverse group of bioactive compounds, are dimers of phenyl propanoids (C6-C3) linked by the central carbon of their side chains. Biepoxy lignans and cyclolignans, occurring in oil seeds- sesame and flax seeds – are known for their strong antioxidant property, both in aqueous and non-aqueous medium [2]. Sesame lignans – sesamin, sesamolin and sesamol, the degradation product of sesamolin, are traditionally known for their antioxidant, anti-aging, antidiabetic and anti-cancerous properties [3,4]. Sesamol, a thermally stable compound, is formed during processing (roasting and decolorization) of oil from sesamolin at high temperature and in presence of moisture [5]. Sesamol (3,4-methylenedioxyphenol) has a benzodiol group that is responsible for its antioxidant activity [6]. Sesamol could exhibit its antioxidant property by radical scavenging activity, metal chelation activity, by electron donation and by acting as chain breaker during the lipid peroxidation process [7]. Sesamol is shown to exhibit anti-inflammatory activity by inhibiting delta-5 desaturase [3], and lipopolysaccharide induced cytokine production [8]. It is also a very potent inhibitor of mushroom tyrosinase, a copper containing dioxygenase [9] and reported to have anti-carcinogenic property [10].

Products released due to the activation of mammalian oxygenases (lipoxygenase, cyclooxygenase and xanthine oxidase) reactions are the major physiological sources of free radicals or reactive oxygen species (ROS) as oxygen is one of the substrates in the reaction [11]. Microbial infection, chemical irradiation and stress also results in ROS generation [12]. ROS, in excess amounts than the enzymatic antioxidants (superoxide dismutase and catalase) in the physiological system, cause imbalance resulting in damaged lipids, proteins and DNA due to increased cellular oxidative stress. Oxidative damage ultimately results in inflammatory pathological conditions like cardiovascular diseases, cancer and neurodegenerative diseases [1,13].

Inflammation, a physiological response to cell damage, is a necessary event, to clear the causative agent and repair the damage caused due to oxidative stress. Improper regulation of the inflammatory process leads to development of chronic inflammatory diseases [14].

Lipoxygenases (LOX) are non-heme iron–containing enzymes that catalyze the stereospecific incorporation of molecular oxygen into polyunsaturated fatty acids with 1,4-*cis, cis* pentadiene moieties [15]. Mammalian lipoxygenases are classified as 5-LOX, 8-LOX, 11-LOX 12-LOX and 15-LOX, based on position of oxygen insertion [16]. Arachidonic acid is metabolised by lipoxygenase to hydrox-

* Corresponding author.
E-mail addresses: ssingh98@yahoo.com, sridevi@cftri.res.in (S.A. Singh).

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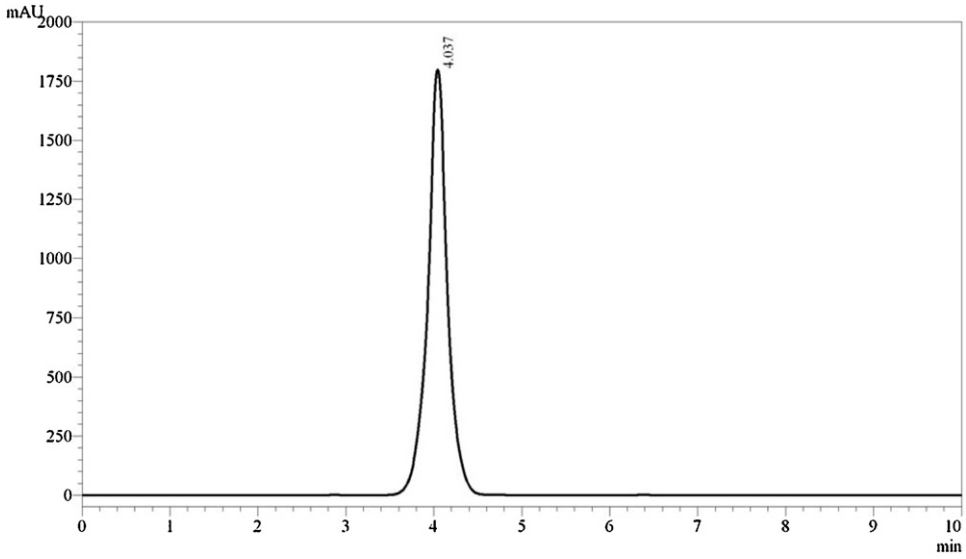

Fig. 1. HPLC profile of sesamol on C_{18} column eluted using mixture of methanol: water (70:30), flow rate 1 mL/ min, detection wavelength 290 nm.

yeicosatetraenoic acid (HETE) and subsequently to leukotrienes, potent proinflammatory mediators that are ligands for G protein coupled receptors and nuclear hormone receptors, which regulate pathways involved in inflammation [17]. Implication of lipoxygenase in pathophysiology of asthma, atherosclerosis, cancer, arthritis and other diseases [18] have provoked the search for effective natural inhibitors of lipoxygenase.

In the present study, the effect of sesamol on the inflammatory oxygenase- lipoxygenase is investigated. Soy lipoxygenase is used as a model inflammatory enzyme to elucidate the mechanism of inhibition by sesamol in detail.

2. Materials and methods

Sesamol, soy lipoxygenase, ferrozine were purchased from Sigma-Aldrich (St. Louis, MO). Purity of sesamol was confirmed by HPLC analysis. Linoleic acid was purchased from Nu-Chek-Prep (Elysian, USA). All other reagents were of analytical grade.

2.1. Homogeneity of sesamol

Sesamol was solubilized in water, filtered through 0.2 μ filter and its homogeneity was analyzed using Shimadzu UFLC (model LC-20AD) equipped with PDA detector. Isocratic mode of elution was employed on Water's reversed phase C_{18} column (250 mm × 4.6 mm, 5 μm) using methanol and water (70:30) as mobile phase at a flow rate of 1 mL/ min. Elution was monitored at 290 nm.

2.2. Assay of lipoxygenase-1 and inhibition by sesamol

Lipoxygenase activity was assayed according to Axelrod et al. [19]. Increase in absorbance at 234 nm, due to formation of

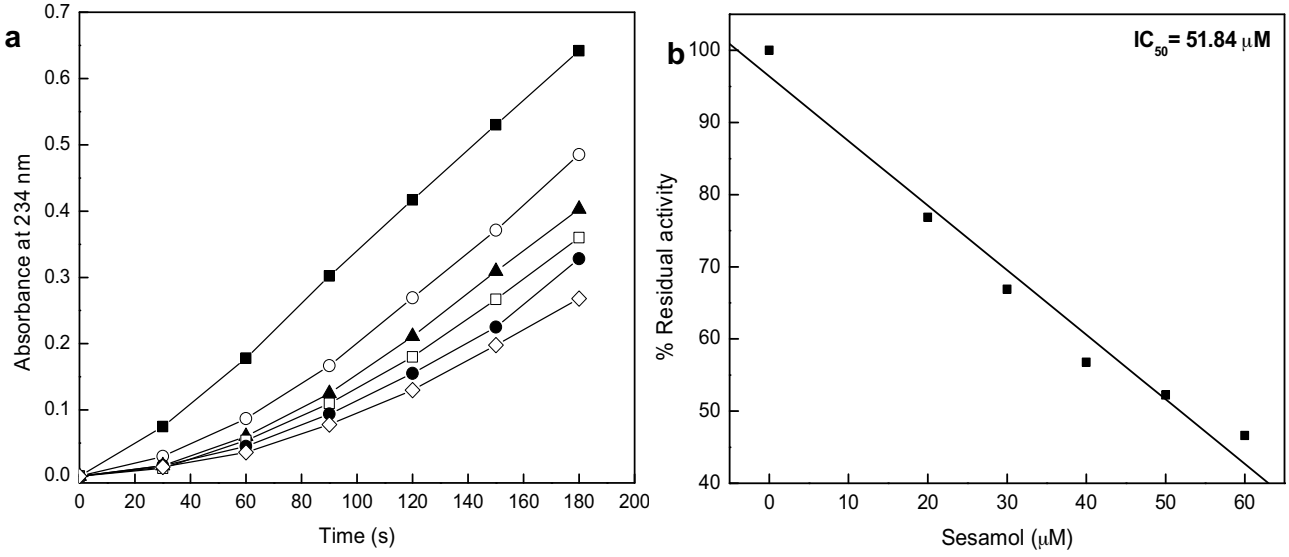

Fig. 2. (a) Time course of lipoxygenase catalysed reaction. Sesamol was incubated with LOX for 3 min in 0.2 M borate buffer, pH 9.0. The reaction was started by addition of 100 μM of linoleic acid and enzyme activity was followed spectrophotometrically at 234 nm. The concentration of sesamol were 0 μM (—■—), 20 μM (—○—), 30 μM (—▲—), 40 μM (—□—), 50 μM (—●—) and 60 μM (—◊—). (b) Inhibition of lipoxygenase by sesamol. LOX was incubated with sesamol for 3 min in 0.2 M borate buffer, pH 9.0. The reaction was started by addition of 100 μM of linoleic acid and LOX activity was followed spectrophotometrically at 234 nm. Sesamol concentrations of 0–60 μM were used for determination of IC_{50} value for LOX inhibition. IC_{50} value of sesamol was found to be 51.84 μM (R^2 = 0.99). Data was obtained as mean values of 3 independent tests.