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Research paper

Interaction of sesamol (3,4-methylenedioxyphenol) with tyrosinase and its effect on melanin synthesis

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ABSTRACT

Sesamin, sesamolin (lignans) and sesamol – from sesame seed (*Sesamum indicum* L.) – are known for their health promoting properties. We examined the inhibition effect of sesamol, a phenolic degradation product of sesamolin, on the key enzyme in melanin synthesis, viz. tyrosinase, *in vitro*. Sesamol inhibits both diphenolase and monophenolase activities with midpoint concentrations of 1.9 μM and 3.2 μM, respectively. It is a competitive inhibitor of diphenolase activity with a K_i of 0.57 μM and a non-competitive inhibitor of monophenolase activity with a K_i of 1.4 μM. Sesamol inhibits melanin synthesis in mouse melanoma B16F10 cells in a concentration dependant manner with 63% decrease in cells exposed to 100 μg/mL sesamol. Apoptosis is induced by sesamol, limiting proliferation. This study of the chemistry and biology of lignans, in relation to the mode of action of bioactive components, may open the door for drug applications targeting enzymes.

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1. Introduction

The major sesame lignans, sesamin and sesamolin, as well as, the degradation product of sesamolin – sesamol, are phenolic and known for their antioxidant [1], anti-inflammatory [2], anti-ageing [3] properties and role in the inhibition of enzymes involved in fatty acid metabolism [4]. The health benefits of lignans are empirically known with little scientific evidence. Sesamol (3,4-methylenedioxy phenol), an excellent antioxidant, has a phenolic and benzodioxole group in its structure and is soluble in both aqueous and organic phase. The benzodioxole group is known to scavenge hydroxyl radicals leading to the formation of 1,2-hydroxybenzene. Continuous or pulsed UV exposure in aqueous solutions is known to generate radicals of the type sesamolyl, benzoquinone anion, cyclohexadienyl in addition to dimer radicals. Sesamol is converted by UV irradiation to a sesamolyl radical or a dimer radical. The antioxidant activity of sesamol is attributed to the reactivity or stability of the sesamolyl radical [5]. The structure of sesame lignans and sesamol is given in Fig. 1.

Melanogenesis, a physiological process, results in the synthesis of melanin pigment, which absorbs free radicals generated in the cytoplasm and shields the host from various types of ionizing radiation that may lead to skin carcinogenesis. In humans and other mammals, the biosynthesis of melanin takes place in melanocytes, which contain the enzyme tyrosinase [6]. The hydroxylation of L-tyrosine, the initial step in melanin synthesis, is of considerable importance, as it controls melanin levels. Tyrosinase, an oxidase, is the rate limiting enzyme for the controlled production of melanin. It is mainly involved in two distinct reactions of melanin synthesis – the hydroxylation of a monophenol and the conversion of an *o*-diphenol to the corresponding *o*-quinone [7]. *o*-Quinone is transformed through a series of non – enzymatic reactions to melanin.

Tyrosinase (EC 1.14.18.1), also known as polyphenol oxidase (PPO) [8,9] is a copper containing mixed-function oxidase, widely distributed in microorganisms, animals and plants. Mushroom tyrosinase, a tetramer with a molecular mass of 120 kD, is composed of two H subunits (43 kD) and two L subunits (13 kD) and contains two active sites, each containing two copper atoms as the cofactors [10]. Tyrosinase is shown to have a type III site, similar to, though not identical with hemocyanin [11]. Tyrosinase has 3 existing forms: '*met*' (E_m)'*deoxy*' (E_d) *and* '*oxy*' (E_o). Both E_m and E_d forms can catalyze diphenol substrates while the E_o form can also catalyze monophenol substrates. E_d can combine with oxygen to form E_o [12]. A structural model, explaining the above, has been proposed for the active site of these 3 forms of tyrosinase [13].

Abbreviations: MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide; UV, ultraviolet; (PPO), Polyphenol oxidase; MBTH, 3-methyl-benzthiazolinone-2-hydrazone; MEM, minimal essential medium; l-DOPA, l-3,4-dihydroxyphenylalanine; DMEM, Dulbecco's modified Eagle's medium.

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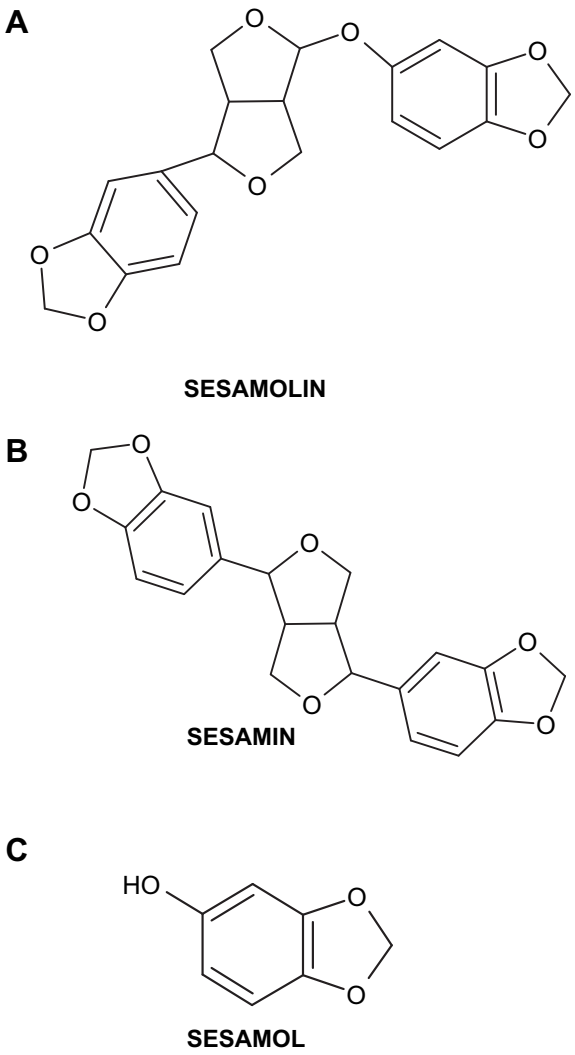

Fig. 1. Chemical structure of sesame lignans and sesamol.

Uncontrolled tyrosinase activity, during melanoma, results in increased melanin synthesis [14]. Several flavonoids, substrate analogues, free radical scavengers and copper chelators have been reported to inhibit tyrosinase [15–18]. In recent times, considerable efforts have been concentrated towards research for the discovery of natural bioactive products (including tyrosinase inhibitors) and their exploitation in medicine and cosmetic formulations for treatment of skin disorders [15]. We have been interested in understanding the effect of sesamin, sesamolin and sesamol on melanin synthesis [19].

In the present study, we have looked into the nature and mechanism of inhibition of tyrosinase activity by sesamol, a phenolic compound formed in roasted sesame oil. The effect of sesamol on tyrosinase activity from mushroom has been used as a model to establish its scheme of action in comparison to mammalian tyrosinase. This is probably one of the early reports of inhibitory effect of lignans on the activity of tyrosinase, which may help to use these naturally occurring lignans as novel tyrosinase inhibitors.

2. Materials and methods

Mushroom tyrosinase (T3824), Sesamol, MBTH, MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide), Acridine orange, Ethidium bromide, L-DOPA and Tyramine hydrochloride used for the bioassay was purchased from Sigma Chemical Co. (St. Louis, MO. USA). All other reagents were of analytical grade.

2.1. Purification of sesamin and sesamolin

Extraction and purification of sesame lignans – sesamin and sesamolin – from commercial white sesame seeds by modification of reported methods [20] was carried out. Sesamin and sesamolin obtained from sesame oil were quantified by analytical RP-HPLC employing a C_{18} (250 × 4.6 mm, 5 μM) column with isocratic elution using methanol: water (70:30 v/v) flow rate: 1 ml/min and detection at 290 nm. The purity of the preparations was ascertained by HPLC as well as GC–MS. Sesamin and sesamolin were recovered from sesame oil to >97% purity. The purity was checked by HPLC and confirmed by GC–MS. The retention times of sesamin and sesamolin on C_{18} column were 13 and 17 min, respectively. Commercially available sesamol (>99% purity) was used for the study. The retention time on the above C_{18} column was 4.2 min. The GC–MS pattern for sesamin, detected the molecular ion radical (M^+) at an m/z of 354 and an intensity of 12%. The main positive fraction ion was generated from electron impact was 149, being the base peak. Similarly, the molecular ion radical of sesamolin was detected at m/z of 370 with an intensity of 15%. The main positive fragment ion generated from electron impact was at 135 as the base peak. These correlated well with reported data [20] and confirmed that the isolated compounds from sesame oil were sesamin and sesamolin.

2.2. Enzyme assay

2.2.1. Diphenolase activity

Diphenolase activity of tyrosinase on L-DOPA was determined by measuring the dopachrome (2-carboxy 2-3-dihydro-indole-5,6-Quinone) accumulation at 475 nm [21]. The assay was performed as 3 mL assay mixture containing 2.0 mM L-DOPA solution in 0.1 M phosphate buffer (pH 6.8) and incubated at 25 °C for 5 min. 30 μL of the aqueous solution of the mushroom tyrosinase (1.9 μg) were added to the mixture to immediately measure the initial rate of linear increase in optical density at 475 nm ($\varepsilon = 3700\ M^{-1}\ cm^{-1}$) using an UV visible spectrophotometer (Shimadzu UV1601 model, Japan). One unit of enzymatic activity was defined as the amount of enzyme increasing 0.001 absorbance at 475 nm in this condition.

2.2.2. Monophenolase activity

Monophenolase activity of tyrosinase on L-tyramine hydrochloride was determined in the presence of 2 mM MBTH [21]. One ml of the reaction mixture consisting on 1 mM tyramine as substrate was incubated with tyrosinase (1.9 μg) in 0.1 M phosphate buffer (pH 6.8) and 25 °C for 5 min. The *o*-quinone formed during the oxidation of tyramine catalyzed by tyrosinase is trapped by MBTH. The reaction was followed at 500 nm ($\varepsilon = 27200\ M^{-1}\ cm^{-1}$). Steady state rate was defined as the slope of linear zone of the product accumulation curve. One unit of monophenolase activity of tyrosinase was defined as the amount of enzyme that produces 1 μM of dopachrome per minute.

2.3. Protein estimation

Protein content was determined by the method of Bradford [22] using bovine serum albumin as standard.

2.4. Cell cultures

B16F10 mouse melanoma cells were cultured in minimum essential medium (MEM) with 10% fetal bovine serum and penicillin/streptomycin (100 IU/50 mg/mL) in a humidified atmosphere containing 5% CO_2 in air at 37 °C.

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