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Enzymatic synthesis of (*R*)-modafinil by chloroperoxidase-catalyzed enantioselective sulfoxidation of 2-(diphenylmethylthio) acetamide

Fengqin Gao[a,b], Limin Wang[a], Yan Liu[a], Shengjie Wang[a], Yucheng Jiang[a,c,*], Mancheng Hu[a,c], Shuni Li[a,c], Quanguo Zhai[a,c]

[a] School of Chemistry & Chemical Engineering, Shaanxi Normal University, Xi'an 710062, PR China
[b] College of Chemistry and Chemical Engineering, Xianyang Normal University, Xianyang 712000, PR China
[c] Key Laboratory of Macromolecular Science of Shaanxi Province, Shaanxi Normal University, Xi'an 710062, PR China

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ABSTRACT

A one-step asymmetric bio-synthesis of (*R*)-enantiomer of the racemic drug modafinil was achieved by chloroperoxidase (CPO)-catalyzed enantioselective sulfoxidation of 2-(diphenylmethylthio) acetamide. Ionic liquids, quaternary ammonium salts or polyhydroxy compounds were introduced into the reaction media to improve productivity. The (*R*)-modafinil yield of 40.8% and high enantiomeric excesses of 97.3% was obtained at pH 5.5 and room temperature in the presence of [EMIM][Br] (V_{ILs}/V_{buffer} = 10%), and a low enzymatic concentration (0.013 mmol L^{-1}) was required. UV–vis and circular dichroism spectral indicated that the α-helix of CPO was strengthened and the heme became more exposed for easier access of substrate to the active site in CPO in the presence of the above additives, and moreover, enzymatic kinetic data showed that the affinity and the specificity of CPO to substrate was improved.

1. Introduction

Modafinil is a very efficient pharmaceutical agent for the treatment of excessive sleepiness caused by narcolepsy, sleep disorders and obstructive sleep apnea [1]. In comparison to modafinil, the (*R*)-enantiomer of the racemic drug has a long half-life due to its slower metabolism and excretion, resulting in greater drug exposure time and consequently a longer action duration [2].

Several methods for the enantioselective preparation of modafinil (sulfoxides) have been reported. The separation of the diastereomeric salts of modafinil acid and the determination of the absolute stereochemistry of (+)- and (−)-modafinil were initially reported by Prisinzano et al. in 2004 [3], but the route was very tedious. A continuous chiral chromatography separation method was developed recently [4]. However, it was abandoned as the cost of the crystallization process was high [5]. Using metal or metal compounds as catalysts can be an alternative [6,7], but most of these methods suffered from a number of disadvantages, including the use of expensive oxidants or the reagents that would cause serious environmental problem [8]. Moreover, some catalysts suffered from low catalytic efficiency [9]. Microbial method [10] was also employed to synthesize chiral sulfoxides. Though the use of whole-cell microorganisms was more practical, one of the problems frequently encountered was the occurrence of side reactions as the coexistence of several enzymes.

Enzymatic method has established itself as a scalable and green technology for the preparation of a broad range of pharmaceutical [11]. Enzymatic processes are usually conducted under mild conditions (close to ambient temperature and atmospheric pressure) in water, with high rates and selectivity. Furthermore, enzymatic synthesis generally obviates the need for functional group protection and/or activation, generates less wastes and is more energy efficient than conventional organic syntheses [12]. In this work, an enantioselective synthesis protocol was presented by chloroperoxidase (CPO) catalyzed sulfoxidation of 2-(diphenylmethylthio) acetamide. CPO is recognized as a versatile catalyst for various organic transformations including halogenation, oxidation, peroxidation, epoxidation and hydroxylation with high enantioselectivity

* Corresponding author at: School of Chemistry& Chemical Engineering, Shaanxi Normal University, Xi'an, No. 620 West Chang'an Road, Chang'an District 710119, PR China. Tel.: +86 29 81530763; fax: +86 29 81530727.
E-mail addresses: gaofq08@snnu.edu.cn (F. Gao), limin.w@163.com (L. Wang), liuyan2012@stu.snnu.edu.cn (Y. Liu), wangshengjie@snnu.edu.cn (S. Wang), jyc@snnu.edu.cn (Y. Jiang), hmch@snnu.edu.cn (M. Hu), lishuni@snnu.edu.cn (S. Li), zhaiqg@snnu.edu.cn (Q. Zhai).

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Nomenclature

CPO	chloroperoxidase
R_z	enzyme purity
MCD	monochlorodimedon
TBHP	*tert*-butyl hydrogen peroxide
H_2O_2	hydrogen peroxide
PEG400	polyethylene glycol 400
PEG600	polyethylene glycol 600
TMABr	tetramethylammonium bromide
TEABr	tetraethylammonium bromide
TPABr	tetrapropylammonium bromide
TBABr	tetrabutylammonium bromide
[EMIM][Br]	1-ethyl-3-methylimidazolium
[PMIM][Br]	1-propyl-3-methylimidazolium
[BMIM][Br]	1-butyl-3-methylimidazolium
[AMIM][Br]	1-amyl-3-methylimidazolium
AD-H	chiral column type
ILs	ionic liquids
QAS	quaternary ammonium salts

[13–16], especially, the epoxidation and sulfoxidation catalyzed by CPO cause great interests [17,18]. However, the actual technical application of CPO was limited because CPO is a highly hydrophilic enzyme, but the poor solubility of organic substrates in aqueous solution prohibits the yield. Three kinds of additives were introduced into the reaction media in order to enhance the yield. The influence of these additives was investigated in detail. The strategy of this one-step enzymatic-catalyzed enantioselective sulfoxidation to prepare (*R*)-modafinil assisted by additives would have potential application in pharmaceutical industry.

2. Material and methods

2.1. Enzyme and chemicals

Caldariomyces fumago was cultured according to the method established by Morris and Hager, but chloroperoxidase was isolated from the growth medium of the fungus using acetone rather than ethanol in the solvent fractionation step. Then, CPO was further purified by DEAE-Sephadex A-50 ion exchange column chromatography (Xi'an shengtai, Co. Ltd). The amounts of CPO was defined by concentration ($\mu mol\,L^{-1}$), which was determined based on its characteristic absorption at 398 nm which was caused by the π–π^* electron transition of the porphyrin in active site, and using a molar extinction coefficient of 91,200 $L\,mol^{-1}\,cm^{-1}$. The molecular mass of CPO is approximately 42 kDa [19]. The enzyme solution was concentrated to 11.8 $mg\,mL^{-1}$ CPO with R_z 1.20 (R_z = purity standard = A_{398}/A_{280} = 1.40 for pure enzyme) and activity of 7928 U/mL based on the standard monochlorodimedon (MCD) assay [20].*tert*-Butyl hydrogen peroxide (TBHP), hydrogen peroxide (H_2O_2 30% in aqueous solution), polyethylene glycol 400 (PEG400), polyethylene glycol 600 (PEG600), tetramethylammonium bromide (TMABr), tetraethylammonium bromide (TEABr), tetrapropylammonium bromide (TPABr), tetrabutylammonium bromide (TBABr) were from Sinopharm Chemical Reagent Co. Ltd.; Nuvigil (standard sample) was from medicine sample provider; 2-(diphenylmethylthio) acetamide,1-ethyl-3-methylimidazolium [EMIM][Br], 1-propyl-3-methylimidazolium [PMIM][Br], 1-butyl-3-methylimidazolium [BMIM][Br], 1-amyl-3-methylimidazolium [AMIM][Br], were purchased from Aldrich. All chemicals are of analytical grade unless otherwise indicated.

2.2. Procedure of synthesis of (R)-modafinil

2-(Diphenylmethylthio) acetamide (0.05 mmol) was put into 3.0 mL 0.1 $mol\,L^{-1}$ aqueous phosphate buffer (pH 5.5) in a 5.0 mL test tube and magnetically stirred at 25 °C for about 30 min. CPO (0.04 μmol) was added into the solution and stirred continually. Then, oxidant TBHP (0.15 mmol) was added one-time whereas H_2O_2 (0.15 mmol) was added step-wise in small aliquots. The reaction was quenched by a saturated sodium sulfite solution and extracted 3 times by methylbenzene. Combined organic extracts was purified by rotary evaporation, and dried by magnesium sulfate. The procedure was the same in the presence of additive.

2.3. HPLC and LC–MS analysis

Chiral liquid chromatography analyses were performed on a LC-20 AT high performance liquid chromatograph equipped with AD-H (0.46 cm × 25 cm, 5 μm) chiral column (Daicel chemical industries, Co. Ltd.) and UV detector at 225 nm. The column temperature was maintained at 30 °C. The eluent was HPLC grade methanol with a flow rate of 0.50 $mL\,min^{-1}$. The products were resolved by methanol for analysis by Agilent 1100-Esquire 6000 liquid chromatography–mass spectrometry (LC–MS) (Genmany Bruker).

Both chemical yields and enantiomeric excesses of modafinil were determined in a single chromatogram based on their consistent elution order during HPLC analysis compared with the standard *R*-modafinil by external standard method.

2.4. Measurements of enzymatic kinetic parameters

Kinetic assay for CPO – catalyzed sulfoxidation of 2-(diphenylmethylthio) acetamide was carried out over the substrate amount of 0.05–10.00 mmol, while TBHP amount was kept constant as 15 mmol in 3.0 mL reaction solution. The reaction showed Michaelis–Menten kinetics characteristics. The kinetic parameters including Michaelis constant (K_m), catalytic turnover frequency (k_{cat}), and second-order rate constants (k_{cat}/K_m) were measured in pure buffer and in the presence of additives by linear regression analysis of the double-reciprocal Lineweaver–Burk plots [21].

The activation energy E_a value was obtained through the k_{cat} determined at 288 K, 293 K, 298 K and 303 K respectively.

$$v^{-1} = \left\{\frac{k_m}{V_{max}}\right\}[S]^{-1} + V_{max}^{-1}$$

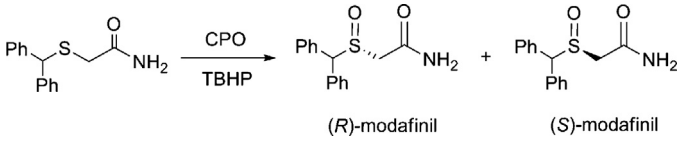
$$V_{max} = k_{cat}[E_0]$$

where E_0 was the initial concentration of CPO.

3. Results and discussion

3.1. Synthesis of (R)-modafinil in buffer solution

The one-step synthetic strategy of (*R*)-modafinil is described as Scheme 1. HPLC spectrum (Fig. 1) indicated two configurations of modafinil were well separated and the retention time was respectively 10.2 min and 13.3 min, which were characteristic retention time of (*R*)- and (*S*)-modafinil. The predominant enantiomer produced was (*R*)-modafinil compared with standard sample. The



Scheme 1. Sulfoxidation of 2-(diphenylmethylthio) acetamide catalyzed by CPO.