Journal of Catalysis 315 (2014) 33–40

Contents lists available at ScienceDirect

Journal of Catalysis

journal homepage: www.elsevier.com/locate/jcat

Indigo dye production by enzymatic mimicking based on an iron(III)porphyrin

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ARTICLE INFO

Article history:
Received 10 February 2014
Revised 15 April 2014
Accepted 18 April 2014
Available online 15 May 2014

Keywords:
Indigo
Indole
Iron(III)porphyrin
Oxidation
Catalysis
Hydrogen peroxide
Mechanism
Ethanol

ABSTRACT

A novel indigo synthesis is based on a simple and cost-effective model system of the enzymes involved in the natural and biocatalytic productions. The method considers the oxidation of indole by hydrogen peroxide, being catalyzed by an iron(III)porphyrin in ethanol, as solvent, and no other additives. The yields of indigo and of the other oxidized indolinoid derivatives were found to be dependent on the metalloporphyrin system used and on the control of the oxidation conditions. Significant reductions of the environmental impact relatively to the present industrial production and of the costs relatively to the biocatalytic methodologies were obtained. The enhanced indigo production in the presence of the iron(III)porphyrin-ethanol catalytic system relatively to the manganese(III)porphyrin-acetonitrile system can be rationalized by the formation of different active species in the two systems.

1. Introduction

Indigo is a historical dye used by almost all the ancient civilizations and with relevance until our days, mainly due to its incidence as the primary color of blue jeans [1]. Other indigoid compounds are also prominent targets in textile industries and for alimentary, medicinal and cosmetic purposes [2–4].

Until the late 19th century, indigo was obtained from natural sources and, with the advent of the modern chemical industry, became one of the first natural molecules to be synthesized [5]. Today, almost all the traded indigo is produced synthetically and, in general, the branded industrial production is based on variations of the Pflegers's method. In the largely used process, the synthesis involves the reaction of aniline, formaldehyde and hydrogen cyanide, affording phenylglycinonitrile that is then hydrolyzed to *N*-phenylglycine. Subsequently, the *N*-phenylglycine is treated with molten mixtures of caustic soda and sodamide at 200 °C to afford indoxyl, which undergoes further oxidative dimerization to form indigo [6]. The processes are highly energy demanding, result in the production of high amounts of toxic waste products, and require sophisticated purification processes [7].

The high production rates and the amount of hazardous wastes have been raising significant environmental concern that have stimulated the reediting of the natural production [8], and since the work of Ensley et al. in 1983, the microbial productions have been considered as an important eco-sustainable alternative [9]. These authors reported an *Escherichia coli* mutant encoding the enzyme naphthalene dioxygenase (NDO) that was able to synthesize indigo from indole. Several other mutant strains have been tested in order to improve the methodology [10,11], which is still expensive and has significant drawbacks in obtaining pure indigo from the culture media (fermentation broth). Enzymatic systems were also tested, such as cytochrome P450 monooxygenases [12], non-heme oxygenases such as NDO [13], and engineered mutants [14,15]. Recently, myoglobin mutants were developed and succeed to directly activate hydrogen peroxide; the system allowed to avoid the use of expensive co-factors, NADPH or NADH, required for the activation of molecular oxygen, thus affording a more practical and green indigo production [16]. The best indigo yield obtained was 12%, based on the consumed indole [16]. However, the cost and purification drawbacks in enzymatic synthesis also need to be considered and, furthermore, significant quantities of

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http://dx.doi.org/10.1016/j.jcat.2014.04.012
0021-9517/

indigo structural isomers, namely indirubin, are produced concomitantly.

Alternative processes using chemical methodologies are scarce; one of these approaches refers to the indigo synthesis with an organometallic catalyst and an organic hydroperoxide, in an anhydrous media [17]; another strategy is the non-catalytic oxidation of indole by *meta*-chloroperoxybenzoic acid (*m*-CPBA) [18]. However, both processes led to the introduction of high amounts of expensive reactants in the reaction mixture, hindering product isolation, and the eco-sustainability of the processes, in terms of by-products release, cost and energy consumption was still compromised.

In the context of cytochrome P450 mimicking by metalloporphyrin catalytic systems, several novel and relevant synthetic routes have been opened, using mild conditions [19–22]. Simultaneously, an improved understanding on the reactivity patterns of the catalytic and biological systems has been achieved, specifically the nature of the porphyrin nucleus, the central metal, and the reaction conditions (protic/aprotic solvent and the type of additives) showed a prominent effect on the reactivity pattern of the catalytic system [23,24], which was rationalized by the tuning of the active species formed [25].

The recently reported oxidation of indole in the presence of different Mn(III)porphyrins, using acetonitrile as solvent and a co-catalyst, afforded a very poor indigo yield that, after optimization, reached only 3% [26]. In the present work, a complimentary Fe(III)porphyrin catalytic system was developed for indole oxidation by hydrogen peroxide. In addition, ethanol was considered as a protic and eco-sustainable solvent [27–29]; furthermore, ethanol is less expensive relatively to other organic solvents, can be obtained from biomass, is biodegradable, and can be used as a fuel.

2. Materials and methods

2.1. Chemicals

Aqueous hydrogen peroxide 30% (w/w) [$H_2O_{2(aq)}$], urea–H_2O_2 adduct compound (UHP), and *m*-chloroperoxybenzoic acid (*m*-CPBA, 77%) were purchased from Aldrich. All other reagents (>95%) and solvents (*p.a.*) were of high purity and used as received. Silica gel 60 (0.063–0.200 mm) was used for column chromatography, and thin layer chromatography (TLC) was performed using silica gel 60 with F_{254} indicator.

2.2. Instruments

Electronic spectra were recorded on a range cell Cary 50 BIO spectrometer. NMR spectroscopy was performed on a Brucker Avance III spectrometer at a frequency of 400.15 and 100.63 MHz for ^{1}H and ^{13}C NMR, respectively. The mass spectra were acquired with electrospray or fast atom bombardment ionization in positive modes, ES^+ or FAB^+, respectively, at Unidad de Masas, Universidad de Santiago de Compostela. GC/MS analyses were carried out on a Finnigan Trace GC/MS (Thermo Quest instruments) using helium as the carrier gas (35 cm/s), a DB-5-type fused silica capillary column (30 m, 0.25 mm i.d., 0.25 μm film thickness), and the mass spectra were acquired with electron impact ionization at 70 eV.

2.3. Metalloporphyrin synthesis

The metalloporphyrin catalyst chloro[5,10,15,20-tetrakis(4-dimethylamino-2,3,5,6-tetrafluorophenyl)porphyrinate]iron(III), denominated as FeP (Fig. 1), was prepared using an adapted literature procedure [30]. The synthesis of the 5,10,15,20-tetrakis(pentafluorophenyl)porphyrin ligand (H_2TPFPP) was performed under microwave heating, in acetic acid as the reaction solvent, replacing the usual hazardous mixture of nitrobenzene and acetic acid. The complexation of the porphyrin was obtained by the reaction with iron(II) chloride at reflux in DMF and in the presence of pyridine [26]. MS FAB^+ [FeP], *m/z* $[M-Cl]^+$ = 1128.2 (Fig. S1).

2.4. Indigo production in the presence of FeP under optimized conditions

Indole (35.1 mg, 0.3 mmol) and the catalyst (1.0 mg, 0.3%) were placed on a round-bottom flask and dissolved in 2 mL of ethanol. The mixture was left under magnetic stirring at room temperature (~20 °C) and the hydrogen peroxide was progressively added in a total amount of 0.6 mmol for 20 min [adequate solutions of H_2O_2 in ethanol (1:10 v/v) were prepared to allow the accurate addition]. The reaction was stopped by removing the excess of catalyst and H_2O_2 by passing the reaction mixture through a small plug of silica gel and washing it with hexane/ethyl acetate (1:1). The catalyst, a colored polar metalloporphyrin is not eluted through the silica column in these conditions and is retained in the top of the column. The collected mixture was evaporated to dryness under vacuum and the residue diluted in an exact volume of dimethyl sulfoxide (DMSO). Since indigo is insoluble in common HPLC reversed-phase solvents, the amount of indigo was determined by UV–Vis spectrophotometry in comparison with a calibration curve obtained with standard solutions of indigo in DMSO.

Otherwise, the precipitation of indigo was obtained through immersion of the reaction mixture in water, followed by filtration through filter paper and washing with water and then with ethanol to afford pure indigo. The resulting water and ethanol filtrate fractions were evaporated to dryness and fractionated by preparative TLC using a mixture of hexane/ethyl acetate (2:1) as eluent; the isolated products were characterized by NMR spectroscopy and mass spectrometry (Supporting Information).

2.5. Catalytic reactions in the presence of the MnP–CH_3CN system

Indole (35.1 mg, 0.3 mmol), 1.0 mg of catalyst (0.3%), and 40 mg of ammonium acetate were placed in a round-bottom flask and dissolved in 4 mL of acetonitrile. The mixture was left under magnetic stirring at room temperature (~20 °C) and the hydrogen peroxide was progressively added in a total amount of 1.2 mmol for 20 min (adequate solutions of H_2O_2 in acetonitrile (1:10 v/v) were prepared to allow the accurate addition). The reactions were left to proceed for 10 min. Quantification of indigo was obtained as described for the FeP–EtOH system.

2.6. Non-catalytic oxidation with m-CPBA

Indole (35.1 mg, 0.3 mmol) and K_2CO_3 (19 mg) were placed in a round-bottom flask and dissolved in 4 mL of solvent. When ethanol was used as solvent, the system was denominated *m*-CPBA–EtOH; when the solvent was dichloromethane, the system was denominated *m*-CPBA–CH_2Cl_2. The mixture was left under magnetic stirring at room temperature (~20 °C) and 103 mg of *m*-CPBA (0.6 mmol) were added. The reaction was stopped after 20 min and the quantification of indigo was obtained using the same protocol described for the catalytic oxidation in the presence of the FeP–EtOH system.

2.7. ^{1}H NMR of the total reaction mixture

In order to determine the yields of the other reaction products, the reactions were performed following the appropriate procedure, and after passing through the short silica column, the eluate was evaporated to dryness and the residue was dissolved in DMSO-d_6