Archives of Biochemistry and Biophysics 544 (2014) 18–26

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

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

Review

Heme-dependent dioxygenases in tryptophan oxidation

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ARTICLE INFO

Article history:
Available online 1 December 2013

Keywords:
Metalloprotein
Oxygen activation
Peroxide reaction
Reactive oxygen species
High-valence iron
Free radical

ABSTRACT

L-Tryptophan is an essential amino acid for mammals. It is utilized not only for protein synthesis but also for the biosynthesis of serotonin and melatonin by the serotonin pathway as well as nicotinamide adenine dinucleotide by the kynurenine pathway. Although the kynurenine pathway is responsible for the catabolism of over 90% of L-tryptophan in the mammalian intracellular and extracellular pools, the scientific field was dominated in the last century by studies of the serotonin pathway, due to the physiological significance of the latter's catabolic intermediates and products. However, in the past decade, the focus gradually reversed as the link between the kynurenine pathway and various neurodegenerative disorders and immune diseases is increasingly highlighted. Notably, the first step of this pathway, which is catalyzed by heme-dependent dioxygenases, has been proven to be a potential target for immune regulation and cancer treatment. A thorough understanding of the intriguing chemistry of the heme-dependent dioxygenases may yield insight for the drug discovery of these prevalent illnesses. In this review, we survey enzymatic and mechanistic studies, initially started by Kotake and Masayama over 70 years ago, at the molecular level on the heme-dependent tryptophan dioxygenation reactions.

Introduction

Hemoproteins perform a wide range of biochemical functions including oxygen transport and storage, gas sensing, electron transfer, and chemical catalysis. The utilization of heme iron for dioxygen activation and oxygen insertion into organic substrates is prevalent in nature, with the most well-known examples being the heme-dependent monooxygenation reactions catalyzed by cytochrome P450s. Notably, hemoproteins rarely express dioxygenase activity as the native biological function. Thus far, only a few examples have been identified in lipid metabolism (fatty acid α-dioxygenase, prostaglandin H synthase, and linoleate diol synthase), tryptophan oxidation (tryptophan 2,3-dioxygenase and indoleamine 2,3-dioxygenase), and natural rubber degradation (rubber oxygenase). Heme-dependent dioxygenases are distinctive members of the dioxygenase family in that they utilize a histidine-coordinated heme rather than a non-heme iron or manganese to facilitate dioxygen activation and oxygen insertion reactions.

Tryptophan 2,3-dioxygenase (TDO)[1] is the first functionally defined heme-dependent dioxygenase [1–3]. It was initially referred to by several different names: tryptophan peroxidase-oxidase, tryptophan pyrrolase, and tryptophan oxygenase. TDO employs a *b*-type ferrous heme to catalyze the oxidative cleavage of the indole ring of L-tryptophan (L-Trp), converting it to *N*-formylkynurenine (NFK) (Scheme 1). In mammals, TDO is mainly a hepatic enzyme that participates in the initial and rate-limiting step of the kynurenine pathway, which is the primary route of L-Trp degradation [4–9]. The kynurenine pathway constitutes the major part of the *de novo* biosynthesis of nicotinamide adenine dinucleotide (NAD), an essential life-sustaining redox cofactor, in eukaryotic organisms and in some bacterial species [10,11]. In addition to mammals, TDO is also present in other sources such as insects and bacteria [3,10,12–14].

Hayaishi et al. discovered an isozyme of TDO in 1967 [15,16]. This enzyme is named indoleamine 2,3-dioxygenase (IDO) because it exhibits a much broader substrate-specificity than TDO. While TDO is highly specific for L-Trp, IDO can tolerate a collection of indoleamine derivatives, including D-Trp, tryptamine, and serotonin [17–21]. IDO participates only in the kynurenine pathway of mammals and is ubiquitously distributed in all tissues except the liver [20–22]. Although TDO and IDO were identified decades ago, their crystal structures were not solved until recently [14,23–25]. IDO is crystallized as a dimer with a disulfide bond connecting the two monomeric units [23], whereas TDO consists of four subunits arranged in a dimer of dimer quaternary structure [14,24,25] (Fig. 1). The two enzymes share only ~10% sequence identity but exhibit similar active-site architectures [14,23–25]. Recently, a potential TDO/IDO superfamily has been proposed upon incorporating another heme-dependent tryptophan-utilizing enzyme, PrnB, which possesses a common structural core as TDO and IDO [26].

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[1] *Abbreviations used:* TDO, tryptophan 2,3-dioxygenase; IDO, indoleamine 2,3-dioxygenase; L-Trp, L-tryptophan; 1-Me-L-Trp, 1-methyl-L-tryptophan; NFK, *N*-formylkynurenine; NAD, nicotinamide adenine dinucleotide; ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid); cmTDO, *Cupriavidus metallidurans* TDO; xcTDO, *Xanthomonas campestris* TDO; dmTDO, *Drosophila melanogaster* TDO; hTDO, human TDO; H-bonding, hydrogen-bonding; DFT, density functional theory; MD, molecular dynamics; QM/MM, quantum mechanics/molecular mechanics; •NO, nitric oxide; WT, wild-type; 2MI, 2-methylimidazole; IPNS, isopenicilin *N* synthase.

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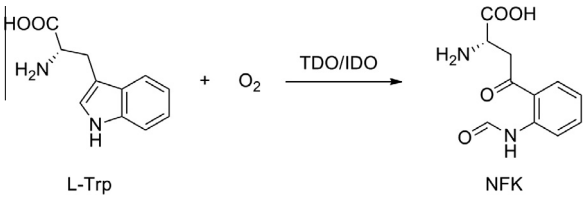
http://dx.doi.org/10.1016/j.abb.2013.11.009



Scheme 1. The chemical reaction catalyzed by TDO and IDO.

During the past decade, TDO and IDO have attracted enormous attention because of their physiological importance. IDO is inducible by interferon-γ as a result of inflammation and thus is highly related to immune functions [27–29]. It is proposed that local depletion of cellular L-Trp by IDO inhibits the growth of certain pathogens, contributing to innate host immune response [27–30]. However, in contrast, a growing body of evidence demonstrates that increased expression of IDO is frequently linked to host immunosuppression. It can promote immune tolerance under various physiological and pathophysiological conditions, which causes serious problems including maternal fetal tolerance and immune escape of cancer [20,27,29–36]. In recent years, TDO has been found to be expressed in tumor cells, and the expression of TDO has been shown to play an immune-regulatory role in many cancer systems via preventing tumor rejection, much like the reports for IDO [35,37]. These findings make it extremely beneficial to characterize the biochemical properties and elucidate the catalytic mechanism of TDO and IDO for inhibitor design and drug discovery.

The catalytic mechanism of oxygen activation and insertion for P450-type monooxygenases is well studied. It features a compound I intermediate, which is a ferryl species (Fe(IV)=O) coupled with a cation porphyrin radical [21,38]. The compound I intermediate is a catalytically competent oxidant and is able to insert the ferryl oxygen into organic substrates [39]. It should be noted, however, heme-dependent monooxygenation consumes electrons (from NADH/NADPH) and protons with one of the atoms of O_2 being reduced to water. In contrast, the TDO/IDO reaction does not consume any electrons or protons from external sources. Thus, the dioxygenation reaction is fundamentally distinct from those monooxygenation reactions in terms of oxygen reduction. This review seeks to synthesize recent findings on the mechanistic studies of TDO and IDO and share our perspectives on several critical aspects of the catalytic properties of these two isozymes, including their reactivity towards hydrogen peroxide, the involvement of high-valence ferryl species in the reaction cycle, and the catalytic roles of a distal histidine residue in TDO.

Reactivities towards hydrogen peroxide and physiological relevance

P450-type monooxygenases exhibit a "peroxide shunt" pathway in which the ferric form of enzymes can interact with single-oxygen donors such as peroxides, leading to direct formation of the compound I intermediate [21,38] (Scheme 2A). Compared to the native Fe(II)- and O_2-dependent reaction pathway, this alternative pathway allows the catalytic cycle to be completed without the participation of electron donors and associated electron transfer proteins.

The ferrous heme of TDO and IDO is the catalytic center that binds and activates dioxygen. Like many other Fe(II)-dependent enzymes, TDO and IDO become auto-oxidized in aerobic environments when the substrate L-Trp is absent. For quite a long time, the reactions involving hydrogen peroxide and the resting ferric state of TDO and IDO received little attention, despite several very interesting phenomena reported from discrete studies. For

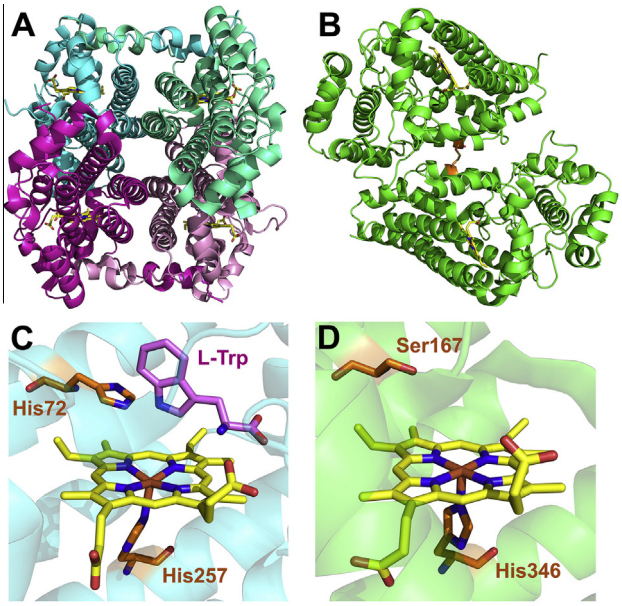


Fig. 1. Crystal structures of TDO and IDO. (A) The quaternary structure of TDO is tetrameric, arranged in a dimer of dimer pattern (PDB entry: 2NOX). Each subunit contains a *b*-type heme that is labeled in yellow. (B) IDO is crystallized as a dimer with a disulfide bond connecting the two monomeric units (PDB entry: 2DOT). The hemes are labeled in yellow and the two cysteine residues of the disulfide bond are labeled in orange. (C) The active-site architecture of substrate-bound TDO (PDB entry: 2NW8). The heme center is coordinated to a proximal histidine residue (His257 in *Cupriavidus metallidurans* TDO (cmTDO) amino acid numbering). The enzyme-bound L-Trp (labeled in pink) is H-bonded to a distal histidine residue (His72 in cmTDO amino acid numbering). (D) The active-site architecture of substrate-free IDO (PDB entry: 2DOT). The distal histidine residue present in TDO is replaced by a serine residue (Ser167) in IDO.

example, it was reported over 60 years ago that hydrogen peroxide is able to activate the resting ferric state of TDO in the presence of L-Trp [9]. This observation was later confirmed by independent studies from different laboratories and further proven by the observation that the activation effect is inhibited by catalase [3,40,41]. More than 30 years ago, IDO was found to possess peroxidase activity [18] and H_2O_2-dependent monooxygenase activity [42], but no studies regarding whether these activities occur in a physiologically meaningful context ensued. It was not until recently, when considerable attention was attracted to these research directions, that significant progress was made. As detailed below, a H_2O_2-mediated enzyme reactivation mechanism has been proposed in TDO based on intensive biochemical and spectroscopic investigations [43]. Several consecutive mechanistic studies have highlighted the versatile activities of IDO towards H_2O_2, which have revealed the fundamental differences between TDO and IDO in their peroxide reactions [44–47].

Hydrogen peroxide-mediated enzyme reactivation pathway in TDO

Although the activation of ferric TDO by H_2O_2 in the presence of L-Trp was discovered in 1950 [9], the mechanism of this phenomenon remained a mystery. We have recently demonstrated via unequivocal spectroscopic (optical and Mössbauer) evidence that ferrous TDO can be produced upon addition of H_2O_2 to ferric TDO in the presence of L-Trp [43]. Through an enzymatic assay with carbon monoxide (CO) as an inhibitor, the freshly generated ferrous enzyme is proven to be the catalytically competent species that gives rise to the observed dioxygenase activity [43]. As shown in Scheme 2B, a two-phase enzyme reactivation mechanism is proposed to illustrate how ferric TDO is reductively reactivated by