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Biochemical and Biophysical Research Communications 338 (2005) 12–19
BBRC
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Review

Biochemical and medical aspects of the indoleamine 2,3-dioxygenase-initiated L-tryptophan metabolism ☆

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Received 11 August 2005
Available online 15 September 2005

Abstract

Indoleamine 2,3-dioxygenase (EC 1.13.11.42) is a heme-containing dioxygenase which catalyzes the first and rate-limiting step in the major pathway of L-tryptophan catabolism in mammals. Much attention has recently been focused on the dioxygenase because this metabolic pathway is involved not only in a variety of physiological functions but also in many diseases. In this review, the discovery and unique catalytic properties of dioxygenase are described first, and then the recent findings regarding the dioxygenase-initiated tryptophan metabolism are summarized, with special emphasis on the detrimental role of dioxygenase in side effects of interferon-γ and interleukin-12 (by systemic tryptophan depletion), the escape of malignant tumors from immune surveillance (by immunosuppression caused by tryptophan depletion), several neurodegenerative disorders including Alzheimer's disease (by an aberrant production of neurotoxin, quinolinic acid), and age-related cataract (due to "Kynurenilation," a novel post-translational modification of lens proteins with tryptophan-derived UV filters).

Keywords: Heme-containing dioxygenase; Tryptophan; Interferon-γ; Immunosuppression; Dendritic cells; Quinolinic acid; Alzheimer's disease; Age-related cataract; Kynurenilation; Neurotoxin; Metabolism; Indoleamine dioxygenase

Although it is not easy to evaluate individual scientific achievements without bias, nobody doubts that one of the biggest breakthroughs in understanding of biological oxidation is the discovery of "Oxygenases" by Profs. Hayaishi [1] and Mason [2] in 1955. Thanks to this discovery, we now know that many different varieties of living matters can transform diverse biological constituents into appropriate materials by the direct incorporation of either one or two oxygen atoms of atmospheric dioxygen into the substrates of such biological constituents. In mammals, oxygenases are involved in the synthesis of vital compounds including prostaglandins, leukotrienes, steroid hormones, and nitric oxide. They also work in the synthesis of neurotransmitters (i.e., serotonin and catecholamine). In this tribute to the celebration of the discovery of oxygenases 50 years ago, I will highlight indoleamine 2,3-dioxygenase (IDO) discovered by Prof. Hayaishi and his co-workers in 1967. In the last decade, the IDO-initiated tryptophan metabolism has been attracting much interest because of its great potential for understanding, treating, and preventing human diseases, as pointed out by Prof. Raymond Brown in the closing remarks in the 10th meeting of the International Study Group for Tryptophan Research (ISTRY) held at Padova in Italy in 2002 [3]. I quote his words to explain why IDO is so important in human diseases as noted below.

> "In these several sessions we heard exciting new data which indicate the critical role of induced IDO activity in several important areas. The activation of dendritic cells (macrophages, astrocytes, etc.) results in IDO induction with the depletion of tryptophan levels locally

☆ *Abbreviations:* IDO, indoleamine 2,3-dioxygenase; TDO, L-tryptophan 2,3-dioxygenase; IFN, interferon; Kyn, L-kynurenine; 3HKyn, 3-hydroxy-L-kynurenine; 3HKG, 3-hydroxy-L-kynurenine glucoside, AHBG, 4-(2-amino-3-hydroxypheny)-4-oxobutanoic acid glucoside; XA, xanthurenic acid; QA, quinolinic acid; DC, dendritic cells.

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0006-291X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2005.09.032

or systemically. This seems to be the mechanism by which interferon inhibits the growth of certain bacteria, intracellular parasites, and viruses. Most exciting were the reports that tryptophan depletion also inhibits T lymphocyte replication which results in immunosuppression and tolerogenicity. This has far reaching implications in many fields of medicine, including fetal rejection, and organ transplant survival. However, in some cases the effects of the IDO activity may be the result of elevated kynurenine pathway metabolites rather than the depletion of tryptophan. For example, many studies have described the excitatory and toxic effects of quinolinic acid on neuronal activity. Furthermore, the depletion of tryptophan leads to decreased levels of serotonin which may produce a wide range of effects. In closing, I would like to ask us all to recognize Professor Osamu Hayaishi for his brilliant, pioneering work in elucidating the tryptophan metabolism, particularly for his important finding of indoleamine dioxygenase (IDO) and for induction by interferon-c. These findings now allow us to reinterpret many older empirical observations of the tryptophan metabolism in animals and man, which has now opened up a whole new era of better understanding the role of tryptophan in the fields of immunology, AIDS, organ transplant, autoimmune diseases, cancer, and mental functions. The next few years will, I am sure, see unbelievable advances in these areas of biology and medicine.''

In these three years after the ISTRY 2002 meeting, as predicted by Prof. Raymond Brown, there have been marked advances in the IDO-initiated tryptophan metabolism in many areas. As one of the IDO researchers, it is an honor for me to describe the discovery, and the unique catalytic properties of IDO, and the recent exciting findings about the IDO-initiated tryptophan metabolism, which is so relevant to the treatment of many human diseases.

Discovery of IDO

L-Tryptophan (Trp) is the least abundant of the essential amino acids for mammals. It is utilized not only for protein synthesis but also for the synthesis of a neurotransmitter, serotonin. A small part of serotonin is further converted into melatonin in the pineal body in the brain. It is well known that only a tiny amount (about 1%) of Trp from food is converted to serotonin and most (more than 95%) dietary Trp is metabolized along the kynurenine (Kyn) pathway, thus leading to the biosynthesis of NAD or the complete oxidation of the amino acid (Fig. 1). Some of the metabolites in the Kyn pathway are biologically active as described later. In 1937, Kotake and Masayama [4] first found the existence of an enzyme in the liver of mammals which catalyzes the first step of the Kyn pathway, namely, the conversion of Trp to *N*-formyl Kyn. The liver enzyme is purified and it was identified as a heme-containing dioxygenase [5]. This enzyme is highly specific to the L-isomer

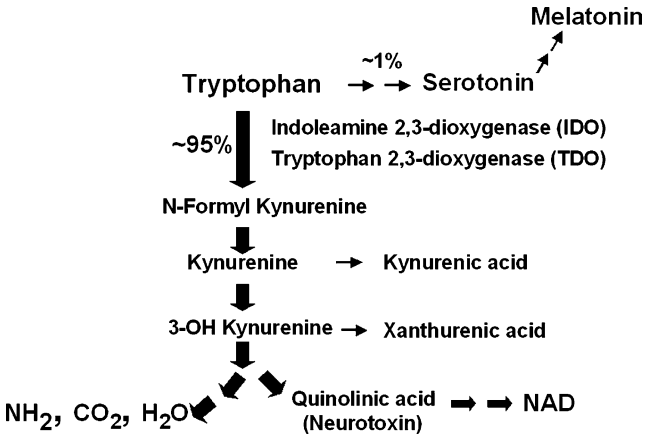

Fig. 1. The major metabolic pathway of L-tryptophan in humans.

of Trp [5,6] and therefore it is called L-tryptophan 2,3-dioxygenase (TDO) (EC 1.13.1.12). TDO is expressed in the liver but not in the other tissues [7], except for the mouse early concepti [8] and rat skin [9]. On the other hand, the finding by Kotake and Ito in 1937 [10] that rabbits fed D-Trp excreted D-Kyn in the urine suggested that an enzyme occurs in the extrahepatic tissues which catalyzes the same oxidative ring cleavage of D-Trp. In 1967, Higuchi, Yamamoto, and Hayaishi [11,12] discovered such an enzyme capable of catalyzing the conversion of D-Trp to *N*-formyl-D-Kyn in the rabbit intestine [11,12]. In 1978, the new enzyme was purified from the rabbit intestine and it was identified as a heme-containing dioxygenase similar to TDO [12,13]. The enzyme was named indoleamine 2.3-dioxygenase (IDO) since the enzyme exhibits a broad substrate specificity: it oxidized not only D-Trp but also L-Trp [12], D- and L-5OH-Kyn, tryptamine, and serotonin [13]. Although TDO is almost completely localized in the liver, IDO is expressed in various rabbit tissues such as the lung, small and large intestine, colon, spleen, kidney, stomach, and brain [14].

Properties of human IDO

In 1985, the existence of human IDO was examined in various human tissues obtained from 9 corpses within 10 h after death and from the fresh term placenta after delivery [15]. As seen in the rabbit, IDO was expressed in many human tissues and relatively high activities were detected in the lungs and intestines. In addition, the highest expression of IDO was found in the term placenta [15]. In 1988, human IDO was purified from the placenta and it was demonstrated to have a similar broad substrate specificity to that of rabbit IDO, except that the human enzyme was inactive for serotonin [16].

Structure of IDO

A cDNA encoding human [17,18], mouse [19], or rat IDO [20] has been cloned and the deduced primary

structure of each IDO was obtained. Human IDO cDNA encodes a protein of 403 amino acids with a molecular weight of about 45 kDa [17,18]. Mouse and rat IDO cDNA encodes a 407 amino acid protein [19]. The primary sequence of human IDO shows either 57% or 58% identity to that of mouse IDO or rat IDO, respectively, but there is no sequence homology between human IDO and rat TDO [21], even though they catalyze the same oxygenation reaction. Human IDO is a hemoprotein but there is no sequence identity or similarity to other mammalian heme-containing proteins such as hemoglobin, myoglobin, or cytochrome P-450.

The catalytic properties of IDO

Purified IDO is a ferric form (Fe^{3+}) of hemoprotein which is completely inactive for oxygenation reaction in vitro, and it therefore requires an artificial reducing system to maintain its active reduced (Fe^{2+}) form [22]. The reducing system routinely used in vitro is a combination of methylene blue (a redox dye) with ascorbic acid [12], and ascorbic acid is replaced with xanthine oxidase [12] or glutathione reductase [23]. In these systems, superoxide anion (O_2^-), which is generated as a result of a reduction of molecular oxygen by the reduced form of methylene blue (leuko-methylene blue), plays a role in the reduction of the ferric form of IDO to the ferrous form because superoxide dismutase, a scavenger of superoxide anion, partially inhibits the IDO activity [23–25]. The residual activity of IDO that is not inhibited by superoxide dismutase is due to the direct reduction of ferric IDO to the ferrous one by leuko-methylene blue.

Is IDO a scavenger of superoxide anion?

It has been suggested [26] that IDO functions as an anti-oxidant because it utilizes a superoxide anion which reduces the inactive ferric form to an active ferrous form in vitro [22]. However, the ferrous form is easily oxidized to the ferric form again, thus resulting in the release of superoxide anion [22]. As a result, IDO never decomposes superoxide anion like superoxide dismutase and therefore it does not work as an anti-oxidant enzyme.

Endogenous reducing system for IDO

The active ferrous IDO is rapidly autooxidized to the inactive ferric form (Fe^{3+}) in the absence of a reducing system [22]. Dihydroflavin mononucleotide [27] and tetrahydropteridine [28] have been proposed as endogenous cofactors for the reduction of IDO based on an in vitro study. However, their role in the IDO reduction in vivo has not yet been assessed. Based on the fact that in erythrocytes a reducing enzyme system is present which maintains the ferrous active form of hemoglobin [29], a similar reducing system specific for IDO has been suggested to exist. Iwamoto and Kido [30] examined the possible occurrence of such an endogenous IDO reducing system in the human term placenta, where IDO is expressed at a high level [15], and they found the existence of IDO activator in the extract of the placenta [30]. However, the molecular properties of the placental activator remain to be elucidated.

Implication of IDO in the defense mechanism against various infectious pathogens

The physiological function(s) of IDO expressed in many tissues remained unknown for more than 10 years after its discovery in the rabbit intestine in 1967. However, in the late 1970s, Yoshida and Hayaishi [31] discovered that IDO in the mouse lung is markedly induced (up to 100-fold) during either bacterial endotoxin shock or during an influenza virus infection [32]. Such IDO induction caused an enhancement of Trp degradation along the Kyn pathway in the mouse body, which was indicated by a parallel elevation of the blood Kyn level and urinary Trp metabolite (i.e., xanthurenic acid) with the induction of IDO in various mouse tissues during endotoxin shock [33]. These findings suggested first that the degradation of Trp by IDO thus plays a role in the defense mechanism against bacterial and viral infections. In this context, in 1984 Pfefferkorn [34] found that Trp depletion caused by IDO induction is a mechanism of the anti-proliferative activity of IFN-γ against *Toxoplasma gondii* in human fibroblasts. After this discovery, the anti-parasitic or anti-viral activity of IFN-γ against various pathogens has been shown to be explained by the same mechanism, namely, the IDO-mediated Trp depletion from human cultured host cells. The pathogens sensitive to intracellular Trp depletion in human cells in vitro are *Chlamydia* (*C.*) *psittaci* [35], *C. trachomatis* [36], *C. pneumoniae* [37], group B *Streptococci* [38] and *Enterococci* [39], *Staphylococcus aureus* [40], herpes simplex virus [41], cytomegalovirus [42], and measles virus [43]. These results indicate that one of the physiological functions of IDO is the suppression of various intracellular pathogens through Trp depletion.

Detrimental effects of the systemic IDO induction

In most cases of infection with viruses (e.g., influenza virus) and parasites (e.g., *Chlamydia*), the induction of IDO is limited to the tissues infected with the pathogens. For example, during a pulmonary infection with an influenza virus, the IDO induction occurred in the lung [32] and such a local induction of IDO seems to effectively suppress the growth of pathogens through IDO-mediated tryptophan deprivation from the infected area, as suggested from the in vitro studies with cultured cells where IDO induced by IFN-γ was able to suppress the intracellular growth of pathogens as described above. However, a systemic induction of IDO occurs during endotoxin shock [33], and this may thus cause a systemic tryptophan deprivation from the body. In fact, as shown in Fig. 2, when

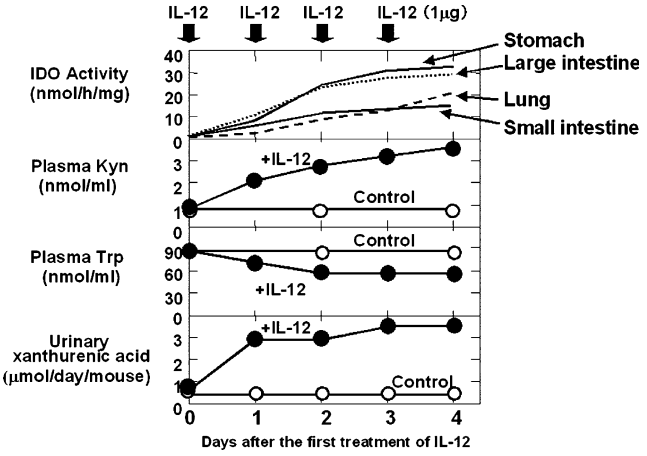

Fig. 2. The systemic Trp depletion caused by treatment of mice with interleukin-12 into mice. Male 8-week-old C57BL/6J mice (5 mice per point) were treated with interleukin-12 (1 μg/mouse/0.2 ml) intraperitoneally, and at the indicated times, the IDO activities in the lung, small intestine, large intestine, and seminal vesicle, plasma Trp and Kyn, and urinary XA were determined as described previously [33]. Interelukin-12 (Genzyme) was dissolved in phosphate-buffered saline containing 0.1 mg bovine serum albumin/ml. Control mice were treated with the vehicle. Each value represents the mean value.

IDO in C57BL/6 mice was systemically induced by an intraperitoneal injection of interleukin-12 (IL-12), which is well known to activate a subset of T-cells and natural killer cells thereby producing a large quantity of IFN-γ [44], a significant decrease in the levels of tryptophan due to a systemic Trp catabolism was observed with a concomitant increase in the blood Kyn level and the urinary level of xanthurenic acid (XA), the major metabolite of blood Kyn in C57BL/6J mouse [33]. This IDO induction and the enhancement of Trp catabolism were not seen when IFN-γ-deficient mice were used, thus indicating that the effects of IL-12 are mediated by IFN-γ (data not shown). A similar unhealthy and dangerous decrease (by about 50%) in the level of serum tryptophan associated with an increase in the urinary excretion of tryptophan metabolites was demonstrated for the patients bearing tumors treated with an intravenous injection of IFN-γ as a clinical trial [45]. Under this condition, it is most likely that the decrease in blood Trp results in a decrease in the synthesis of serotonin in the human brain stem and gut enterochromaffin cells. This phenomenon may also help to explain some of the side effects of IFN-γ, such as depression, anxiety, and diarrhea [46], and these side effects may limit the therapeutic application of IFN-γ only to renal caner [47] and mycosis fungoides [48].

Immunosuppressive role of IDO in immune system

The fundamental function of the immune system is to discriminate between self and non-self. The question remains as to why the maternal immune system cannot recognize the genetically different (allogeneic) fetus as non-self in the placenta. The molecular mechanisms underlying this maternal-tolerance toward the allogeneic fetus are still unknown. In 1998, Munn et al. proposed the hypothesis that the placental IDO prevents the rejection of the fetus. This was based on the finding that a pharmacological inhibition of the placental IDO rejected the fetus in mice [49]. This hypothesis was supported by their subsequent findings that either macrophages or a subset of dendritic cells (DC), which express IDO, were shown to suppress the T-cell responses by depriving T-cells of Trp in vitro [50,51]. The immunosuppressive activity of IDO was then applied to inhibit the T-cell-mediated rejection of allografted pancreas islets in mice and such IDO-expressing islets have also been demonstrated to show a significant prolongation of graft survival [52]. Similarly, it has recently been shown that the T-cell-mediated experimental asthma was inhibited by the up-regulated pulmonary IDO [53]. Based on these findings in vitro and in experimental mouse systems, the concept that cells expressing IDO can suppress the T-cell responses and induce tolerance has thus emerged as a new paradigm in immunology [51].

Escape of tumor cells from the immune surveillance by IDO expression

Immune escape is a crucial property of cancer progression. In 2003, Uyttenhove et al. [54] discovered that most of human malignant tumor cells express IDO, and using a mouse model system, they showed that tumors expressing IDO at a high level effectively escape the immune surveillance of the host by degrading local Trp, which thus inhibits T-cell responses as described above. In the model system, the same tumor, but expressing little or no IDO, was easily recognized by the immune system and rejected from the host animals [54]. These findings suggested that the extent of IDO expression in tumor cells can thus be used for the prognosis of such tumors. In this context, Okamoto et al. [55] have recently examined the possible relationship between the extent of IDO expression in human serous-type ovarian cancer and overall survival, and have found that the 50% survival of patients classified as sporadic (IDO^{low}), focal ($IDO^{moderate}$), and diffuse (IDO^{high}) was 41, 17, and 11 months, respectively. This result clearly shows that IDO is a marker for a poor prognosis of serous-type ovarian cancer.

Production of neurotoxin, quinolinic acid in neurodegenerative disorders

Some of intermediate metabolites of the Kyn pathway of Trp metabolism (Fig. 1) are neuroactive [56]. Of these metabolites, much attention has been focused on quinolinic acid (QA) because it causes neural death by direct intracerebral injection [57] or when applied to neurons in culture in vitro [58]. This toxicity is due to the activation of the subpopulation of neural glutamate receptors sensitive to *N*-methyl-D-aspartate (NMDA) [59]. The accumulation of QA within the brain occurs in a broad spectrum of patients

or experimental animals with inflammatory neurologic diseases such as the acquired immunodeficiency (AIDS) dementia complex caused by an infection with immunodeficiency virus type 1 (HIV-1) [60], poliovirus brain infection [61], cerebral malaria [62], and ischemic brain disease [63]. The cerebrospinal fluid levels of QA were correlated well with the severity of the neurological deficits [60], thus suggesting that QA plays a direct role in the pathogenesis of neurodegenerative disorders. The production of QA closely reflects the local induction of the first and rate-limiting enzyme, IDO, in the Kyn pathway within the central nervous system [60]. It is therefore most likely that the up-regulation of IDO in the brain results in the accumulation of QA and the resultant neural degenerations.

IDO induction and QA accumulation in Alzheimer's disease

Alzheimer's disease (AD) is the most common form of dementia that affects millions of people around the world. As AD increases with the increasing age of the population, it is estimated that the number of AD patients will reach as many as 16 million in United States alone [64]. Although the precise etiology of AD is still unknown, quite recently, in 2005, using immunostaining techniques Guillemin et al. [65] demonstrated the Kyn pathway to be up-regulated in the AD brain, thus leading to increases in neurotoxin QA. In the hippocampus of the AD brains, which is one of the vulnerable regions in AD, both IDO expression and QA accumulation were detected in the cortical microglia, astrocytes, and neurons [65]. Considering that IDO is induced in various types of inflammation, as described above, and that multiple and complex inflammation occurs in AD progression [66], the observed up-regulation of IDO and the accumulation of QA are thus considered to be quite feasible. As a result, QA may be involved in the neurodegeneration in AD. This conclusion is supported by the fact that a soluble oligomer of amyloid β peptide ($A\beta_{1-42}$), which has been considered to play a central pathogenic role in AD [67], has been shown to activate the microglia in vitro while also inducing IDO expression and QA production in the cells [68].

Synthesis of UV filters in human lens

The function of the primate lens is not only to transmit and focus light, but also to filter a UV light between 300 and 400 nm to protect the retina from the UV light. The UV filter compounds in the human lens are Kyn, 3-hydroxy-L-kynurenine (3HKyn), 3-hydroxy-L-kynurenine glucoside (3HKG), and 4-(2-amino-3-hydroxypheny)-4-oxobutanoic acid glucoside (AHBG) (Fig. 3). These UV filters are synthesized locally from tryptophan in the lens epithelial cells [69] and they are also present at a high level in the lens. For example, the major filter, 3HKG, is present at the millimolar level [70] and AHBG is found at 0.2–1.3 mM [71]. AHBG is derived from 3HKG by the slow deamination of the α-amino group of 3HKG and subsequent reduction of the α,β-unsaturated intermediate of

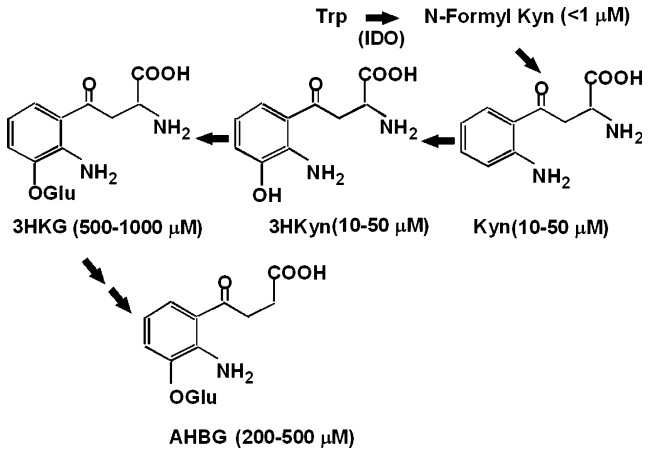

Fig. 3. Biosynthesis of UV filters in the human lenses.

3HKG [71]. IDO is thus the first, rate-limiting enzyme in the UV filter biosynthetic pathway in the human lens [72].

Is Kynurenilation of lens proteins with UV filters involved in age-related cataract?

With age, the human lens becomes progressively more yellow and fluorescent, and such yellowing is much more prominent in age-related nuclear cataract, resulting in an irreversible loss of vision [73]. The underlying cause for this age-related disease has been poorly understood for a long time. However, the pivotal role of the tryptophan-derived UV filter pathway in the age-related lenticular change has been elucidated over the past 5 years by Truscott's group in Australia. Their new finding is based on the discovery that the deaminated 3HKG binds covalently to lens proteins (crystallins) in 1999 [74]. With little or no protein turnover in the lens [75], the UV filter adduct tends to accumulate with age. In fact, the 3HKG-protein adducts, which are yellow and fluorescent, are also detected at low levels in young lenses (20–30 years) but they increase dramatically (5–10 times) with age (over 60 years) [74]. Their levels from 60- to 85-year-old lenses are 728 ± 437 pmol/mg of lens proteins ($n = 16$) [74]. Kyn, which is present at 10–50 μM in the lens, has also been found to form yellow and fluorescent adducts with lens proteins by a reaction mechanism similar to 3HKG. The Kyn adducts also increase with age and the level in aged lenses is of the order of 500–800 pmol/mg of lens protein [76]. 3HKyn, which is present at a level similar to that of Kyn, can react with lens proteins and produce yellow fluorescent adducts [77], but the amount of 3HKyn adducts in lenses has not yet been determined because of the complex reaction of 3HKyn with lens protein [78]. On the other hand, yellow and fluorescent products attributable to the Maillard reaction between reducing sugars and lens proteins have been proposed to be implicated in the yellowing of the human lens [79]. Indeed, vesperlysine A (LM-1) [80] and pentosidine [81], the products of the Maillard reaction, are found in lens proteins. The amounts of these compounds

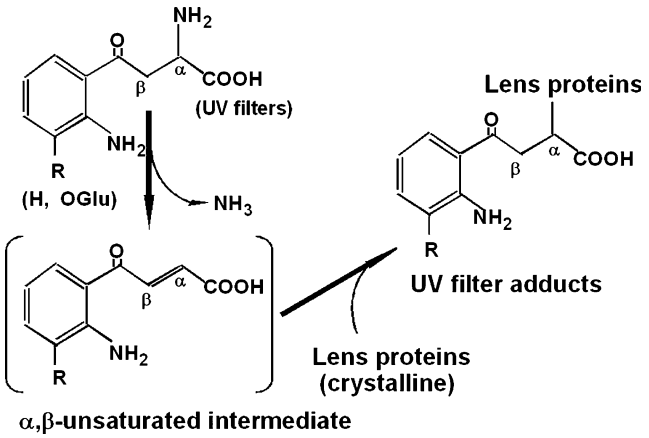

Fig. 4. The mechanism of Kynurenilation of lens proteins with UV filters.

gradually increase with aging, but even in 80-year-old lenses, their levels are 2–20 pmol/mg of protein, being 30–500 times less than the UV filter adducts. Furthermore, 3HKyn is highly autooxidizable and it also generates H_2O_2 during the process of oxidation [82,83]. Oxidation is a hallmark of age-related nuclear cataract [84–86]. Considering that the level of H_2O_2 in the aqueous humor is low or undetectable (less than 5 μM) [87], 3HKyn is a potential internal source of H_2O_2 in the lens and it may also be involved in the oxidation of crystallins in the cataractous lens. Based on these findings, the UV filter adduct formation designated as "Kynurenilation" and the oxidation of lens proteins possibly by H_2O_2 generated from 3HKyn seem to play an important role in the yellowing of the lens and they may also play a crucial role in age-related cataract. It may therefore be possible to prevent or reduce the Kynurenilation and oxidation of lens proteins by lowering the levels of UV filters in the lens. Since IDO is the first and rate-limiting enzyme in the UV filter synthetic pathway and the expression of IDO is unchanged even in the aged lenses [72], IDO inhibition may therefore reduce lens levels of the reactive UV filters (Kyn, 3HK, and 3HKG) (Fig. 4).

Conclusions

IDO plays an important physiological role in the defense mechanism against a variety of infectious pathogens, in the regulation of T-cell function by macrophages and a subset of DC, and in the synthesis of UV filters in human lenses. However, serious problems arise from the unregulated over-expression of IDO, which often results in a deleterious systemic Trp depletion and/or the accumulation of neurotoxin, QA in the brain. The IDO expression in malignant tumors helps them to avoid the immune surveillance through a local Trp depletion. The "Kynurenilation" of the lens protein with UV filters thus appears to be the major cause of age-related cataract. As a result, IDO plays both a physiological and pathological role in our body. Since IDO is a rate-limiting enzyme of the Kyn pathway leading to these diseases, IDO is therefore considered to be an ideal therapeutic target for all of these diseases.

Acknowledgments

I thank Profs. Hayaishi, Que, and Yamamoto for giving me this opportunity to contribute to this memorial volume celebrating the discovery of "Oxygenases" 50 years ago [1,2].

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