Journal of Photochemistry & Photobiology, B: Biology 198 (2019) 111546

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

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

Comparative studies on picosecond-resolved fluorescence of D-amino acid oxidases from human with one from porcine kidney. Photoinduced electron transfer from aromatic amino acids to the excited flavin

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A B S T R A C T

Fluorescence dynamics of human D-amino acid oxidase (hDAAO) and its five inhibitors have been studied in the picoseconds time domain, and compared with one in D-amino acid oxidase from porcine kidney (pkDAAO) reported. The fluorescence lifetimes were identified as 47 ps in the dimer, 235 ps in the monomer, which are compared with those of pkDAAO (45 ps–185 ps). The fluorescence lifetimes of the hDAAO did not change upon the inhibitor bindings despite of modifications in the absorption spectra. This indicates that the lifetimes of the complexes are too short to detect with the picosecond lifetime instrument. Numbers of the aromatic amino acids are similar between the both DAAOs. The fluorescence lifetimes of hDAAO were analysed with an ET theory using the crystal structure. The difference in the lifetimes of the dimer and monomer was well described in terms of difference in the electron affinity of the excited isoalloxazine (Iso*) between the two forms of the protein, though it is not known whether the structure of the monomer is different from the dimer. Three fastest ET donors were Tyr314, Trp52 and Tyr224 in the dimer, while Tyr314, Tyr224 and Tyr55 in the monomer, which are compared to those in pkDAAO, Tyr314, Tyr224 and Tyr228 in the dimer, and Tyr224, Tyr314 and Tyr228 in the monomer. The ET rate from Trp55 in hDAAO dimer was much faster compared to the rate in pkDAAO dimer. A rise component with negative pre-exponential factor was not observed in hDAAO, which are found in pkDAAO.

1. Introduction

D-Amino acid oxidase (DAAO) exists in a wide range of species from yeasts to human [1–5]. DAAO contains flavin adenine dinucleotide (FAD) as a cofactor, of which function is to oxidize D-amino acids to the corresponding imino acids, producing ammonia and hydrogen peroxide. Mammalian DAAO has been demonstrated to connect with the brain D-serine metabolism and to the regulation of the glutamatergic neurotransmission [6,7]. Various novel inhibitors to human DAAO have been found [8–10].

Human DAAO (hDAAO) consists of 340 amino acids and one FAD per monomer. Crystal structure of hDAAO has been determined by Kawazoe et al. [11]. Characteristics of hDAAO was compared to those of porcine kidney DAAO (pkDAAO) [12]. The hDAAO forms dimer in solution, though tetramer in crystal, while pkDAAO is in an equilibrium state between monomer and dimer in relatively dilute solution [13]. Apo hDAAO is also dimer [12], while monomer in pkDAAO [14].

Time-resolved fluorescence spectroscopy of pkDAAO in picoseconds time domain has been reported by Nakashima et al. [15–17]. Fluorescence lifetimes of dimer and monomer of pkDAAO are 45–185 ps, which are much shorter than one of free FAD (2.3–2.5 ns) [18,19]. The shorter lifetimes are ascribed to fast photoinduced electron transfer (ET) from aromatic amino acids as tryptophanes (Trps) and tyrosines (Tyrs) [20–22]. The ET rates are closely related to conformational changes of flavoproteins near isoalloxazine (Iso) ring of flavins [23–31].

Comparing to pkDAAO, physico-chemical property of hDAAO is little known. It is very important to examine sub-microscopic structure of hDAAO. In the present work we have studied a picosecond-resolved fluorescence of free hDAAO and its complexes with five kinds of inhibitors, and analysed the ET rates from aromatic amino acids to the excited Iso (Iso*), using its crystal structure, and compared with those of pkDAAO.

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https://doi.org/10.1016/j.jphotobiol.2019.111546
Received 31 December 2018; Received in revised form 20 June 2019; Accepted 28 June 2019
Available online 19 July 2019
1011-1344/