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Enzyme assay for D-amino acid oxidase using optically gated capillary electrophoresis-laser induced fluorescence detection☆

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ABSTRACT

Because D-amino acids (AAs) play an essential role in the regulation of many processes in living cells, detection of D-AAs and assay of D-amino acid oxidase (DAAO) activity are of vital importance in bioanalytical science. However, the reliability and accuracy of DAAO assays could be interfered due to the facts that DAAO presents broad substrate activity towards different D-AAs and there could be abundant L-AA enantiomers in biological samples. In this study we presented the first application of optically gated capillary electrophoresis with LIF detection (OGCE-LIF) for efficient assay of DAAO activity. High repeatability of the OGCE-LIF assay of amino acids (AAs) was achieved with relative standard deviation (RSD) (n = 15) less than 1.5% and 2.7% for migration time and peak height, respectively. Under the optimal OGCE-LIF conditions, five pairs of D/L-AA enantiomers were efficiently separated in less than 1 min with low limit of detection of 1.3 μM. Enzymatic assays of DAAO were successfully achieved by detection the substrate consumption with OGCE-LIF, for either single or mixed AA substrates. Kinetic analysis of the parallel oxidation reactions of two different substrates was performed, which was in good agreement with the experimental results. Our study indicates OGCE-LIF can perform rapid and efficient separation of mixed pairs of AA enantiomers and is a promising method for quantitatively assaying DAAO catalyzed reaction with the presence of L-AA enantiomers in the sample. Our study would pave the way for accurate determination of D-AAs and DAAO enzymes in complicated biological samples.

1. Introduction

D-Amino acid oxidase (DAAO, EC 1.4.3.3) is a flavoprotein that catalyzes the oxidative deamination of D-amino acids (D-AAs) to the corresponding α-keto acids, hydrogen peroxide and ammonia (Scheme 1) [1,2]. It is now realized that D-AAs play an important role in the regulation of many processes in living cells. Recent progresses in the detection of D-AAs have linked DAAO to aging, neural signaling and hormone secretion such as schizophrenia, epilepsy, Alzheimer's disease, and renal disease [3–5]. In addition, reliable and efficient determination of enzymatic activity of DAAO is of vital importance since the DAAO activity can be used as a possible cancer diagnostic test for some organs [6,7].

Common protocols used for determination of DAAO activity are based on the measurement of products (hydrogen peroxide, a-keto acids or ammonia) of the DAAO-catalyzed oxidation of D-AAs [8–12]. This approach could suffer from two fatal drawbacks. Firstly, there could be large possibility that the same products could be present in the biological samples derived from abundant L-AAs or other substances via various pathways [13]. Secondly, DAAO enzyme displays broad substrate specificity, which can deaminate several alkaline, neutral or nonpolar D-AAs [14,15]. This indicates that D-AAs in mammalian tissues can be simultaneously reacted with DAAO, thus the determined products could be partially produced from the conversion of other D-AAs rather than the investigated target D-AA. These drawbacks lead to a severe limitation to evaluate the activity and kinetics of DAAO by measuring reaction products.

The detection of consumption of the substrates D-AAs can be used as an alternative method, which could avoid of the aforementioned drawbacks for DAAO activity assay. The key to this approach is efficient enantioseparation of AAs. This could be a challenging task because there are different D-AAs as well as large amount of L-AA enantiomers in complex biological fluid. Many methods,

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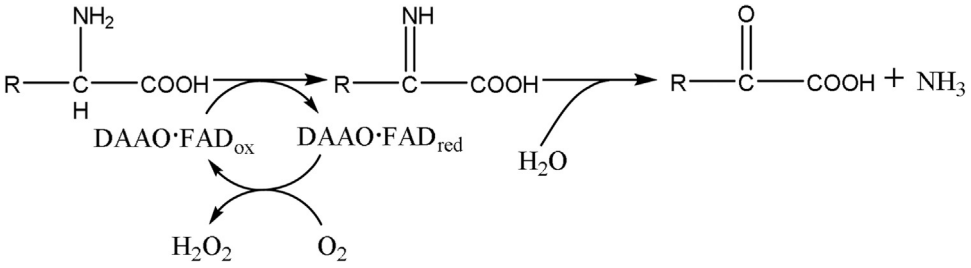


Scheme 1. Oxidative deamination of D-amino acids (D-AAs) catalyzed by DAAO.

such as gas chromatography (GC) [16–20], high performance liquid chromatography (HPLC) [21–25] and capillary electrophoresis (CE) [26–32] have been developed for enantioseparation of AAs. Among them, CE has been demonstrated as an efficient approach, due to its unique advantages of high separation efficiency, small sample requirement and economical equipment [32,33]. By employing amino acid ionic liquid as the chiral ligand, several studies have been reported on assays of DAAO activity and inhibition via measuring the substrate using the chiral ligand exchange capillary electrophoresis (CLE-CE) method [34–36]. While relative long analysis time of tens of minutes was required for AAs enantioseparation, these studies demonstrated the feasibility and advantages for DAAO enzyme assay by determining the consumption of substrates D-AAs.

To further reduce analysis time and improve assay efficiency, we present, for the first time, the application of optically gated capillary electrophoresis with LIF detection (OGCE-LIF) for assay of DAAO enzymatic activity. OGCE-LIF can achieve sequential analysis with rapid and trace amount (less than picoliter) sample injection, thus is capable to perform CE analysis with high temporal resolution and high separation efficiency [37–41]. In this study, we show that efficient enantioseparation of AA enantiomers can be accomplished by using OGCE-LIF, with excellent repeatability and low limit of detection. Based on this, we carry out enzymatic assays of DAAO by detection of the substrate consumption using OGCE-LIF, for either single or mixed D/L-AAs samples. We also perform analysis of kinetic equations of parallel oxidation reactions with two different substrates, the results of which is in good agreement with the experimental observations. To the best of our knowledge, this is the first report for the study of DAAO kinetic with multiple D-AAs. The results indicate that OGCE-LIF is a promising method for detection of D-AAs and assay of DAAO catalyzed reactions.

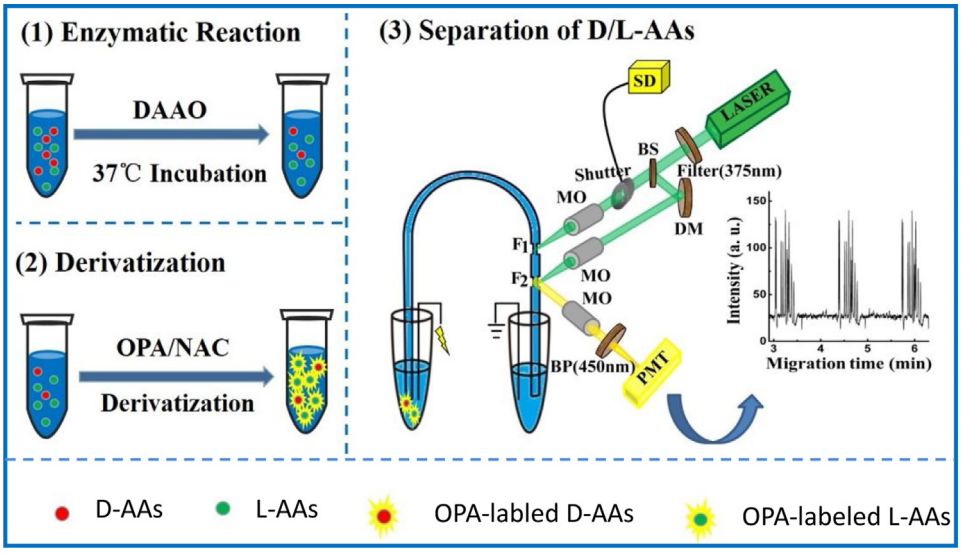
2. Experimental section

2.1. Chemicals

DAAO (EC 1.4.3.3) from porcine kidney, N-acetyl-L-cysteine (NAC), and o-phthalaldehyde (OPA) were purchased from Sigma Chemical Co. (St. Louis, MO). Chiral AAs (D/L-arginine (D/L-Arg); D/L-phenylalanine (D/L-Phe); D/L-valine (D/L-Val); D/L-methionine (D/L-Met); D/L-glutamine (D/L-Glu)) and 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) were purchased from Beijing Dingguo Biotechnology (Beijing, China). Deionized water was produced by a micropure water purification system (Milli-Q Water System, Millipore, Bedford, MA, USA). All solvents and solutions were filtered using 0.22 μm PES membrane filters prior to use.

2.2. Sample preparation and amino acids derivatization

Stock solutions of borate buffer (100 mM) and phosphate buffer (30 mM) were prepared in deionized water, and their pH values were adjusted by adding 1 M NaOH into the solution. Stock solutions of D- and L-AAs (50 mM) were prepared in deionized water and were diluted by 10 mM phosphate buffer (pH 8.0) to the desired concentration prior to use. Stock solution of NAC (500 mM) was prepared in 100 mM borate buffer (pH 9.5). OPA/NAC stock solution (20 mM) was prepared by dissolving 2.0 mg of OPA in a mixture of



Scheme 2. Schematic process of enzyme assay for DAAO using OGCE-LIF detection instrument. (1) DAAO catalyzed reaction; (2) Derivatization of unreacted substrate D/L-AAs; (3) In-house constructed OGCE-LIF system and the sequential analysis of the fluorescently labeled samples. SD: Shutter Drive; BS: Beam Splitter; MO: Microscope Objective; BP: Band-Pass filter; DM: Dichroic Mirror; PMT: PhotoMultiplier Tube.

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