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研究課題名（和文）Pyridoxamine could be a candidate drug for Parkinson`s disease: Mechanistic study to reduce dopamine-induced toxicity

研究課題名（英文）Pyridoxamine could be a candidate drug for Parkinson`s disease: Mechanistic study to reduce dopamine-induced toxicity

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研究成果の概要（和文）：ドパミン（DA）の酸化体であるドパミンキノン（DAQ）はパーキンソン病（PD）の主な原因として知られている。ピリドキサミン（PM）は、酸化・カルボニルストレスによる慢性疾患の治療薬候補として注目されている。そこで本研究では、DAQ捕捉の観点でPMの効果を精査した。DAとPMの反応では、DAの酸化によるDAQがPMに捕捉され、ピリドキサール(PL)-DA付加体が生成する事を明らかにした。次に細胞内環境に近い実験でPMのDAQ捕捉及びDA由来のα-シヌクレイン酸化阻害効果を確認した。よって、PMのPD予防薬としての可能性に加え、PL-DA付加体の酸化ストレスマーカーとしての可能性も示した。

研究成果の学術的意義や社会的意義
今回、神経毒性化合物のDA酸化体（DAQ）とPMの反応機構、反応生成物、およびは細胞内環境に近い条件でのDAQ捕捉、α-シヌクレイン酸化阻害効果を精査した。これらの知見からPMは、糖尿病、酸化・脂質化の関与する様々な慢性疾患（高脂血症、老化など）のみならず、PD予防薬としての可能性が示された。本研究の進展により、PD予防法の確立、長期的なDA補充療法に伴う問題（ウェアリング・オフ現象やジスキネジアの発生）の解決、ドラッグデザインへの応用等が期待される。

研究成果の概要（英文）：The cytotoxic effects of dopamine (DA) involve its oxidation to DA o-quinone (DAQ), which has profound implications in Parkinson’s disease (PD). Pyridoxamine (PM) is a drug candidate for diabetic complications because of its scavenging effects against reactive oxygen/carbonyl species. PM can also inhibit lipid hydroperoxide-derived protein damage by trapping lipid-derived aldehydes. In the reaction of DA and PM, pyridoxal (PL)+DA−H2O was produced. Its structure was characterized, and the reaction mechanism was proposed as follows: DA initially oxidizes to DAQ, which reacts with PM to produce PL after hydrolysis. PL then reacts with DA to form PL-DA adduct. PM was shown to scavenge DAQ in the presence of tyrosinase and glutathione. PM also inhibited DA-induced alpha-synuclein (Syn) oxidation. Therefore, PM could prevent DA-induced dopaminergic cell death and aggregation of Syn involved in PD, and the resulting PL-DA adduct can be used as a dosimeter for oxidative stress/DA oxidation.

研究分野：Clinical analytical chemistry, Chemical toxicology

キーワード：Pyridoxamine　Dopamine　Parkinson`s disease　Oxidative stress

１．研究開始当初の背景

(1) Parkinson's disease (PD) is an age-related neurodegenerative disorder which affects about 1–2% of people over the age of 65. PD is primarily characterized by a progressive degeneration of dopaminergic neurons in the *Substantia Nigra pars compacta* resulting in reduced levels of dopamine (DA). Additionally, PD is characterized by the presence of cytosolic filamentous inclusions known as Lewy bodies whose main component is fibrillar α-synuclein (α-Syn). In PD, L-DOPA (a precursor of DA) therapy is a standard approach, as it is designed to replenish the loss of DA. However, it has been reported that a long-term use of L-DOPA causes adverse effects such as motor fluctuations, dyskinesia, and psychiatric symptoms. These effects are attributed in part to the formation of DA *o*-quinone (DAQ) under various oxidative stress conditions. DAQ reacts with cellular thiols to form 5*S*-glutathionyl- and 5*S*-cysteinyl-DA, which has been shown to be neurotoxic. DAQ can also covalently modify L-cysteine residues in proteins to irreversibly alter and/or inhibit protein function, and consequently cause cytotoxicity in dopaminergic neurons. Current studies suggest that an interaction between α-Syn and DA leads to the selective neuronal cell death and the accumulation of misfolded α-Syn. Although the exact mechanism is not fully defined, DA oxidation is considered a key mechanism. Therefore, a DAQ-quenching molecule should prevent DA-induced pathogenicity in PD, such as death of dopaminergic neurons and aggregation of α-Syn.

(2) Pyridoxamine (PM) is one of vitamin B_6 vitamer and functions as a coenzyme in enzymatic transaminations *in vivo*. PM is also a promising pharmacological agent for the treatment of diabetic complications and other chronic conditions. This is based on its multiple inhibitory effects, including (i) inhibition of advanced glycation end products formation by chelation of metal ions with phenol and aminomethyl group, (ii) inhibition of advanced lipoxidation end products formation by scavenging of toxic reactive carbonyl species, and (iii) trapping of reactive oxygen species with phenol group. Recently, we have demonstrated that PM can inhibit lipid hydroperoxide-derived damage to proteins by trapping 4-oxo-2(*E*)-nonenal (ONE), the most abundant and reactive lipid-derived aldehyde. The potential role of PM-ONE adducts as biomarkers of oxidative stress have also been suggested.

(3) Liquid chromatography (LC)/electrospray ionization (ESI)-mass spectrometry (MS) analysis of the reaction between PM and DA revealed the formation of a PM-DA adduct. It corresponded to PM+DA−H_3O (PL+DA−H_2O), and the structure was tentatively characterized by MS and tandem mass spectrometry (MS/MS) analyses. As the PM concentration increased, dose-dependent elevation in the production of a PM-DA adduct was observed. Therefore, PM could inhibit DA-induced neurotoxicity by directly scavenging DAQ, and the PM-DA adduct can serve as novel biomarker of oxidative stress.

２．研究の目的

(1) To characterize a novel PM-DA adduct
(2) To investigate inhibition effects of PM on DA-induced neurotoxicity and α-Syn modification/aggregation that are significantly associated with PD
(3) To evaluate a PM-DA adduct as a biomarker of oxidative stress/DA oxidation.

３．研究の方法

(1) PM was reacted with DA or structural analogs of DA to elucidate the reaction mechanism. The resulting pyridoxal (PL)-DA adduct was characterized by LC/ESI-MS, MS/MS, and NMR analyses.
(2) DA was reacted with PM in the presence of tyrosinase and/or glutathione (GSH) to mimic the intracellular conditions. Reaction time-/tyrosinase/PM dose-dependent changes in levels of DA and PL-DA adduct were monitored.
(3) DA was reacted with α-Syn followed by enzymatic digestion and LC/ESI-MS/MS analysis to identify modification pattern/sites. PM dose-dependent changes in modification levels were monitored.

4．研究成果

(1) Characterization of PM-DA adduct:

- In the reaction of DA and PM, the PL-DA adduct was formed together with PL. The adduct corresponded to PL+DA−H_2O was also produced in the reaction of DA and PL. Its structure was characterized to be a 1-[3-hydroxy-5-(hydroxymethyl)-2-methylpyridin-4-yl]-1,2,3,4-tetrahydroisoquinoline-6,7-diol formed through a Schiff base by MS/MS, UV, and NMR analyses.
- Based on the results from the reaction of DA analogs and PM, the mechanism for the formation of PL-DA adduct was proposed. Thus, DA initially oxidizes to DA quinone (DAQ), which reacts with PM to produce PL after hydrolysis. PL then reacts with DA to form PL-DA adduct (Figure 1).
- As the PM concentration increased, dose-dependent elevation of a PL-DA adduct was observed.

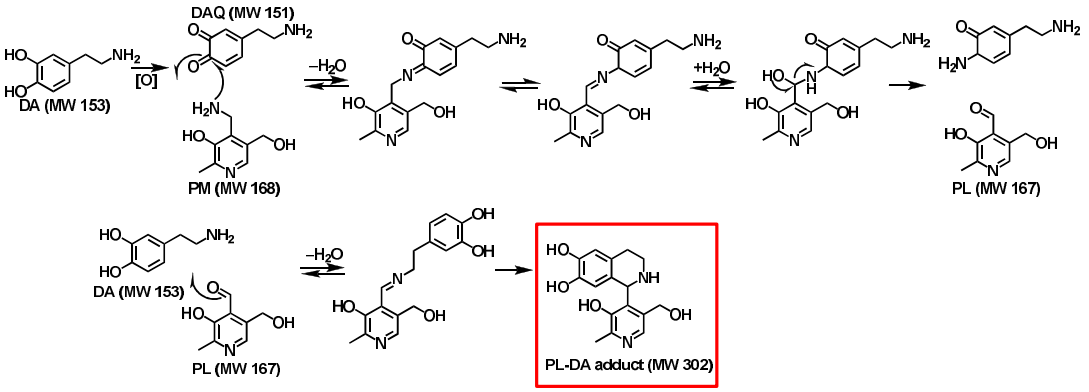

Figure 1. Proposed mechanism for the formation of PL-DA adduct.

(2) Inhibition effect of PM on DA-induced neurotoxicity:

- DA was reacted with PM in the presence of tyrosinase to confirm the scavenging effect of PM against DAQ. Tyrosinase can convert DA to DAQ by the diphenolase activity. Reaction time- and tyrosinase concentration-dependent decrease in DA and increase in PL and PL-DA adduct were observed. This result indicates that PM can scavenge DAQ that is produced by enzymatic oxidation of DA (Figure 2).
- DA was reacted with PM in the presence of GSH, an antioxidant that can neutralize reactive oxygen species and free radicals. It is present in the cytosol and organelles at mM level and protects cells. From the reaction, the formations of GSSG, PL, GSH-DA, and PL-DA were observed. DAQ was formed via the autooxidation of DA and reacted with GSH as well as PM to produce GSH-DA and PL-DA adducts. The level of PL-DA adduct was increased in a PM dose-dependent manner, but not GSH-DA adduct. The same results were obtained when the reaction was carried out in the presence of GSH and tyrosinase.
- These results confirmed PM can scavenge DAQ in the presence of tyrosinase and GSH, the intracellular-like conditions. And the PL-DA adduct level can reflect the degree of oxidative stress/DA oxidation.

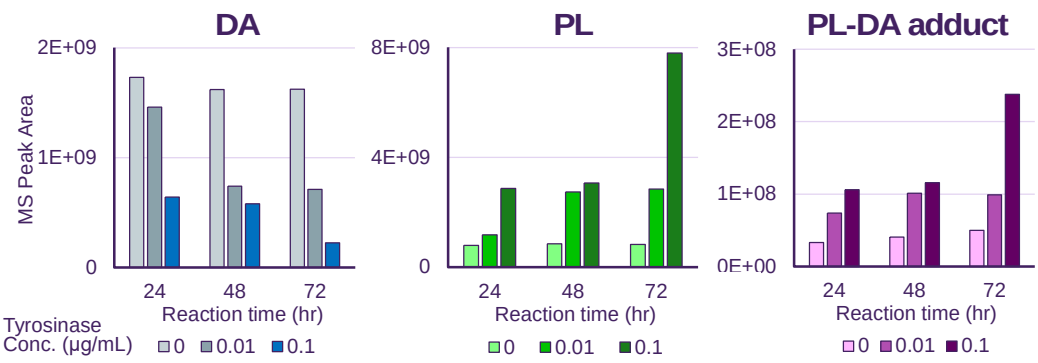

Figure 2. Changes in DA, PL, and PL-DA adduct in reaction of DA and PM in the presence of tyrosinase.

(3) Inhibition effect of PM on DA-induced α-Syn modification:

- α-Syn was digested with three different enzymes, trypsin, V8, and chemotrypsin. Sequence coverage (%) of each enzyme was 72.9, 83.6, and 38.9, respectively. Sum of those enzymatic peptides covered 100% sequence of Syn, which is important to identify modification sites on α-Syn.
- In the reaction of α-Syn with DA, multiple modifications on α-Syn were identified as follows: +DAQ, K43, 45, 96, 97, and 102; +DAQ–H_2O, K21; oxidation, M1, 5, 116, and 127.
- Met oxidation was increased in a DA dose-dependent manner. When α-Syn was incubated with DA in the presence of PM, a PM dose-dependent decrease in oxidation levels were observed for M116 and 127, located in C-terminal calcium binding domain.
- These results indicate that PM can prevent the aggregation of α-Syn by quenching DAQ.

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掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.ab.2021.114327	査読の有無 有
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６．研究組織

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７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関