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研究課題名（和文）神経ペプチド上の過酸化脂質由来の化学修飾解析:神経変性疾患への新規アプローチ

研究課題名（英文）Lipid hydroperoxide-derived modifications to neuropeptides: a novel approach for neurodegenerative diseases

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研究成果の概要（和文）：アンジオテンシン II と脂質過酸化由来アルデヒドとの反応を精査し、4,5-エポキシ-2(E)-デセナールあるいは4-ヒドロペルオキシ-2(E)-ノネナールでは主にN末端とHis6 付加体、4-オキソ-2(E)-ノネナールではN末端α-ケトアミド体の生成を明らかにした。更にα-ケトアミド体では、ピリドキサミンからのアミノ基転移によるアミノ酸への変換も見出した。またヒドロキシラジカルとの反応では、N末端α-ケトアミド体と環化体を確認した。これらの修飾による代謝への影響も明らかにした。ちなみにN末端α-ケトアミド化とそのアミノ基転移、環化反応は、神経ペプチドでも観察され、病態との観点で興味深い。

研究成果の概要（英文）：In the reactions of angiotensin (Ang) II with lipid peroxidation-derived 4,5-epoxy-2(E)-decenal or 4-hydroperoxy-2(E)-nonenal, the major modifications have been identified at the N-terminus and His6 of Ang II. 4-Oxo-2(E)-nonenal was shown to introduce an α-ketoamide moiety at the N-terminus of Ang peptides containing not only N-terminal Asp but also Ala, Arg and Val. The resulting N-terminal α-ketoamide is converted to the D- and L-amino acids by transamination in the presence of pyridoxamine. The reaction of Ang II and the hydroxyl radical produced N-terminal cyclized-Ang II (Ang C) as well as pyruvamide-Ang II (Ang P). Ang P and Ang C were not further metabolized by aminopeptidase A, which converts Ang II to Ang III. Formation and transamination of N-terminal α-ketoamide, and N-terminal cyclization were also observed in neuropeptides including amyloid beta 1-11. Analytical conditions were optimized to detect N-terminal α-ketoamide Ang peptides in human plasma.

研究分野：　医歯薬学

キーワード：　Oxidative stress　Lipid peroxidation　Chemical modification　Mass spectrometry　Angiotensin peptides　Neuropeptides

１．研究開始当初の背景

(1) Lipid hydroperoxide-derived reactive aldehydes covalently modify cellular macromolecules, which can lead to mutation and apoptosis. In settings of oxidative stress, the production of reactive oxygen species and the enzymes such as cyclooxygenases and lipoxygenases are up-regulated, which mediate the formation of lipid hydroperoxides. In the presence of transition metal ions or ascorbic acid (AscA), lipid hydroperoxides can undergo homolytic decomposition to highly reactive aldehydes such as 4-oxo-2(*E*)-nonenal (ONE), 4-hydroxy-2(*E*)-nonenal (HNE), 4,5-epoxy-2(*E*)-decenal (EDE), and 4-hydroperoxy-2(*E*)-nonenal (HPNE).

(2) We have identified the major ONE- and HNE-derived modifications to the N-terminus, Asp^1, Arg^2, and His^6 of Ang II (DRVYIHPF). Ang II has been implicated in various cardiovascular diseases of which oxidative stress is considered as a central mechanism. From the reactions of Ang II with ONE or HNE, the following modifications have been identified; pyruvamide-Ang II (Ang P, $CH_3COCONH$-RVYIHPF), the 4-ketoamide form of [N-ONE]-Ang II, the Arg-modified [Arg^2(ONE − H_2O)]-Ang II, and the dehydrated Michael addition products of [His^6(HNE−H_2O)]-Ang II.

(3) Our recent studies revealed that biological activities of Ang II can be significantly altered when its N-terminus undergoes oxidative modification. Ang P can be produced from Ang II by a novel ONE-derived oxidative decarboxylation of N-terminal aspartic acid. To test the biological activity of novel Ang peptides, we have developed a stable-isotope dilution mass spectrometry (MS)-based label free binding assay for Ang II type 1 (AT_1) receptor, which mediates most of physiological effects of Ang II. Competition-binding experiments using our assay revealed that the affinity of Ang P (K_i = 42 nM) towards AT_1 receptor was much lower than Ang II (K_d = 0.96 nM).

２．研究の目的

(1) To characterize modifications to Ang II derived from EDE and HPNE
(2) To characterize formation, trans-amination and cyclization of N-terminal α-ketoamide peptides
(3) To characterize lipid hydroperoxide-derived modifications to neuropeptides
(4) To test biological activities of modified Ang peptides
(5) To develop the mass spectrometry (MS)-based analytical methodology to detect α-ketoamide Ang peptides in human plasma

３．研究の方法

(1) Ang II was allowed to react with lipid hydroperoxide-derived EDE or HPNE.
(2) Each of Ang peptides and neuro peptides were allowed to react with ONE, hydroxyl radical or pyridoxamine (PM).
(3) Reactions were monitored by liquid chromatography (LC)-UV and/or LC/ electrospray (ESI)-MS in different reaction times and concentrations. The modifications were characterized by LC-ESI/MS and tandem MS.
(4) Further reactions such as sodium borohydride reduction or reaction with model amino acids were carried out for additional structural information.
(5) Conditions for derivatization, clean-up and LC/ESI-MS were optimized for the analysis of α-ketoamide Ang peptides.

４．研究成果

(1) Investigation on the Ang II modification by EDE and HPNE

- EDE and HPNE preferentially modified the N-terminus and His^6 of Ang II.
- In addition to the N-substituted pyrrole of [N-C_4H_2]-Ang II and Michael addition products of [His^6(EDE)]-Ang II, hydrated forms were detected as major products. Substantial amounts of [N-(EDE−H_2O)]-Ang II isomers were also formed.
- The main HPNE-derived products were [His^6(HPNE)]-Ang II and [N-(HPNE−H_2O)]-Ang II. However, ONE, HNE, and malondialdehyde-derived modifications were dominant, because HPNE is a precursor of these aldehydes.
- A mixture of 13-HPODE and [$^{13}C_{18}$]-13-HPODE (1:1) was then used to determine the major modifications derived from linoleic acid (LA) peroxidation. The characteristic doublet (1:1) observed in the mass spectrum and the mass difference of the $[M+H]^+$ doublet aided the identification of Ang P (N-terminal α-ketoamide), [N-ONE]-Ang II (4-ketoamide), [Arg^2(ONE−H_2O)]-Ang

II, [His^6(HNE)]-Ang II (Michael addition product), [N-C_4H_2]-Ang II (EDE-derived N-substituted pyrrole), [His^6(HPNE)]-Ang II, [N-(9,12-dioxo-10(*E*)-dodecenoic acid)]-Ang II, and [His^6(9-hydroxy-12-oxo-10(*E*)-decenoic acid)]-Ang II as the predominant LA-derived modifications.

(2) Investigation on the formation and transamination of N-terminal α-ketoamide peptides

- ONE can also introduce an α-ketoamide moiety at the N-terminus of peptides containing N-terminal residues other than Asp such as Ang A (ARVYIHPF), Ang III (RVYIHPF), and Ang IV (VYIHPF).
- Hydroxyl radical-mediated reaction was much more efficient for the formation N-terminal α-ketoamide peptides.
- The resulting N-terminal α-ketoamide is converted to the D- and L-amino acids by transamination in the presence of PM. The formation of epimeric N-terminus depended on the incubation time and the concentration of PM, and increased further upon addition of Cu(II) ions.
- The ONE- and hydroxyl radical-derived formation of N-terminal α-ketoamide and its transamination in the presence of PM were observed in amyloid beta 1-11 (A$\beta_{1\text{-}11}$, DAEFRHDSGYE).
- Other neuropeptides neurokinin B, cholecystokinin-8 and melanin-containing hormone were also converted to their N-terminal α-ketoamide form upon reaction with ONE or hydroxyl radical.

(3) Investigation on the cyclization of N-terminal α-ketoamide peptides

- The reaction of Ang II and the hydroxyl radical generated by the Cu(II)/AscA system or UV/hydrogen peroxide system produced N-terminal cyclized-Ang II (Ang C) and Ang P.
- The structure of Ang C was confirmed by MS and infrared spectroscopy, and comparison to an authentic standard.
- The subsequent incubation of isolated Ang P in the presence of Cu(II)/AscA revealed that Ang P was the direct precursor of Ang C.
- The proposed mechanism involves the formation of a nitrogen-centered (aminyl) radical, which cyclizes to form a five-membered ring containing the alkoxy radical. The subsequent β-scission reaction of the alkoxyl radical results in the cleavage of the terminal CH_3CO group. The initial aminyl radical can be stabilized by chelation to the Cu(II) ions.
- The affinity of Ang C toward the Ang II type 1 receptor was significantly lower than that of Ang II or Ang P. Ang P and Ang C were not further metabolized by aminopeptidase A, which converts Ang II to Ang III.
- Hydroxyl radical-mediated N-terminal cyclization was also observed in other Ang peptides (Ang A, Ang III, Ang IV), and A$\beta_{1\text{-}11}$.

(4) Detection of α-ketoamide Ang peptides in human plasma

- Internal standard ([$^{13}C_5$, $^{15}N_1$]-Ang P) was prepared using [$^{13}C_5$, $^{15}N_1$]-Ang II.
- Conditions for derivatization with Girard`s reagents and clean-up using solid phase extraction and ion-exchange chromatography were optimized.
- LC/ESI-selected reaction monitoring/ MS method was developed.
- The optimized methodology will be applied to detect α-ketoamide Ang peptides in human plasma.

５．主な発表論文等
（研究代表者、研究分担者及び連携研究者には下線）

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〔産業財産権〕

○**出願状況（計** 0 **件）**

名称：
発明者：
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○**取得状況（計** 0 **件）**

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東北大学研究者紹介（研究代表者）：
http://db.tohoku.ac.jp/whois/detail/a63434da0ba1befd3ff395e655af195e.html

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