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Formation of 6-nitrotryptophan in purified proteins by reactive nitrogen species: A possible new biomarker

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Abstract. We analyzed products of tryptophan residues in two model proteins which were reacted with reactive nitrogen species. We modified human Cu, Zn-superoxide dismutase, which has a single tryptophan residue and no tyrosine residue, by using two reactive nitrogen species generating systems; peroxynitrite/CO_2 and myeroperoxidase/H_2O_2/NO_2^- systems. We identified 6-nitrotryptophan as a major nitration product along with other oxidized products as the reaction products of tryptophan residue by using LC-MS/MS and HPLC-photodiode array analyses of the tryptic peptides. We modified hen egg-white lysozyme as a model of a simple protein having both tryptophan and tyrosine residues by peroxynitrite/CO_2 system. The modified enzyme lost 89% of the enzymatic activity. Among six tryptophan residues in lysozyme, Trp62, Trp63, and Trp123 were nitrated to form 6-nitrotryptophan, along with the formation of 3-nitrotyrosine in all tyrosine residues. However, the efficiency of nitration was different for each residue. No oxidized product of tryptophan residue was observed in the modified lysozyme. In conclusion, we propose that 6-nitrotryptophan is a unique and major nitrated product of tryptophan residue in proteins reacted with reactive nitrogen species.

Keywords: Reactive nitrogen species; 6-Nitrotryptophan; Tryptophan residue; Cu; Zn-superoxide dismutase; Lysozyme

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1. Introduction

Reactive nitrogen species (RNS), such as peroxynitrite ($ONOO^-$) and nitrogen dioxide ($.NO_2$) have been implicated as causes of various pathophysiological conditions, including inflammation, neurodegenerative and cardiovascular diseases and cancer [1,2]. Peroxynitrite is produced by the very fast reaction between nitric oxide (NO) and a superoxide radical in vivo and is known to have the potential to cause DNA-strand breaks, oxidation and nitration of lipids, nucleotides, proteins, and small biomolecules, such as methionine, tyrosine (Tyr), and tryptophan (Trp) [1–4]. A specific nitration product of tyrosine, 3-nitrotyrosine, has been widely utilized as a biomarker for the RNS production in a wide range of human and animal diseases. Most enzymes are inactivated by tyrosine nitration, but some enzymes are activated [1,2]. In the presence of a physiological concentration of carbon dioxide, $ONOO^-$ is known to form a peroxynitrite-carbon dioxide adduct ($ONOO^-/CO_2$), which also exhibits a very high reactivity to nitrate biomolecules and may be the ultimate chemical species in vivo [5]. Another RNS, $.NO_2$, is produced through one-electron oxidation of nitrite, which can be accumulated under conditions when NO is overproduced. Oxidation of nitrite to $.NO_2$ can be catalyzed by peroxidases in the presence of hydrogen peroxide [6,7]. Myeloperoxidase (MPO) utilizes nitrite (NO_2^-) and hydrogen peroxide (H_2O_2) as substrate to catalyze tyrosine nitration in proteins in vitro and also in vivo [8]. On the other hand, tryptophan and tryptophan residues in proteins could be another target amino acid for RNS. We have reported that human Cu, Zn-superoxide dismutase (SOD), which has no tyrosine residue and only one tryptophan residue in the molecule, showed formation of 6-nitrotryptophan and decreases of 30 and 15% enzymatic activity upon reaction with the $ONOO^-/CO_2$ and $MPO/NO_2^-/H_2O_2$ systems, respectively [9–11]. Recently, formation of 4-, 5-, and 6-nitrotryptophan and 5- and 6-nitrotryptophan was reported on myoglobin and hemoglobin reacted with $ONOO^-/CO_2$, respectively [12]. Formation of 6-nitrotryptophan was also reported as a result of the reaction between bovine serum albumin and $MPO/NO_2^-/H_2O_2$ system [13]. However, no study on the entire products of tryptophan residue reacted with the RNS has been reported [12,13]. Furthermore, determination of the relative reactivity between tryptophan residue and tyrosine residue in proteins for the RNS is also important in order to evaluate the pathophysiological and physiological significance of the tryptophan residue modification. However, as yet no such study has been reported.

In this study, we show the entire reaction products of the tryptophan residue in human Cu,Zn-SOD reacted with $ONOO^-/CO_2$ and $MPO/NO_2^-/H_2O_2$ system. We obtained almost the same spectra of the products including 6-nitrotryptophan as the major nitrated product with other nitrated and oxidized products. In order to learn the relative reactivity of $ONOO^-/CO_2$ with tryptophan and tyrosine residues, we had chosen hen egg-white lysozyme, which contains six tryptophan residues, and three tyrosine residues in the molecule. This enzyme is also a model protein having no prosthetic group. We obtained 6-nitrotryptophan as the major reaction product for the reaction with $ONOO^-/CO_2$ without significant formation of oxidized product. Three solvent-exposed tryptophan residues in lysozyme showed considerable reactivity with $ONOO^-/CO_2$ similar to that of tyrosine residues. A part of this study has been published [11].