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Post-translational modification of indoleamine 2,3-dioxygenase: *N*-terminal modification and nitration

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Abstract. Indoleamine 2,3-dioxygenase (IDO) induction can deplete L-Trp in target cells, and this effect is partially responsible for the antimicrobial, antiviral, and antiproliferative activities of several cytokines. Although a lot of studies have indicated the biological importance of IDO, the post-translational modification of IDO remains unknown. In this study, we analyzed IDO protein by liquid chromatography and tandem mass spectrometry (LC-MS/MS) to find post-translational modifications. LC-MS/MS analysis revealed that: (1) immunoprecipitated-IDO from a human monocyte cell line has been processed to cleave the N-terminal methionine, and the resulting N-terminal alanine is acetylated; (2) peroxynitrite, an NO-derived reactive species, which can modify proteins via formation of 3-nitrotyrosine, induced nitration and inactivation of recombinant IDO, specifically nitrating Tyr15, 345, and 353 residues. We successfully revealed these two types of post-translational modifications of IDO, and further findings of the post-translational modification may shed light on the mechanisms of IDO induction.

Keywords: Indoleamine 2,3-dioxygenase; Post-translational modification; Acetylation; Nitration; Peroxynitrite

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1. Introduction

Indoleamine 2,3-dioxygenase (IDO) is the rate-limiting and first enzyme of the L-tryptophan (L-Trp)-kynurenine pathway that converts L-Trp to *N*-formylkynurenine in mammalian extra hepatic tissues.

Post-translational modification (PTM) is important to understanding of biopharmaceuticals. The majority of approved therapeutic proteins, as well as those currently in development, bear some form of PTM in their natural form [1]. Although numerous studies have noted the importance of IDO activation in disease, the nature of PTMs in IDO has not been described. In this study, we analyzed IDO by two-dimensional liquid chromatography and tandem mass spectrometry (LC-MS/MS) to identify PTMs.

2. Materials and methods

The human monocyte cell line THP-1 was cultivated as described previously [2]. THP-1 cells were stimulated with 5 ng/ml of IFN-γ to induce IDO expression. Immunoprecipitation was performed using Dynabeads Protein G (Dynal Biotech) according to the manufacturer's instructions. Recombinant IDO (rIDO) was expressed as a GST fusion protein using a pGEX plasmid vector (Amersham Biosciences), and a GST tag was removed by cleavage of PreScission Protease as described previously [3]. IDO activity was determined by the methylene blue/ascorbate assay as previously described [4]. LC-MS/MS analysis and Mascot sequence database-searching software (Matrix Sciences) were used for identifying PTMs.

3. Results

3.1. Identification of N-termini and acetylation of IDO

We isolated IDO from IFN-γ-stimulated THP-1 cells by immnoprecipitation. THP-1 cells are known to induce IDO by IFN-γ [5]. Purified IDO was cleaved by trypsin and analyzed by LC-MS/MS. The LC-MS/MS analysis of the singly protonated peptide (m/z 1416.6) corresponding to molecular mass 1415.6 revealed the N-terminal sequence of the IDO (Fig. 1). The fragmentation spectrum shows that the starting methionine has been removed from the N-terminus of IDO and the resulting N-terminal alanine has been acetylated (unpublished data). The N-terminus of IDO was identified only in the cleaved methionine and acetylated form.

3.2. Identification of nitrated residues in peroxynitrite-treated rIDO

Nitration of tyrosine residues in proteins is a PTM associated with oxidative stress and production of peroxynitrite, and there has been increasing interest in the impact of Tyr nitration on protein and enzyme structure–function relationships in diverse clinical pathologies [6]. Peroxynitrite-treated or untreated rIDO was digested with endoproteinase Lys-C and trypsin, and peptides were analyzed by LC-MS/MS. The results indicated the presence of three nitrated peptides (Tyr15, 345, and 353) in the peroxynitrite-treated rIDO