Identify Potential Mechanisms for Oxidative Regulation of Group VI Group VIA Phospholipase A_2 by Mass Spectrometry

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Overview

Purpose: Utilizing a variety of approaches, we thoroughly studied the *in vitro* oxidation of iPLA$_2$β with H_2O_2, NO and HOCl. Mass spectrometry and site directed mutagenesis methods were applied to reveal potential mechanism of iPLA$_2$β inactivation by biological oxidants.

Approach: Phospholipase A2 activity assay, SDS-PAGE and immunoblotting, free thiol assay, site mutagenesis and LC/MS/MS.

•iPLA$_2$β catalytic activity and free thiol content are affected by the oxidants in a dose-dependent manner.

•LC/MS/MS analyses of the oxidant-treated iPLA$_2$β identified 16 oxidation products of methionine, tryptophan, or cysteine residues.

•Site directed mutagenesis of W^{460} with T^{460} or Y^{460} suggested that the inactivation of iPLA$_2$β occurring upon exposure to biological oxidants may have two distinct modes: reversible and irreversible mechanism.

Introduction

Group VIA Phospholipase A_2 (iPLA$_2$β) is expressed in phagocytes, vascular cells, pancreatic islet β-cells, neurons, and other cells and plays roles in transcriptional regulation, cell proliferation, apoptosis, secretion, and other events. A bromoenol lactone (BEL) suicide substrate used to study iPLA$_2$β functions inactivates iPLA$_2$β by alkylating Cys thiols. This suggests that iPLA$_2$β might be subject to redox regulation. Here we report that biological concentrations of H_2O_2, NO, and HOCl inactivate iPLA$_2$β, and this is partially reversible by dithiothreitol (DTT). Oxidant-treated iPLA$_2$β modifications were studied by LC/MS/MS analyses of tryptic digests, and included DTT-reversible events (*e.g.* formation of disulfide bonds and sulfenic acids), and irreversible events (*e.g.* formation of sulfonic acids, Trp oxides, and Met sulfoxides). W^{460} oxidation could cause irreversible inactivation because it is near the lipase consensus sequence ^{463}GTSTG^{467}, and site-directed mutagenesis of W^{460} yields active mutant enzymes that exhibit no DTT-irreversible oxidative inactivation.

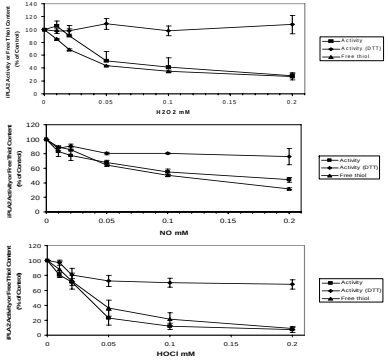
Method

Oxidation Reactions. Stock solutions of H_2O_2, HOCl, and the NO donor compound diethylamine NONOate were prepared, calibrated, and stored at 4°C. Reactions were performed at 37°C in buffer (50 mM Na_2HPO_4, 20 mM imidazole, pH 7.8) containing 0.2 μg/μl iPLA$_2$β with various concentrations of oxidant for various intervals and terminated by adding a 10-fold molar excess of L-methionine.

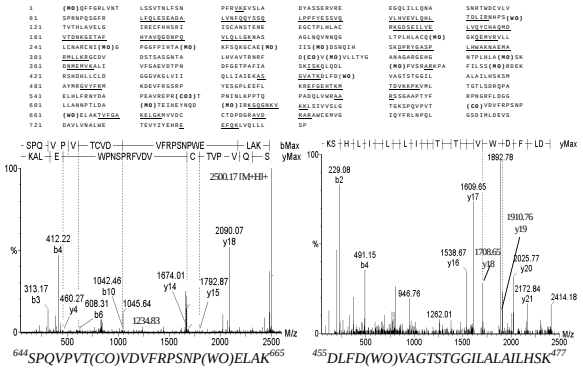
LC/MS/MS: Peptide mixtures were analyzed on a Waters Capillary LC system and the eluant was introduced into the nanoflow source of a Micromass (Manchester, UK) Q-tof Micro mass spectrometer. Switching from survey MS scans to MS/MS scans was performed automatically by a data-dependent algorithm, and the MS/MS data were processed with Masslynx software to generate a peak list file for database searching or manual sequencing.

Results

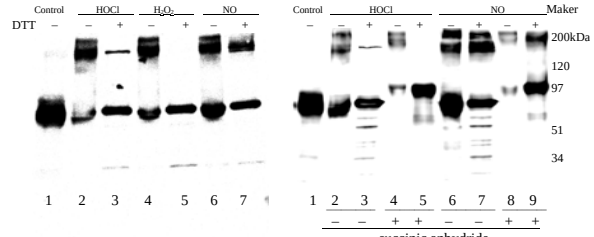
Effects of biological oxidants on iPLA$_2$β catalytic activity and cysteine content



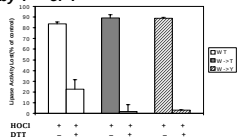
Identification of stable covalent modifications of iPLA$_2$β caused by biological oxidants with LC/MS/MS analyses.



Evidence for formation of intermolecular disulfide bonds upon treating iPLA$_2$β with biological oxidants



DTT completely restores catalytic activity to HOCl-treated iPLA$_2$β mutant proteins in which W^{460} is replaced by T^{460} or Y^{460}



Proposed mechanisms for iPLA$_2$ β oxidative inactivation

Conclusions

•Three cysteine (Cys332, Cys559, Cys651), three tryptophan (W120, W460, W661), and 11 methionine residues of iPLA$_2$β were identified with LC-MS/MS analyses to be covalently modified by biological oxidants.

• Two distinct modes of iPLA$_2$β inactivation occur upon exposure to biological oxidants
  - •One is reversible by DTT and associated with formation of higher molecular weight oligomers that can be reduced by DTT.
  - •Another is not reversible with DTT and could be caused by Trp or Met oxidation or by formation of Cys sulfonic acid derivatives

Acknowledgement:
This work was supported by United States Public Health Service Grants R37-DK34388, RO1-69455, P01-HL57278, P41-RR00954, P60-DK20579, and P30-DK56341.