Available online at www.sciencedirect.com

SCIENCE DIRECT

Free Radical Biology & Medicine 38 (2005) 786–795

www.elsevier.com/locate/freeradbiomed

Original Contribution

Nuclear proteasome activation and degradation of carboxymethylated histones in human keratinocytes following glyoxal treatment

Daniel Cervantes-Laurean, Michael J. Roberts, Elaine L. Jacobson, Myron K. Jacobson*

Department of Pharmacology and Toxicology, College of Pharmacy, Arizona Cancer Center, University of Arizona, 1515 North Campbell Avenue, Tucson, AZ 85724, USA

Received 13 April 2004; revised 16 November 2004; accepted 24 November 2004
Available online 18 December 2004

Abstract

Nuclear DNA damage has been studied in detail, but much less is known concerning the occurrence and fate of nuclear protein damage. Glycoxidation, protein damage that results from a combination of protein glycation and oxidation, leads to the formation of protein-advanced glycation end products (AGE) of which N^{ε}-carboxymethyllysine (CML) is a major AGE. We have used glyoxal, a product of environmental exposures that readily leads to the formation of CML, to study nuclear protein glycoxidation in HaCaT human keratinocytes. Glyoxal treatment that did not affect cell viability but inhibited cell proliferation in a dose-dependent manner that led to accumulation of CML-modified histones. Modified histones were slowly degraded but persisted for more than 3 days following treatment. Preincubation of cells with a proteasome inhibitor following glyoxal treatment led to an increase in CML-modified histones. While glyoxal treatment resulted in a slight decrease in total cellular proteasome activity, a dose dependent increase of up to 4-fold in nuclear proteasome activity was observed. The increase in nuclear proteasome activity was due to both increased nuclear proteasome protein content and increased activity, neither of which were affected by cyclohexamide. The increase also was unaffected by inhibitors of poly(ADP-ribose) polymerases, which have been previously implicated in nuclear proteasome activation by oxidizing agents. Accumulation of CML-modified histones over time may lead to epigenetic changes that contribute to various pathologies including aging and cancer, and upregulation of nuclear proteasome activity under conditions of glyoxidative stress may function to limit such damage.

Introduction

Reactive carbonyl species (RCS)[1] such as glyoxal, methylglyoxal, malondialdehyde, 4-hydroxynonenal, and acrolein are known intermediates in protein damage. Protein damage caused by RCS is involved in a number of pathophysiological conditions including skin aging [1,2], diabetes [3], atherosclerosis [3], and inflammation [4]. RCS are formed during reactions of normal cellular metabolism such as glycolysis, and are derived from nonenzymatic glycation [5,6], lipid peroxidation, and inflammation [4]. Glycation, a nonenzymatic reaction between a reducing sugar and a lysine residue, is an important source of RCS which leads to the formation of protein advanced glycation end products (AGEs) such as N^{ε}-carboxymethyllysine (CML), N^{ε}-carboxyethyllysine (CEL), pentosidine, glyoxal lysine dimers (GOLD), and methylglyoxal lysine dimers (MOLD) [3]. CML is a major AGE that is formed during RCS-mediated glycoxidation and oxidative degradation of glycation products as shown in Fig. 1. The CML content of proteins is increased in actinic dermal aging [7,8] and has been correlated with diabetic complications [2]. Furthermore, CML accumulates in skin lesions of actinic elastosis, a

Abbreviations: AGE products, advanced glycoxidation end products; CEL, N^{ε}-carboxyethyllysine; CML, N^{ε}-carboxymethyllysine; DMEM, Dulbecco's modified Eagle's medium; GOLD, glyoxal lysine dimers; MOLD, methylglyoxal lysine dimers; OPA, *o*-phthaldialdehyde; PARP, poly(ADP-ribose) polymerase; PBS, phosphate-buffered saline; RCS, reactive carbonyl species; Suc-LLVY-AMC, succinyl leucine-leucine-valine-tyrosine 7-amino-4-methyl-3-coumarin; TCA, trichloroacetic acid; TFA, trifluoroacetic acid.

* Corresponding author. Fax: +1 520 626 8567.
E-mail address: mjacobson@pharmacy.arizona.edu (M.K. Jacobson).

0891-5849/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.freeradbiomed.2004.11.030

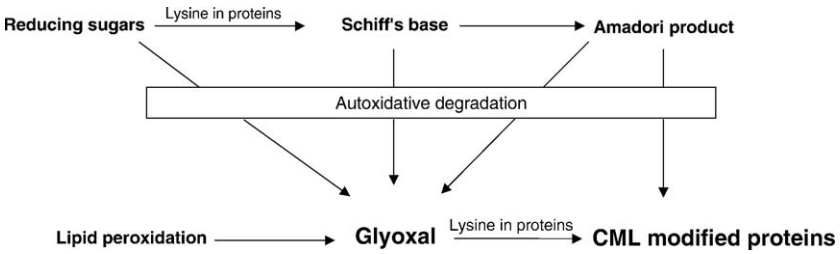

Fig. 1. Metabolic sources of protein CML adduct formation. Shown are the two sources for the formation of CML adducts on proteins, autoxidative degradation of glycation product intermediates and the direct modification of proteins by glyoxal formed by lipid peroxidation.

hyperplasia of the upper dermis caused by chronic exposure to sunlight [1,8]. The CML content of proteins can be used as a biomarker for intracellular glyoxidative stress [5].

The occurrence and fate of nuclear proteins damaged by RCS is not well studied but oxidatively damaged histones are degraded by a nuclear 20S proteasome in an ATP- and ubiquitin-independent manner [9,10]. The nuclear 20S proteasome is a 700-kDa multicatalytic, multisubunit protein, whose basal activity is higher in tumor cells than in nonmalignant cells [10,11]. Furthermore, the nuclear proteasome is regulated in cancer cells by poly(ADP-ribose) polymerase-1 (PARP-1) activity [10]. Proteasome regulation by phosphorylation in yeast [12] and by oxidation in maize roots has been reported [13].

The fate of histones modified by glycoxidation is potentially important since the alteration of amino acid side chains may interfere with the histone code, a variety of reversible posttranslational modifications of the amino terminal regions of these DNA binding proteins. These modifications include acetylation, phosphorylation, methylation, poly(ADP-ribosy)lation, and ubiquitination [14], all of which are involved in regulatory functions in the processing of genetic information. Here we report investigations of the nuclear protein targets of glycoxidation and cellular responses in cultured human keratinocytes exposed to glyoxal as a model toxicant. Nuclear protein damage was assessed by measuring CML adduct formation on histones, and the fate of the CML-modified histones was monitored in conjunction with nuclear proteasome activity following glyoxal treatment.

Experimental procedures

Chemicals

The 20S proteasome, clasto β-lactone lactacystin, Suc-LLVY-AMC, and lactacystin were from BIOMOL. Histones H1, H2A, H3, H2B, H4, SP-Sephadex C25, Dowex 2X8-100, OPA, glyoxal, and cycloheximide were from Sigma Co. Reversed-phase HPLC columns (μBondapak C18 and Xterra C18) were from Waters. A Microsorb-MV C4 reversed-phase column was from Varian. Anti-proteasome 20S alpha-type 1 subunit antibody, Human (rabbit) was from Calbiochem. Goat peroxidase-conjugated anti-rabbit IgG antibody was from Jackson research laboratories.

Cell culture

The established cell line of human epidermal keratinocytes (HaCaT cells), a gift from Dr. Norbert Fusenig (German Cancer Research Center, Heidelberg, Germany), was routinely cultured in DMEM containing 10% fetal bovine serum and kept in a humidified atmosphere containing 5% CO_2 at 37°C. Cells were maintained in exponential growth by subculture twice weekly at a split ratio of 1:10. Cells used for experiments were of passage number 1–30.

Exposing HaCaT keratinocytes to glyoxal

Exponentially growing HaCaT cells in 75 cm^2 flasks were exposed to 10 mM glyoxal in DMEM for 30 min, the medium was removed, the cells were washed twice with PBS, and fresh medium was added. Appropriate flasks were preincubated in DMEM in the presence of 10% FCS containing 13 μM clasto β-lactone lactacystin for 1 h before the addition of glyoxal. The clasto β-lactone lactacystin was also present throughout the 30 min exposure to glyoxal. Fresh medium was applied to cells prior to further study.

Histone separation

Reversed-phase HPLC experiments were performed using a Varian ProStar 230 HPLC. Histone separation was performed on a Varian Microsorb-MV C4, 5-μm spherical particle size (300 Å pore size) column using a described procedure [15] with some minor modifications. The histones were subjected to chromatography at a constant flow rate of 1.3 ml/min. A two gradient buffer system was used for histone separation as reported previously [15] with modifications. Buffer A was 0.1% trifluoroacetic acid and buffer B was 0.1% trifluoroacetic acid in acetonitrile. Buffer A decreased from 71 to 59% (10 min), 59 to 47% (13 min), and 47 to 38% (3 min); 38% was maintained for 3 min, and them went from 38 to 8% (1 min) and 8 to 71% (4 min). Detection employed a 208-nm filter (Pharmacia LKB: Uvicord SII). Fig. 2 shows a representative chromatographic