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# Proteomic profiling of acrolein adducts in human lung epithelial cells☆

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ABSTRACT

Acrolein (2,3-propenal) is a major indoor and outdoor air pollutant originating largely from tobacco smoke or organic combustion. Given its high reactivity, the adverse effects of inhaled acrolein are likely due to direct interactions with the airway epithelium, resulting in altered epithelial function, but only limited information exists to date regarding the primary direct cellular targets for acrolein. Here, we describe a global proteomics approach to characterize the spectrum of airway epithelial protein targets for Michael adduction in acrolein-exposed bronchial epithelial (HBE1) cells, based on biotin hydrazide labeling and avidin purification of biotinylated proteins or peptides for analysis by LC-MS/MS. Identified protein targets included a number of stress proteins, cytoskeletal proteins, and several key proteins involved in redox signaling, including thioredoxin reductase, thioredoxin, peroxiredoxins, and glutathione S-transferase π. Because of the central role of thioredoxin reductase in cellular redox regulation, additional LC-MS/MS characterization was performed on purified mitochondrial thioredoxin reductase to identify the specific site of acrolein adduction, revealing the catalytic selenocysteine residue as the target responsible for enzyme inactivation. Our findings indicate that these approaches are useful in characterizing major protein targets for acrolein, and will enhance mechanistic understanding of the impact of acrolein on cell biology.



## 1. Introduction

Acrolein (2,3-propenal) is a common indoor and outdoor pollutant, produced by combustion of fossil fuels, wood burning, heating cooking oils, and cigarette smoking, and is considered one of the greatest non-cancer health risks of all organic air pollutants [1–3]. Cigarette smoking represents the primary source of acrolein exposure to humans, and mainstream cigarette smoke contains up to 90 ppm acrolein [4]. Acrolein can also be produced endogenously during several

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biological processes, such as lipid or amino acid oxidation during conditions of inflammation [5,6], oxidative deamination of spermine [7,8], or drug metabolism [9]. Measurements of acrolein in airway secretions from smoking subjects indicate its presence at concentrations of 1–10 μM [10,11], concentrations that exert major effects on cellular function [12,13]. Because of its considerable chemical reactivity, acrolein is thought to play an important role in many of the adverse health effects associated with smoking, such as chronic bronchitis or lung cancer [13], [14].

The biological effects of acrolein are related to the presence of two reactive groups: a carbonyl group which can form Schiff bases with primary amines, and an adjacent α,β-unsaturated bond with strong electrophilic character, which is highly reactive with nucleophilic cellular targets by Michael addition at the β-carbon. Primary reactions of acrolein with biological molecules are predominated by reactions with nucleophilic amino acid side chains in proteins, primarily cysteine thiol residues or the ε-amino group lysine, and the imidazole nitrogen of histidine [9,12], as well as DNA bases [14], and small nucleophiles such as GSH [15]. Many of the reported cellular effects of acrolein, such as the activation of stress responses, (in) activation of transcription factors, and effects of cell proliferation and cell death pathways, are closely associated with changes in cellular GSH [16–18] and are often attributed to increased oxidative stress as a result of GSH depletion [19–22]. Because the acrolein reaction with GSH is an important detoxification mechanism, catalyzed by glutathione S-transferases (GSTs) [23,24], and serves to minimize interactions of acrolein with other critical cell constituents, the biological effects of acrolein are most likely mediated by direct interactions with critical protein targets or DNA bases. Examples of these include proteins involved in activation of transcription factors such as NF-κB or Nrf2 [25–28], caspases that regulate apoptotic cell death [16,18,19], protein tyrosine phosphatases [29], or proteins involved in regulating redox signaling [30,31]. Such chemical interactions of acrolein are analogous to those of other biologically relevant α,β-unsaturated carbonyls, such as the lipid oxidation product 4-hydroxynonenal (HNE) [32,33] or the cyclopentenone prostaglandins [34–36], which have been extensively studied [37,38]. However, differences in reactivity and cellular distribution may account for some observed differences in cell signaling effects of acrolein compared to more lipid-soluble electrophiles such as HNE [39].

In an attempt to understand the general mechanisms by which acrolein affects cell function, various global genomic and proteomic approaches have been employed to delineate alterations in gene or protein expression levels in response to acrolein [40,41]. However, such approaches have as yet not been applied to identify direct protein targets of acrolein, information that would be critical to better understand the impact of acrolein on specific biological processes or signaling pathways. Several previous reports have described global strategies to identify protein targets of other electrophiles such as HNE, using immunopurification of HNE-protein adducts with specific antibodies [42]. Such approaches have the limitation that they would only recognize specific stable adducts that are selectively recognized by these antibodies [5,42–46], and ignore other potentially important adducts. More recent strategies to identify protein targets for electrophiles have employed the use of e.g. biotin-tagged electrophiles [47–50], or the use of click chemistry to label proteins for their purification and analysis by MS [51]. Such approaches are, however, not applicable to acrolein because of its small molecular size and the absence of suitable sites for labeling without significant impact on its chemical properties or cellular distribution.

Alternative strategies based on derivatization of protein-bound carbonyls with hydrazides, such as dinitrophenylhydrazide (DNPH) [52,53] or biotin hydrazide [19,54], have been described. In the present study, we describe the use of biotin hydrazide labeling as a means to characterize protein targets for acrolein in intact bronchial epithelial cells, based on avidin purification of labeled proteins and proteomic analysis using LC-MS/MS. Secondly, we demonstrate more detailed proteomic analysis of one prominent target, thioredoxin reductase (TrxR), in order to identify the consequence of specific modifications within this selenoprotein for enzymatic activity. Overall, these combined approaches are useful to demonstrate direct targeting by acrolein of specific proteins in complex biological samples, and will guide more directed approaches to link specific protein modifications to the biological effects associated with acrolein exposure.

## 2. Materials and methods

### 2.1. Reagents

Cell culture materials and media were purchased from Invitrogen. Biotin hydrazide and monomeric avidin were obtained from Pierce. Sequencing grade trypsin was purchased from Promega. Streptavidin-HRP, TrxR1 primary antibody and all secondary antibodies were obtained from Sigma. Primary antibodies against Trx1 and Prx1 were purchased from Abcam and GSTπ from Abnova. Full length murine mitochondrial TrxR (mTrxR-GCUG) and a truncated form (mTrxRΔ3, lacking the terminal three amino acids: cysteine (C), selenocysteine (U) and glycine (G)) were generated and purified as previously described [55]. All other chemicals were purchased from either Sigma or Fisher.

### 2.2. Cell culture and acrolein treatment

Experiments were performed with human bronchial epithelial HBE1 cells, which were cultured at 37 °C under 5% $CO_2$ in DMEM/F12 medium supplemented with 50 units/mL penicillin, 50 μg/mL streptomycin, 10 ng/mL cholera toxin, 10 ng/mL epidermal growth factor, 5 μg/mL insulin, 5 μg/mL transferrin, 0.1 μM dexamethasone, 15 μg/mL bovine pituitary extract and 0.5 mg/mL BSA, as described previously [56]. Cells were plated at a density of 500,000 cells per well in a 6 well plate and grown to 80–90% confluence prior to acrolein treatment. At least 30 min prior to acrolein treatment, media was replaced by Hank's balanced salt solution (HBSS) containing 1.3 mM $CaCl_2$ and 0.5 mM MgCl. Acrolein was prepared as a stock solution in water (immediately prior to use) and added to the HBSS to give a final concentration up to 30 μM (equivalent to 60 nmol/$10^6$ cells). Following incubation with acrolein, HBSS was removed and cells were washed on ice with 5% dextrose and