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ARTICLE

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Toluene Diisocyanate Reactivity with Glutathione Across a Vapor/Liquid Interface and Subsequent Transcarbamoylation of Human Albumin

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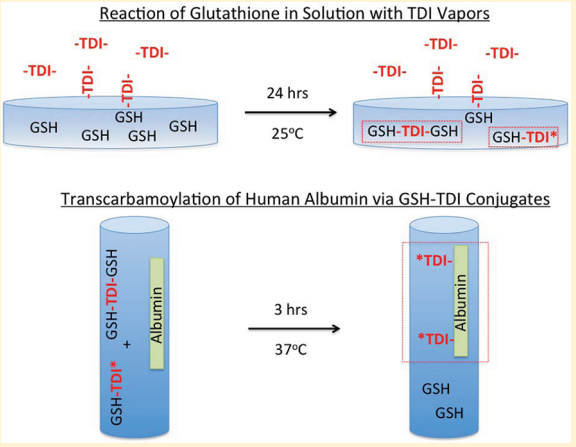
ABSTRACT: Glutathione has previously been identified as a reaction target for toluene diisocyanate (TDI) in vitro and in vivo, and has been suggested to contribute to toxic and allergic reactions to exposure. In this study, the reactivity of reduced glutathione (GSH) with TDI in vitro was further investigated using a mixed phase (vapor/liquid) exposure system to model the in vivo biophysics of exposure in the lower respiratory tract. HPLC/MS/MS was used to characterize the observed reaction products. Under the conditions tested, the major reaction products between TDI vapor and GSH were S-linked bis-(GSH)-TDI and to a lesser extent mono(GSH)-TDI conjugates (with one N═C═O hydrolyzed). The vapor-phase-generated GSH-TDI conjugates were capable of transcarbamoylating human albumin in a pH-dependent manner, resulting in changes in the self-protein's conformation/charge, on the basis of electrophoretic mobility under native conditions. Specific sites of human albumin-TDI conjugation, mediated by GSH-TDI, were identified (Lys[73], Lys[159], Lys[190], Lys[199], Lys[212], Lys[351], Lys[136/137], Lys[413/414], and Lys[524/525]) along with overlap with those susceptible to direct conjugation by TDI. Together, the data extend the proof-of-principle for GSH to act as a "shuttle" for a reactive form of TDI, which could contribute to clinical responses to exposure.



INTRODUCTION

The health hazards of TDI exposure are well recognized.[1−3] Airborne concentrations >2.5 ppm are considered immediately dangerous to life or health on the basis of animal studies, while concentrations <20 parts/per billion (ppb), below the short-term permissible exposure limits set by regulatory agencies, have been shown to trigger asthma attacks in sensitized individuals.[4−6] Globally, TDI is the second most abundantly produced diisocyanate and is commonly used for making polyurethane foam.[7]

The process by which TDI exposure causes disease remains unclear, in part due to TDI's potent chemical reactivity, which has hampered mechanistic studies.[8,9] A possible role for GSH in TDI asthma pathogenesis is suggested by several lines of evidence. In vitro studies with primary human bronchial cells and in vivo mouse studies have shown that TDI vapors can cause marked decreases in cellular thiol levels and induce oxidative stress.[10−14] Genetic studies in humans have shown an association of TDI asthma and TDI metabolites in urine and serum, with specific GSH *S*-transferase polymorphisms.[15−17] Furthermore, the overall importance of GSH in human asthma caused by other occupational and environmental allergens is increasingly being recognized.[18]

Generally, GSH plays a protective role against exposure to xenobiotics.[19,20] However, for TDI it is hypothesized that GSH plays a pathogenic role by acting as a shuttle that stabilizes a reactive form (of TDI) for delivery to another location, potentially extending the target range for protein modification and immune presentation.[21,22] This hypothesis is supported by in vitro studies demonstrating that GSH can form conjugates with TDI, capable of transcarbamoylating a model peptide.[10] Similar findings have been demonstrated in studies with other monoisocyanates, most notably, 2-chloroethyl-isocyanate, released by the cancer drug 1,3-bis(2-chloroethyl)-1-nitrosourea, methylisocyanate, the cause of the catastrophic industrial disaster in Bhopal India in 1984, and phenyl isocyanate, a sensitizer in animal studies.[23−26] Thus, substantial evidence for the reversible formation of thiocarbamate linkages between isocyanates and GSH suggest that this process might contribute to TDI's toxicity and/or allergenicity.

To date, liquid phase reactions have been used almost exclusively for in vitro studies of TDI and other isocyanates'

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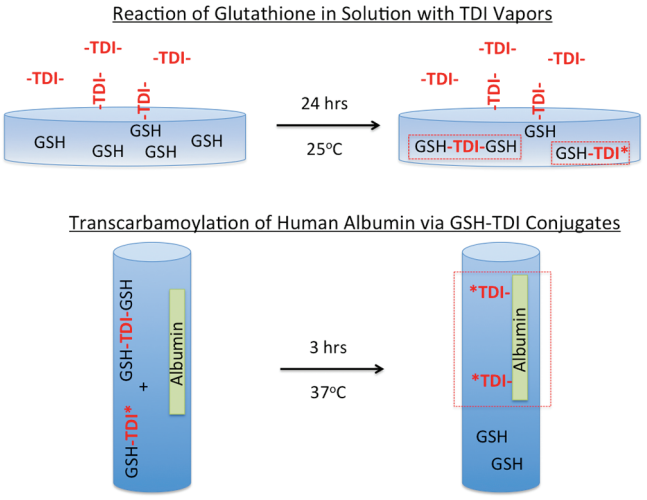

Figure 1. Depiction of methodology used in the study. (Top) Vapors of reactive TDI interacting with GSH in solution. (Bottom) Coincubation of GSH-TDI reaction products with human albumin in solution.

reactivity with GSH.[10,23−27] However, vapor, rather than liquid TDI, is the major phase to which GSH in the lower airway fluid is exposed in vivo.[28] Vapor phase TDI may react with compounds in solution differently than liquid phase TDI, as evidenced by recent studies with human albumin.[9] It remains unclear if differences between the TDI vapor vs the liquid phase are due to biophysics, maximum/minimum achievable TDI concentration, or technical issues, especially (a) TDI's insolubility and tendency to self-aggregate in aqueous solution, creating localized areas of high concentrations and a self-encapsulating physical barrier, as well as (b) TDI's reactivity with water and conversion of its N=C=O groups to NH_2, which may further copolymerize with unhydrolyzed TDI.

In the present study, we investigated the interaction of GSH (in solution) with TDI in its vapor phase to better model exposure biophysics in vivo in the lower respiratory tract. The mixed phase exposure approach alleviated many of the technical challenges previously noted in studying TDI/GSH reactivity in solution, namely, problems with mixing, precipitation formation, and large numbers of reaction products.[10] GSH-TDI conjugates formed via mixed phase exposure and subsequent transcarbamoylation of human albumin (a major carrier protein mediating TDI allergenicity) were further studied through tandem mass-spectrometry approaches. The data support the hypothetical pathogenic role of GSH in response to TDI exposure.

EXPERIMENTAL PROCEDURES

TDI Vapor Phase Exposure of GSH. Ten millimolar solution of reduced or oxidized glutathione (GSH or GSSG respectively) from Sigma (St. Louis, MO) in 20 mM phosphate buffered saline (PBS, pH 7.4) from Gibco (Grand Island, NY) was exposed to room air or TDI vapor (<200 ppb) for 18 h, in 35 × 10 mm Petri dishes obtained through VWR International (Bridgeport, NJ) as depicted in Figure 1 (top). All solutions and reaction products were 0.2 μm filtered (Millipore; Billerica, MA) to ensure sterility. The TDI solution used was an 80/20 mixture of 2,4/2,6-isomers, obtained from Sigma-Aldrich.

Reverse Phase HPLC Analysis of GSH-TDI Reaction Products. Samples were initially fractionated by the Yale Keck Center as previously described, on a Hewlett-Packard 1090 HPLC system equipped with an Isco Model 2150 Peak Separator and a 1 mm × 25 cm Vydac C-18 (5 μm particle size, 300 Å pore size) reverse phase column.[29] Following equilibration with 98% buffer A (0.06% TFA) and 2% buffer B (0.052% TFA, 80% acetonitrile), TDI-GSH reaction products were eluted over the course of 1 h, by increasing buffer B from 2 to 37%.

Mass Spectrometry Analysis of GSH-TDI Reaction Products. GSH-TDI reaction products were initially analyzed by the Yale Keck Center on a Micro Q-Tof MS instrument (Agilent Technologies; Santa Clara, CA). Twenty-five microliters of each HPLC purified sample was diluted with 25 μL of 0.1% formic acid and injected onto a column (RP C_4 micro column: PROTO 300 C_4 5 μm 50 × 0.3 mm) for desalting. The bound analyte was eluted over a 30 min gradient (mobile phase is acetonitrile with 0.1% TFA) directly into the Micro Q-Tof MS instrument where accurate mass was recorded. In other studies, a separate aliquot (2 μL) of the HPLC purified reaction products were diluted in 60 μL of 60% acetonitrile/0.1% formic acid and directly infused via Triversa NanoMate into a Bruker 9.4T FT-ICR MS (Bruker Daltonics; Billerica, MA).[30]

GSH-TDI Mediated Transcarbamoylation of Human Albumin. HPLC purified TDI-GSH reaction products or total (10 mM) GSH solutions exposed to TDI vapors were incubated 1:2 (v/v) with a 5 mg/mL solution of human albumin at 37 °C, as depicted in Figure 1 (bottom). Initial reactions, including those analyzed by native gel (Figure 7) and MS/MS (Table 1) were performed in isotonic saline (140 mM NaCl) buffered to pH 8.8 with 0.1 M carbonate. Subsequent experiments, exploring pH-dependence, were performed using 0.1 M buffers made with citric acid, phosphate, or carbonate (Sigma) to achieve pH levels of 3, 7, and 9, respectively, or varying ratios of mono/dibasic phosphate to achieve pH values ranging from 5.8 to 8.2.

Native Gel and Anti-TDI Western Blot. TDI conjugation to human albumin was detected in native gels on the basis of characteristic changes in electrophoretic mobility as previously described.[31,32] For native protein analysis, samples were prepared in a glycerol buffer, run on 10% polyacrylamide gels, and stained with Imperial protein stain from Pierce (Rockford, IL). For Western blot analysis, samples were electrophoresed under reducing conditions on precast 4−15% gradient gels and a nitrocellulose trans-blot system from BioRad (Hercules, CA). Nitrocellulose membranes were blocked with 3% dry milk in PBS, probed with 1 μg/mL of the anti-TDI mAb 79G7,[33] followed by antimouse IgG1 from Pharmingen (San Diego, CA), and developed with enhanced luminescence reagent from Thermo Fisher Scientific (Rochester, NY).

Reduction, Alkylation, and Trypsin Digestion of Albumin Samples. In preparation for MS/MS analysis of GSH-mediated TDI conjugation sites of human albumin, 50 μL aliquots of GSH-TDI/albumin coculture reactions were treated with tributylphosphine for 30 min at room temperature, followed by alkylation with iodoacetamide for 1 h at room temperature. Alkylation was quenched by further addition of tributylphosphine for 15 min at room temperature, and samples were twice dialyzed against 3 L of 25 mM NH_4HCO_3 using 3500 molecular weight cutoff mini dialysis units (Slide-A-Lyzer, Thermo Scientific, Waltham, MA). Porcine trypsin in 25 mM NH_4HCO_3 was then added at a 40:1 (protein:trypsin) ratio. Samples were incubated overnight at 37 °C with shaking (400 rpm) and finally centrifuged at 14,000*g* in a microcentrifuge (MiniSpin, Eppendorf, Hamburg, Germany) to pellet any insoluble material.

Ultraperformance Liquid Chromatography. Tryptic peptides of albumin were separated on a Waters (Milford, MA) nanoACQUITY ultraperformance liquid chromatography system. Aliquots (1 μL) of the digest mixture were injected and trapped/desalted on a 5 μm SymmetryC_{18} (180 μm × 20 mm) trapping column with 99.5/0.5 A/B (A, 0.1% formic acid; B, 0.1% formic acid in acetonitrile) at a flow rate of 15 μL/min for 1 min. Separation was performed on a 1.7 μm BEH130 C_{18}

Table 1. Sites of TDI Conjugation to Human Albumin Mediated via GSH-TDI

	mono(GSH)-TDI*		bis(GSH)-TDI		
observed TDI conjugation sites	HPLC peak A	HPLC peak B	HPLC peak C	HPLC peak D	total GSH-TDI RXN products
Lys73		×			×
Lys173		×	×	×	×
Lys159	×	×			×
Lys190		×	×	×	×
Lys199	×	×			×
Lys212		×			×
Lys351	×	×		×	×
Lys525	×		×	×	×

(100 μm × 100 mm) analytical column utilizing gradient elution at a flow rate of 400 nL/min and a gradient of 99/1 to 60/40 A/B over 60 min.

Tandem Mass Spectrometry of Transcarbamoylated Albumin Peptides. The eluent from the ultraperformance liquid chromatography system was directed to the nanoelectrospray source of a Waters SYNAPT MS quadrupole time-of-flight (qTOF) mass spectrometer. Positive ion nanoelectrospray was performed utilizing 10 μm PicoTip (Waters) emitters held at a potential of +3.5 kV. The cone voltage was held constant at +40 V for all experiments. Dry N_2 desolvation gas was supplied to the instrument via a nitrogen generator (NitroFlowLab, Parker Hannifin Corp., Haverhill, MA). $[Glu]^1$-Fibrinopeptide B (100 fmol/μL in 75/25 A/B) was supplied to an orthogonal reference probe and the $[M + 2H]^{2+}$ ion (m/z = 785.84265 u) measured as an external calibrant at 30 s intervals. Ultrahigh purity argon was used as collision gas. Spectra were acquired in an MS^e fashion.[34] Alternating one-second mass spectra were acquired. The collision energy was set to 6 eV (1 s low energy scan) and a 15−30 eV ramp (1 s high energy scan).

Data analysis. Data were analyzed with BioPharmaLynx v. 1.2 (Waters), a software program for the analysis of peptide mass maps and identification of sites of modification on known protein sequences. Default peptide mass map analysis criteria of 30 ppm mass error in both low and high collision energy mode were specified. Trypsin was specified as the digestion enzyme, and 2 missed cleavages were allowed. The submitted protein sequence was taken from P02768, "serum albumin precursor, *Homo sapiens*" (www.uniprot.org/uniprot/P02768) excluding the signal and propeptides (residues 1−24). Two custom modifiers were created for TDI. The first (TDI: $C_9H_6N_2O_2$, m/z = 174.0429 u) represents TDI with both isocyanate moieties bound to a peptide via urea bonds. The second (TDI*: $C_8H_8N_2O$, m/z = 148.0637 u) represents one isocyanate moiety bound to a peptide via a urea bond, while the second isocyanate moiety is hydrolyzed to the primary amine. Identification of an isocyanate binding site proceeded via a rigorous procedure that involved the following steps: (1) observation of a potential peptide−isocyanate conjugation product with less than 30 ppm $m/\Delta m$ mass error in the analyte peptide mass map. (2) Comparison of the analyte and control peptide mass map from unmodified human serum albumin shows that observed m/z and chromatographic retention time are unique to the analyte. (3) MS/MS data contains b_n- and y_n-type ions consistent with the assigned sequence and modifier.

RESULTS

TDI Vapor Phase Exposure of GSH and the Purification of Reaction Products. Solutions of GSH that had been exposed to TDI vapors, as depicted in Figure 1 (top), were subjected to reverse phase HPLC, and the UV light absorbance of each fraction of column eluent was measured. Comparison of the HPLC chromatograms of TDI vapor vs room-air-exposed GSH revealed fractions that likely contained GSH-TDI reaction products, on the basis of increases in A_{210} and A_{250} (Figure 2). As highlighted, the bulk of the reaction products were contained in the fractions labeled peaks A through D, which eluted after unreacted GSH (peak Ø), indicating increased hydrophobicity.

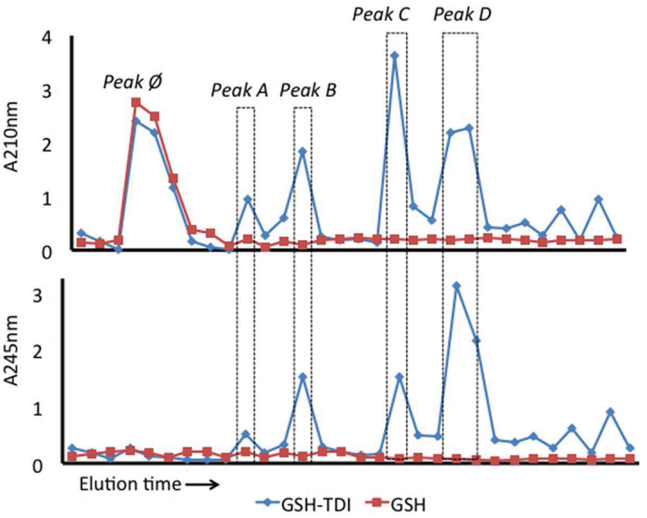

Figure 2. HPLC separation of GSH-TDI reaction products. The absorbance at 210 nm (top) and 245 nm (bottom) was measured (*Y*-axis) for each 1 min eluent sample collected over time (*X*-axis). Samples of TDI vapor (<200 ppb) exposed GSH (10 mM), shown as blue diamonds, were compared with "mock" exposed GSH, shown as red squares, to identify TDI reaction products, highlighted as peaks A−D, and unreacted GSH (peak Ø).

Identification and Characterization of Vapor GSH-TDI Reaction Products by Mass Spectrometry. The specific HPLC fractions with elevated A_{210} and A_{250} were further characterized by MS. As shown in Figure 3, peaks A and B contained primarily a singly charged 456.2187 or 456.2645 m/z species, while peaks C and D predominantly contained the doubly charged 395.2007 m/z and its singly charged species (m/z = 789.4146). Fragmentation of these species yielded the tandem mass spectra shown in Figures 4 and 5, which are consistent with the chemical structures shown in Figure 6. Together, the data indicate HPLC fractions A and B contain the mono(GSH)-TDI conjugate, with one N═C═O group hydrolyzed to a free amine (indicated as

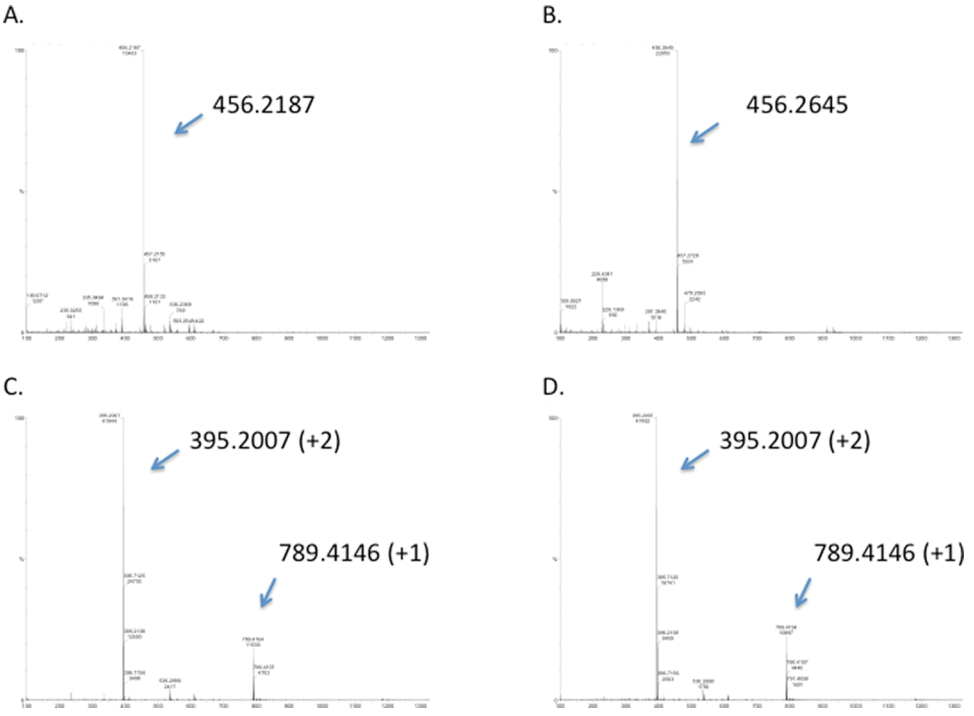

Figure 3. Mass spectrometry analysis of HPLC fractions containing GSH-TDI reaction products. Each panel A through B corresponds to samples from peaks A through D in Figure 2. Tandem MS/MS data (X-axis = m/z) and deduced structures for the singly charged m/z 456.2 and the doubly and singly charged m/z 395.2 and 789.4 are shown in Figures 4−6.

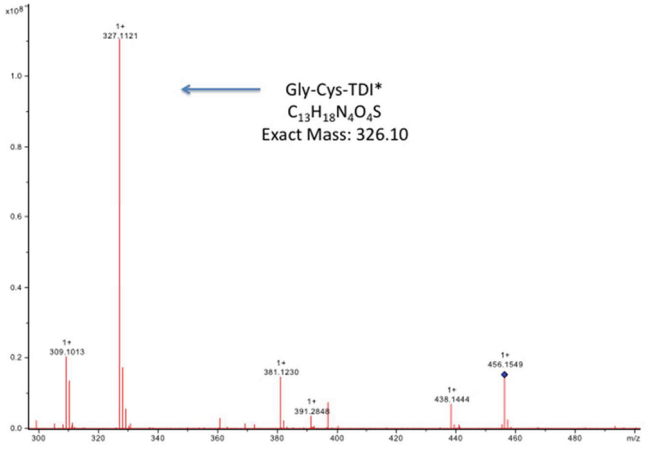

Figure 4. Tandem mass spectrometry analysis of peak A from HPLC purified GSH-TDI reaction products. A sample of peak A from the HPLC fractionation of GSH exposed to TDI vapor was analyzed by MS/MS (X-axis = m/z), focusing on the major m/z 456.2 product. The MS/MS spectrum shows a major fragment with an m/z consistent with the Gly-Cys-TDI* structure shown in Figure 6, suggesting the linkage of TDI via the thiol of Cys, rather than the glutamate amino acid of GSH. Identical results were obtained with peak B from the HPLC fractionation shown in Figure 2.

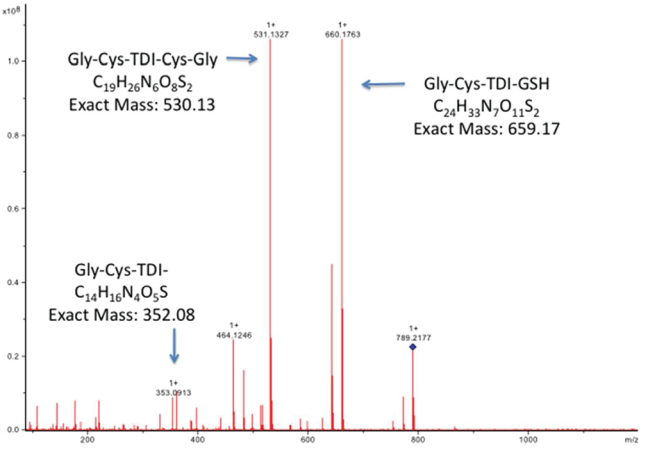

Figure 5. Tandem mass spectrometry analysis of peak C from HPLC purified GSH-TDI reaction products. A sample of peak C from the HPLC fractionation of GSH exposed to TDI vapor was analyzed by MS/MS (X-axis = m/z), focusing on the major m/z 789.4 product. The MS/MS spectrum shows a major fragment with an m/z consistent with the Gly-Cys-TDI-GSH, Gly-Cys-TDI-Cys-Gly, and Gly-Cys-TDI structures shown in Figure 6, suggesting the linkage of TDI via the thiol of Cys, rather than the glutamate amino acid of GDH. Identical results were obtained with peak D from the HPLC fractionation shown in Figure 2.

mono(GSH)-TDI* hereon), while fractions C and D contain bis(GSH)-TDI. As shown in Figure 6, the fragmentation data (along with functional data, see below) are consistent with TDI conjugation via the thiol group of cysteine, rather than the α-amine of the glutamate residue. The reason that GSH-TDI conjugates with identical masses elute from the HPLC in 2 distinct peaks remains unclear; however, the 2 distinct peaks may represent GSH conjugates with different isomers of TDI (2,4 vs 2,6) present in the starting vapors.

Transcarbamoylation of Human Albumin by Vapor GSH-TDI Conjugates. To test the functional ability of GSH-TDI conjugates generated by TDI vapor exposure to

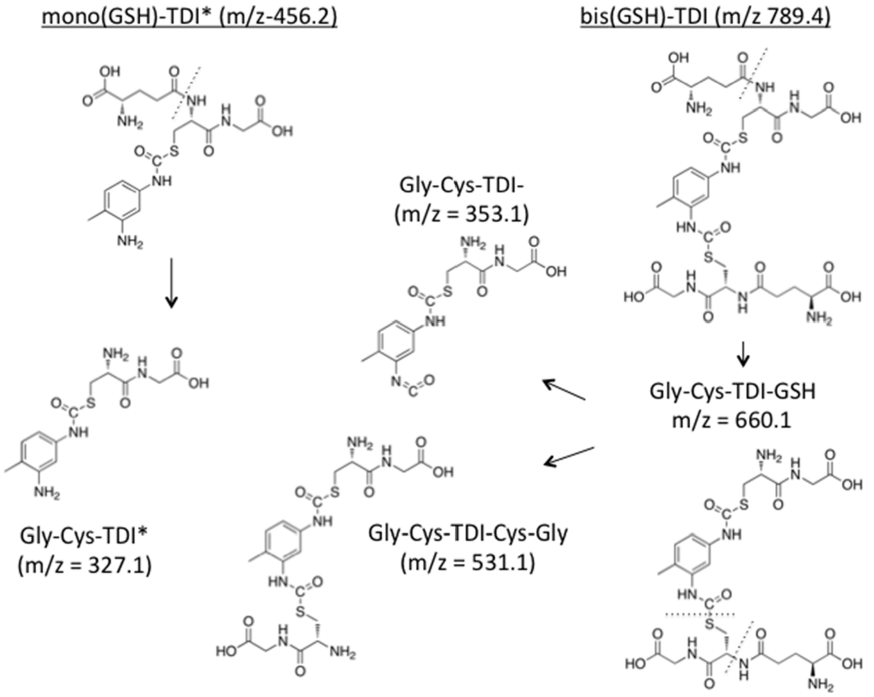

Figure 6. Chemical structures of the major reaction products between TDI vapors and GSH in solution. The structure of the major GSH-TDI reaction products and their predicted ionization fragments, including the *m*/*z* of the singly charged species, consistent with the observed MS/MS data shown in Figures 4 and 5.

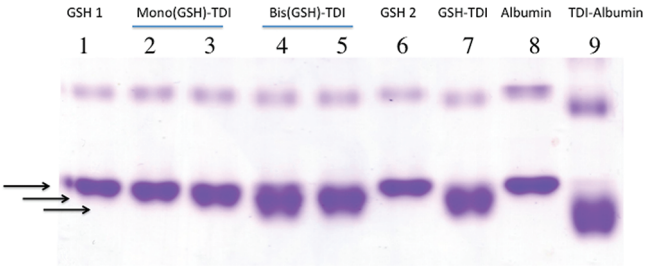

Figure 7. Native gel analysis of human albumin transcarbamoylated by purified GSH-TDI reaction products. Human albumin coincubated with HPLC purified GSH-TDI reaction products or controls (labeled above the lane) was subjected to electrophoresis under native conditions and stained for protein. A downward shift in electrophoresis is consistent with TDI conjugation based on previous published data. All coincubations used albumin in 0.1 M carbonate at pH 8.8, plus the following: lane 1, GSH from HPLC peak Ø; lanes 2 and 3, mono(GSH)-TDI* from HPLC peaks A and B, respectively; lanes 4 and 5, bis(GSH)-TDI from HPLC peaks C and D, respectively; lane 6, mock exposed GSH (unfractionated); lane 7, TDI vapor exposed GSH (unfractionated); lane 8, mock exposed albumin; lane 9, albumin exposed directly to TDI vapors.

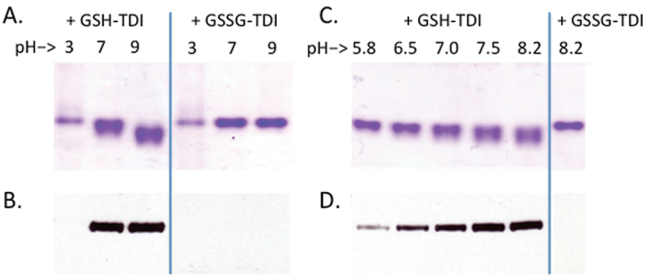

Figure 8. Dependence of human albumin transcarbamoylation by GSH-TDI on pH and free thiol of cysteine. Results of transcarbamoylation reactions of human albumin with total unfractionated GSH-TDI or GSSG-TDI reaction products. Co-incubations were performed at different pH levels (as labeled) and analyzed by gel electrophoresis (panels A and C) or anti-TDI Western blot (panels B and D). Note: A/B and C/D are paired gel/Western blots. Protein-stained gels were run under native conditions to highlight the shift in migration due to conformational/charge differences, while Western blots were run under reducing conditions to maximize anti-TDI mAb binding.

transcarbamoylate another protein, human albumin was coincubated with GSH-TDI conjugates (Figure 1, bottom) and subsequently analyzed by native gel electrophoresis for conformational/charge changes indicative of TDI conjugation. HPLC purified bis(GSH)-TDI, total (unpurified) GSH-TDI, and to a lesser extent mono(GSH)-TDI all caused electrophoretic changes in albumin consistent with TDI transcarbamoylation (Figure 7). As further shown in Figure 8, through native gel analysis as well as anti-TDI Western blot, GSH-TDI mediated transcarbamoylation of human albumin was strongly pH-dependent regardless of the buffer ion (phosphate, citrate, and carbonate) and across a wide range (pH 3−9), with lower activity at acidic pH and higher activity at basic pH levels. Further support of the thiocarbamate-dependent mechanism of TDI transcarbamoylation via GSH is provided by the fact that oxidized GSH (GSSG) did not mediate TDI transcarbamoylation, when vapor exposed and cocultured with albumin under identical conditions.

Identification of Human Albumin'S Specific Sites of TDI Conjugation via GSH-TDI. It was hypothesized that specific amino acid side chains of human albumin would be most

susceptible to TDI conjugation via GSH-TDI conjugates and the identity of these putative targets was evaluated through tandem mass spectrometry analysis. Under alkaline conditions, which yielded maximal transcarbamoylation, 8 different lysine residues were consistently identified as targets of TDI conjugation by mono(GSH)-TDI* or bis(GSH)-TDI in replicate experiments (Table 1). In each case, the second (unbound) N═C═O group of the transferred TDI molecule had been hydrolyzed to the free amine. Overall, the pattern of albumin conjugation via GSH-TDI is qualitatively distinct from that recently reported via direct exposure to low dose TDI in liquid phase titration studies.[35] However, two GSH-mediated TDI conjugation sites (Lys^{199} and Lys^{525}) are also targeted by direct low dose TDI exposure, while the other six identified GSH-mediated TDI conjugation sites (Lys^{73}, Lys^{159}, Lys^{190}, Lys^{212}, Lys^{351}, and Lys^{137}) can become directly conjugated with high enough TDI exposure concentrations.[35]

DISCUSSION

The reactivity of TDI with GSH was studied under mixed (vapor/liquid) phase conditions, as exists in the human airways, an important site of occupational exposure. GSH-TDI reaction products formed readily and were further capable of transferring TDI to albumin, altering the self-protein's native conformation/charge. Specific lysine residues of human albumin were identified as the predominant targets for TDI conjugation, via GSH-TDI conjugates, and overlap with those susceptible to direct conjugation with TDI, a process previously shown to induce antigenic changes.[9] Together, these findings advance our understanding of the complex biochemistry that may connect TDI vapor inhalation with toxicity and/or allergy/asthma and further suggest an important role for GSH in response to occupational TDI exposure.

The identification of bis(GSH)-TDI conjugates as a predominant reaction product via mixed phase exposure implies that reactivity of TDI with the thiol group of GSH, rather than reactivity with water, is a primary step through which the chemical vapor crosses the human airway's fluid phase boundary. The reversible thiocarbamates that TDI forms, via S-linked conjugation with GSH, might shelter TDI from hydrolysis thereby allowing further penetration into the body in a reactive form, increasing toxicity or allergenicity, and creating the potential for systemic effects. Thus, GSH and/or other thiols at major sites of occupational TDI exposure (respiratory tract as well as mucous membranes and skin) could play an important role in toxic and/or allergic responses. Airway fluid pH, which normally ranges from 6.5 to 7.5 and is acidified in some human conditions (e.g., asthma, pneumonia), may further modulate TDI exposure outcomes via its influence of transcarbamoylation reactions involving TDI-GSH conjugates, as demonstrated here.[36]

The present findings agree with the previously published studies of liquid phase TDI reactivity with GSH by Day et al., which also described predominantly bis(GSH)-TDI reaction products, when using 10-fold higher GSH concentrations (100 mM), ~2% v/v TDI, and alkaline pH 7.7.[10] The present study extends these observations, by demonstrating the formation of GSH-TDI under more physiologic reaction conditions as well as the ability of GSH-TDI to transfer TDI to human albumin, an important carrier protein for allergic (IgE) responses to TDI a and potential biomarker of exposure.[9,37,38] GSH-mediated transfer of TDI was shown to occur preferentially on specific lysine residues, some of which are also susceptible to direct conjugation by TDI, and have been associated with antigenic changes induced by other diisocyanates (HDI, MDI).[31,32,35] Thus, the present findings raise new questions, which may be highly relevant to TDI asthma pathogenesis, e.g., the potential competition for isocyanate reactivity in vivo between thiols, such as GSH, and amines, such as those on albumin, as well as potential similarities and/or differences between TDI-albumin resulting from direct reaction with TDI vs transcarbamoylation via GSH.

The present data differ from previous findings that GSH protects human albumin from vapors of another diisocyanate, HDI.[39] Such dual effects could be due to differences in the reaction and hydrolysis/disassociation rates of GSH with (aromatic) TDI vs (aliphatic) HDI, as might be expected on the basis of studies of their corresponding thiocarbamates prepared with cysteine methyl esters (k_d for TDI roughly 100× that of HDI).[21] Differences in the effect of GSH might also relate to exposure conditions, which differed in the study on HDI, particularly the pH/temperature of the transcarbamoylation reaction, and the timing of albumin exposure relative to GSH (e.g., competitive vs sequential). Studies re-evaluating the interactions of GSH, albumin, and HDI vapors, under physiologic conditions (pH 7, temperature of 37 °C) more favorable to thiol-amine isocyanate exchange, such as those used in the present investigation, are underway in our laboratory.

The major strength of the present study is the application of a mixed (vapor/liquid) phase system to investigate the interaction of GSH with TDI vapors. The study design targeted a fixed GSH concentration and pH as starting points for investigations; however, the system should readily permit the evaluation of a more dynamic range of physiologic factors (e.g., glutathione/ion concentration, pH, and temperature) and other volatile occupational/environmental coexposures (amine catalysts and solvents) that may influence TDI reactivity. The vapor/liquid exposure system should be similarly useful for studying other volatile diisocyanates (HDI and MDI) and differences that have been reported in their reactivity/stability with GSH and related thiols.[21,39,40] The basic system could be further developed with underlying human epithelial cell layers and overlying surfactant to more completely model the airway microenvironment. However, animal models or clinical investigations will likely be needed to ultimately determine potential systemic effects of GSH-TDI interactions in vivo.

In summary, it was demonstrated that GSH can mediate TDI vapor uptake across a fluid phase boundary and subsequently transfer TDI onto human albumin, a protein recognized as a major carrier for TDI in vivo. GSH-mediated transfer of TDI onto human albumin occurred on specific lysine residues and altered the self-protein's native conformation/charge, an effect previously associated with TDI's antigenicity. Together, the data extend proof-of-principle for the hypothesis that GSH and potentially other thiols in exposed tissue, act as a primary reaction route for the entry of TDI into the body and could play an important role in pathogenic responses to exposure.

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DISCLOSURE

The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official position of the Centers for Disease Control and Prevention or the National Institute for Occupational Safety and Health.

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ABBREVIATIONS

GSH, reduced glutathione; GSSG, oxidized glutathione; HDI, hexamethylene diisocyanate; MDI, methylene diphenyl diisocyanate; ppb, parts per billion; TDI, toluene diisocyanate.

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