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Article

Sensitive Method for the Identification of Potential Sensitizing Impurities in Reaction Mixtures by Fluorescent Nitrobenzoxadiazole-Labeled Glutathione

Takashi Tokunaga,* Gaku Yamamoto, Teruki Takahashi, Makiko Mukumoto, Masayuki Sato, and Masahiko Okamoto

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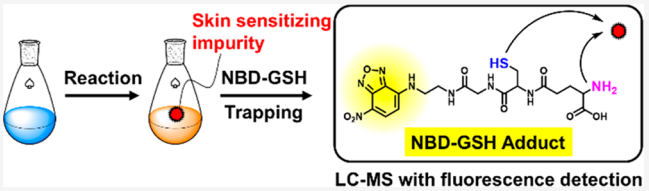
ABSTRACT: Allergic contact dermatitis is a critical issue in the development of new chemicals. Minor impurities with strong skin-sensitizing properties can be generated as byproducts. However, it is very difficult to identify these skin sensitizers in product mixtures. In this study, fluorescent nitrobenzoxadiazole-labeled glutathione (NBD-GSH) was synthesized to identify small amounts of skin sensitizers in reaction mixtures. Twelve known skin sensitizers and three nonsensitizers were reacted with NBD-GSH. Adducts formed only with the skin sensitizers, which allowed for their detection by a fluorescence detector. Liquid chromatography-mass spectrometry (LC-MS) analyses showed that NBD-GSH reacted with the skin sensitizers via its thiol and amino groups. An adduct of NBD-GSH with the strong skin sensitizer 1-chloro-2,4-dinitrobenzene was detected with a limit of detection of 6×10^{-8} mol/L by high-performance liquid chromatography with fluorescence detection. When a reaction mixture from primary alcohol oxidation was incubated with NBD-GSH, a NBD-GSH adduct formed with skin-sensitizing aldehyde impurities and could be specifically detected by LC-MS with fluorescence detection. This method will be useful for detection and identification of small amounts of skin sensitizers in raw materials, intermediates, reaction mixtures, and end products in the chemical industry.



INTRODUCTION

In the chemical industry, the risk of allergic contact dermatitis is a critical issue in the development of new chemicals. For the protection of workers and consumers, skin-sensitizing potentials of chemicals in manufacturing processes, including raw materials, intermediates, and products, must be assessed at an early stage of product development. It is known that some unexpected minor impurities with strong skin-sensitizing properties are generated as byproducts and can contaminate the nonsensitizing target product, which can then give the entire sample strong skin-sensitizing properties.[1] In such cases, it is not easy to identify the component that is the main cause of skin sensitization. In previous research with the commercial dye Sudan III, each component was fractionated by column chromatography and skin sensitization with each fraction was evaluated to identify the main skin sensitizer.[1] Other studies have also used chromatographic separation before evaluation to identify the skin-sensitizing components in a German chamomile (*Matricaria chamomilla*) extract[2] and a *Commiphora myrrha* (myrrh) extract used as a natural skin remedy.[3]

The guinea pig maximization test,[4−6] Buehler test,[4,7,8] and the murine local lymph node assay[9−11] have been validated by the Organization for Economic Co-operation and Development as animal tests for evaluating skin sensitization. In recent years, because of increasing public awareness of animal welfare, the use of test animals for scientific purposes has been restricted.[12] Many alternatives to animal testing have been developed on the basis of the key events from adverse outcome pathways.[13] The direct peptide reactivity assay (DPRA)[14−16] and the amino acid derivative reactivity assay[14,17] can be used to assess the skin-sensitizing potentials of chemicals on the basis of key event 1 of covalent binding of the chemical to skin proteins. These tests evaluate the reactivity of chemicals toward model synthetic peptides as a surrogate for proteins. The KeratinoSens[18,19] and LuSens[18,20] tests have been designed around key event 2, the activation of keratinocytes, to evaluate activation of the Keap1-Nrf2-ARE pathway by oxidative stress in keratinocytes. Another set of tests are based on a third key event, the activation of dendritic cells, and include the human cell line activation test (h-CLAT),[21,22] U-SENS,[21,23] and IL-8 Luc assay.[21,24] All types of testing, including both animal tests and alternative methods, require time-consuming steps for chromatographic fractionation,

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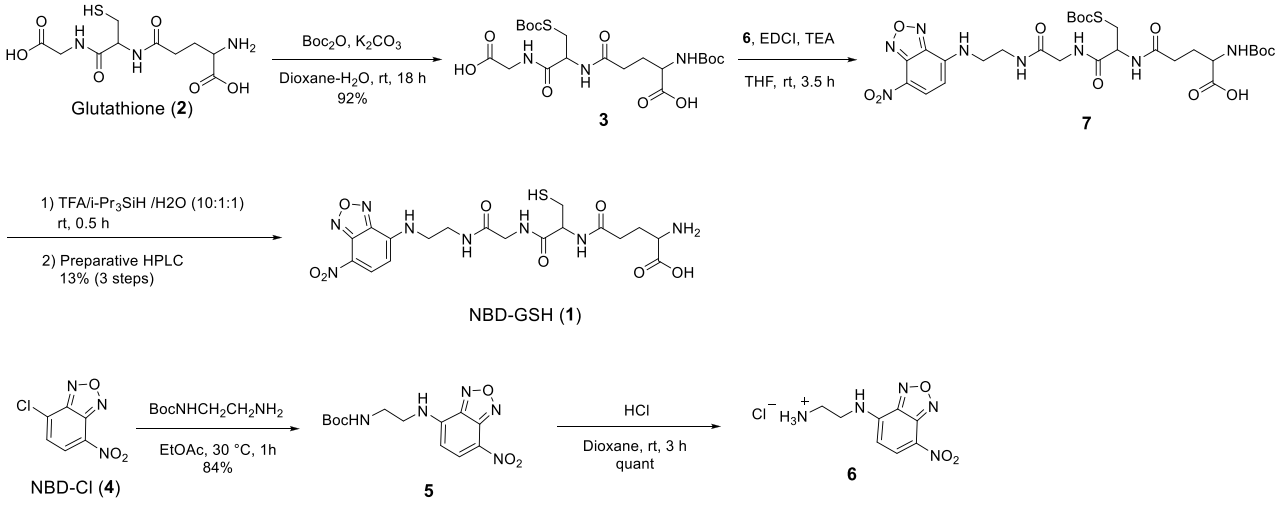

Abbreviations
Boc: *tert*-butoxycarbonyl, EDCI: 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide, TEA: triethylamine, THF: tetrahydrofuran, TFA: trifluoroacetic acid, EtOAc: ethyl acetate

Figure 1. Synthetic scheme for NBD-GSH.

evaluation of the skin-sensitizing potential of each fraction, and identification of the main skin-sensitizing substance.

Some methods that use peptides to trap skin sensitizers in a mixture have been reported. In one study, the synthetic peptide Ac-RFAACAA, the cysteine peptide in DPRA, was incubated with a commercial fragrance (Azurone) and an adduct with unexpected skin-sensitizing impurity was identified by liquid chromatography-mass spectrometry (LC-MS).[25] Glutathione (GSH, γ-glutamyl cysteinylglycine) is an endogenous tripeptide containing a thiol group. It plays a central role in biological detoxication by trapping electrophilic xenobiotics and their metabolites via its thiol group under catalysis by glutathione-*S*-transferase.[26] With use of this property of GSH, a method for screening chemicals for their skin-sensitization potentials was developed on the basis of the detection of GSH adducts of the analytes by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and LC-MS.[27] In addition, dansyl glutathione has been widely used as a trapping agent for identifying reactive metabolites in pharmaceutical drug development.[28] However, this method was designed for trapping soft electrophilic skin sensitizers on thiol groups and are not suitable for hard electrophiles such as aldehydes.

In this study, we designed a new trapping agent to identify a wide variety of skin-sensitizing impurities. For this purpose, we synthesized a fluorescent, nitrobenzoxadiazole (NBD)-labeled GSH (NBD-GSH) with two reactive sites: a thiol group for soft electrophiles and an amino group for hard electrophiles. Because of its fluorescent group, both NBD-GSH itself and its adducts can be selectively detected by a fluorescence detector. This report describes the synthesis of NBD-GSH, reactivity of NBD-GSH with known skin sensitizers, limit of detection of NBD-GSH, and trapping and identification of skin-sensitizing byproducts in a reaction mixture.

EXPERIMENTAL PROCEDURES

Materials. Glutathione (purity ≥99.0%), di-*tert*-butyl dicarbonate (purity ≥97.0%), tetrahydrofuran (purity ≥99.5%), hydrogen peroxide (35% aqueous solution, w/w), ethyl acetate (purity ≥99.5%), 1-chloro-2,4-dinitrobenzene (DNCB, purity ≥99.0%), glutaraldehyde (50% aqueous solution w/w), *p*-phenylenediamine (purity ≥97.0%), maleic anhydride (purity ≥99.0%), 2,4,6-trinitrobenzenesulfonic acid (TNBS) sodium salt dihydrate (purity ≥98.0%), formaldehyde solution (35% solution, w/w), methyl salicylate (purity ≥98.0%), phenol (purity ≥99.0%), sodium dodecyl sulfate (≥95.0%), sodium dihydrogen phosphate (purity ≥99.0%), and sodium cyanoborohydride ($NaBH_3CN$, purity ≥95%) were obtained from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). Acetic acid (purity ≥99.7%), 1,4-dioxane (purity ≥98.0%), distilled water, trifluoroacetic acid (purity ≥98.0%), 0.1 mol/L sodium thiosulfate solution, sodium tungstate dehydrate (purity >98.0%), hydroquinone (purity ≥99.0%), disodium hydrogen phosphate, and heptahydrate (≥99.0%) were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Acetonitrile (purity >99.5%), benzoquinone (purity >98.0%), and *trans*-cinnamaldehyde (purity >97.0%) were obtained from Kanto Chemical Co., Inc. (Tokyo, Japan). Triethylamine (purity >99.0%), triisopropylsilane (purity >98.0%), *N*-(*tert*-butoxycarbonyl)-1,2-diaminoethane (purity >97.0%), 4 mol/L hydrogen chloride in 1,4-dioxane, tetrabutylammonium bromide (purity >98.0%), 2-mercaptobenzothiazole (purity >99.0%), trimellitic anhydride (>98.0%), α-hexylcinnamaldehyde (purity >90.0%), and 2-phenyl-1-propanol (purity >98.0%) were sourced from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Deuterium oxide (99.9% D) and 4-chloro-7-nitrobenzofurazan (purity >98.0%) were purchased from Sigma-Aldrich (St. Louis, MO). 1-Ethyl-3-(3-(dimethylamino)propyl)-carbodiimide was purchased from the Peptide Institute, Inc. (Osaka, Japan).

Instrumentation. Analysis of adducts with known skin sensitizers and nonsensitizers was performed using high-performance liquid chromatography (HPLC, LC-20A, Shimadzu Corporation, Kyoto, Japan) with fluorescence detection (RF-$20A_{XS}$, Shimadzu Corporation). The excitation and emission wavelengths were 470 and 540 nm, respectively. An XBridge C18 column (150 × 4.6 mm i.d., 3.5 μm; Waters Corporation, Milford, MA) was used with a gradient elution with a mobile phase flow rate of 1.0 mL/min. The mobile phase was a mixture of 0.1% acetic acid in water (mobile phase A) and 0.1% acetic acid in acetonitrile (mobile phase B). The initial mobile phase composition was 95% of mobile phase A and 5% of mobile phase B, and the percentage of mobile phase B was increased linearly to 95% over 20 min. An Applied Biosystems QSTAR XL mass spectrometer (Thermo Fisher Scientific, Waltham, MA) was used for LC-MS of adducts of known skin sensitizers. The LC-MS experiments were carried out under the following conditions: electrospray ionization mode, positive; capillary voltage, 5.5 kV; ion source temperature, 300 °C; and mass range, m/z 50−1200. For detecting and identifying minor skin-sensitizing impurities in the reaction mixture, a Waters ACQUITY UPLC with a FLR fluorescence detector

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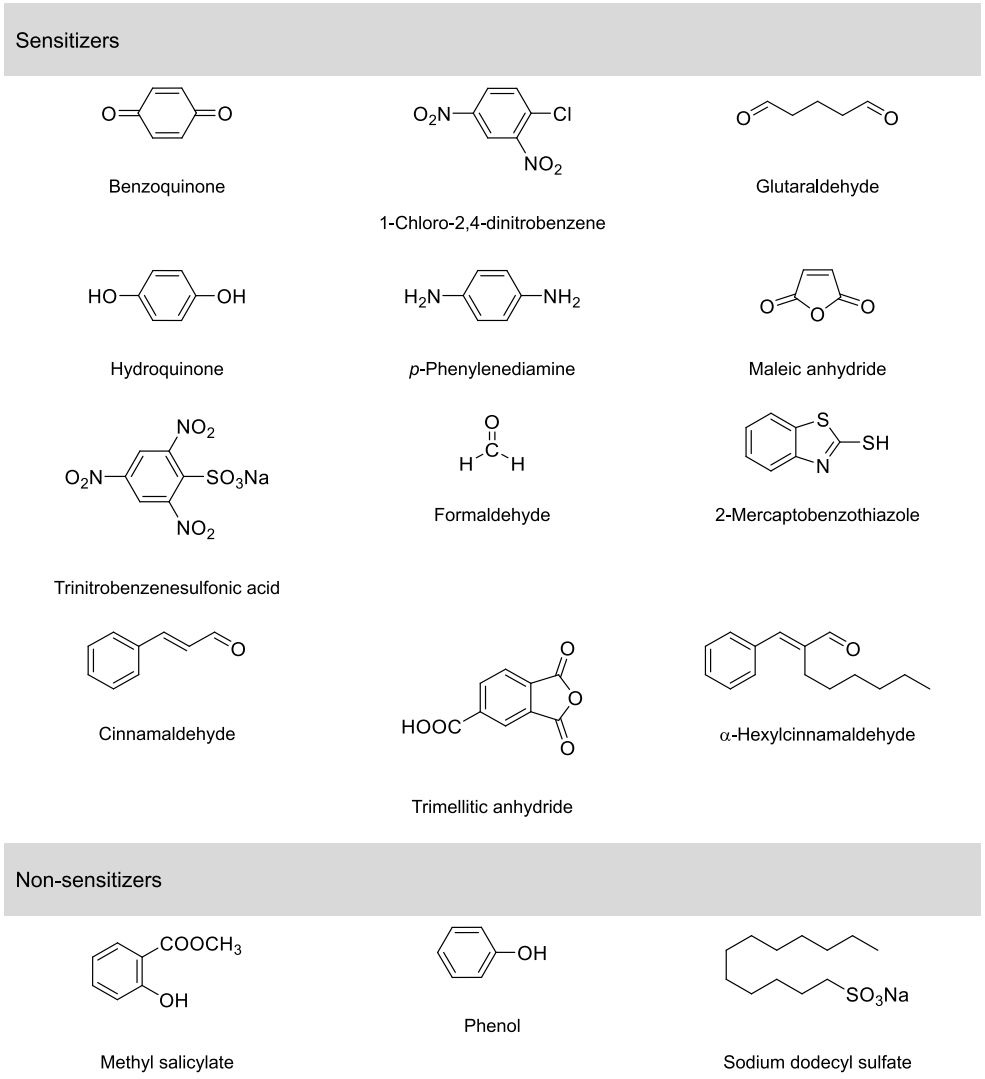

Figure 2. Structures of the tested compounds.

(Waters Corporation, Milford, MA) and ZORBAX Eclipse XDB-C18 column (50 × 3 mm, 1.8 μm; Agilent Technologies, Santa Clara, CA) was directly hyphenated with a Waters ACQUITY TQD mass spectrometer. The composition of the mobile phase of water/acetonitrile was increased linearly from 95:5 (v/v) to 5:95 (v/v) in 4.33 min. The mobile phase flow rate was 0.86 mL/min. The LC flow was split before the mass spectrometer with a split ratio of 4:1. A Waters ACQUITY UPLC H-class and a Waters Synapt G2-Si mass spectrometer equipped with a Waters ACQUITY UPLC BEH C18 column (100 × 2.1 mm, 1.7 μm) were used to obtain high-resolution mass spectra for structure elucidation of the NBD-GSH adducts and skin-sensitizing impurities in the reaction mixture. The chromatographic conditions used for the high-resolution MS were the same as those for LC-MS with fluorescence detection. High-resolution mass spectra were recorded under the following conditions: electrospray ionization mode, positive; capillary voltage, 0.25 kV; ion source temperature, 100 °C; desolvation gas temperature, 450 °C; desolvation gas flow rate, 1200 L/h; and mass range, m/z 50−1500. Masslynx software (version 4.2, Waters Corporation) was used for data analysis. A Bruker AVANCE II 600 MHz NMR spectrometer (Bruker BioSpin Corporation, Billerica, MA) was used for ^{1}H NMR measurements. The ATR FTIR spectra were measured using a Jasco FT/IR-6300 spectrometer (Jasco Corporation, Tokyo, Japan) equipped with a single reflection diamond ATR accessory (ATR Pro One, Jasco Corporation). Aliquots of the samples were placed on the surface of the diamond ATR crystal and pressure was applied using a metal rod to obtain the spectrum. The spectral range was 350−4000 cm^{-1}. A F-2700 fluorescence spectrophotometer (Hitachi High-Tech Science Corporation, Tokyo, Japan) was used. The absorption spectrum of a NBD-GSH solution in water/acetonitrile (1:1, v/v) with the concentration of 4 × 10^{-6} mol/L was obtained from 220 to 730 nm. The excitation wavelength was set to 470 nm and the emission wavelength was scanned from 220 to 730 nm in 0.5 nm increments.

Synthesis of Fluorescent NBD-GSH. NBD-GSH was synthesized from GSH as shown in Figure 1. ^{1}H NMR (600 MHz, D_2O): δ 8.30 (br d, J = 9.0 Hz, 1H, benzofurazan H-5), 6.21 (d, J = 9.0 Hz, 1H, benzofurazan H-6), 4.33 (t, J = 6.6 Hz, 1H, Cys-α-methine), 3.90 (t, J = 6.6 Hz, 1H, Glu-α-methine), 3.78 (s, 2H, Gly-α-methylene), 3.61 (br s, 2H, $-CH_2CH_2-$), 3.51 (br t, J = 6.6 Hz, 2H, $-CH_2CH_2-$), 2.74 (d, J = 6.0 Hz, 1H, Cys-β-methylene), 2.51−2.40 (m, 2H, Glu-γ-methylene), 2.15−2.05 (m, 1H, Glu-β-methylene). ^{13}C NMR (100 MHz, D_2O): δ 174.5, 172.4, 172.2, 171.7, 146.4, 144.2, 143.8, 139.0, 120.5, 99.8, 55.7, 52.8, 42.7, 42.5, 38.0, 30.8, 25.5, 25.2. HRMS (ESI+): calcd for $C_{18}H_{25}N_8O_8S$ [M + H]$^+$, 513.1516; found, 513.1519. IR: 3276, 3072, 2946, 1641, 1618, 1590, 1525, 1413, 1354, 1242, 1187, 1132, 836, 721, 594, 517, 448 cm^{-1}. The detailed procedure for the preparation of NBD-GSH is given in the Supporting Information, section 1.1−1.5.

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Table 1. Comparison of Skin -Sensitizing Activities and NBD-GSH Adduct Formation for Different Compounds[a]

chemical	skin-sensitizing activity			NBD-GSH adduct	references
	guinea pig maximization test	local lymph node assay	DPRA		
benzoquinone	+	+ extreme	+	D	11,36
1-chloro-2,4-dinitrobenzene	+	+ extreme	+	D	11,36
glutaraldehyde	N/A	+ strong	+	D	36
hydroquinone	+	+ strong	+	D	11,36
p-phenylenediamine	+	+ strong	+	D	11,36
maleic anhydride	+	+ strong	+	D	35,36
trinitrobenzenesulfonic acid	N/A	+ strong	+	D	38
formaldehyde	+	+ strong	+	D	11,36
2-mercaptobenzothiazole	+	+ moderate	+	D	11,36
trans-cinnamaldehyde	+	+ moderate	+	D	11,36
trimellitic anhydride	+	+ moderate	+	D	11,36
α-hexylcinnamaldehyde	+	+ weak	−	D	11,36
methyl salicylate	−	−	−	ND	11,36
phenol	N/A	−	N/A	ND	37
sodium dodecyl sulfate	−	−[b]	−	ND	11,36

[a]Symbols and abbreviations: +, sensitizing; −, nonsensitizing; N/A, not available; D, detected; ND, not detected. [b]Characterized as a false positive in LLNA.[39]

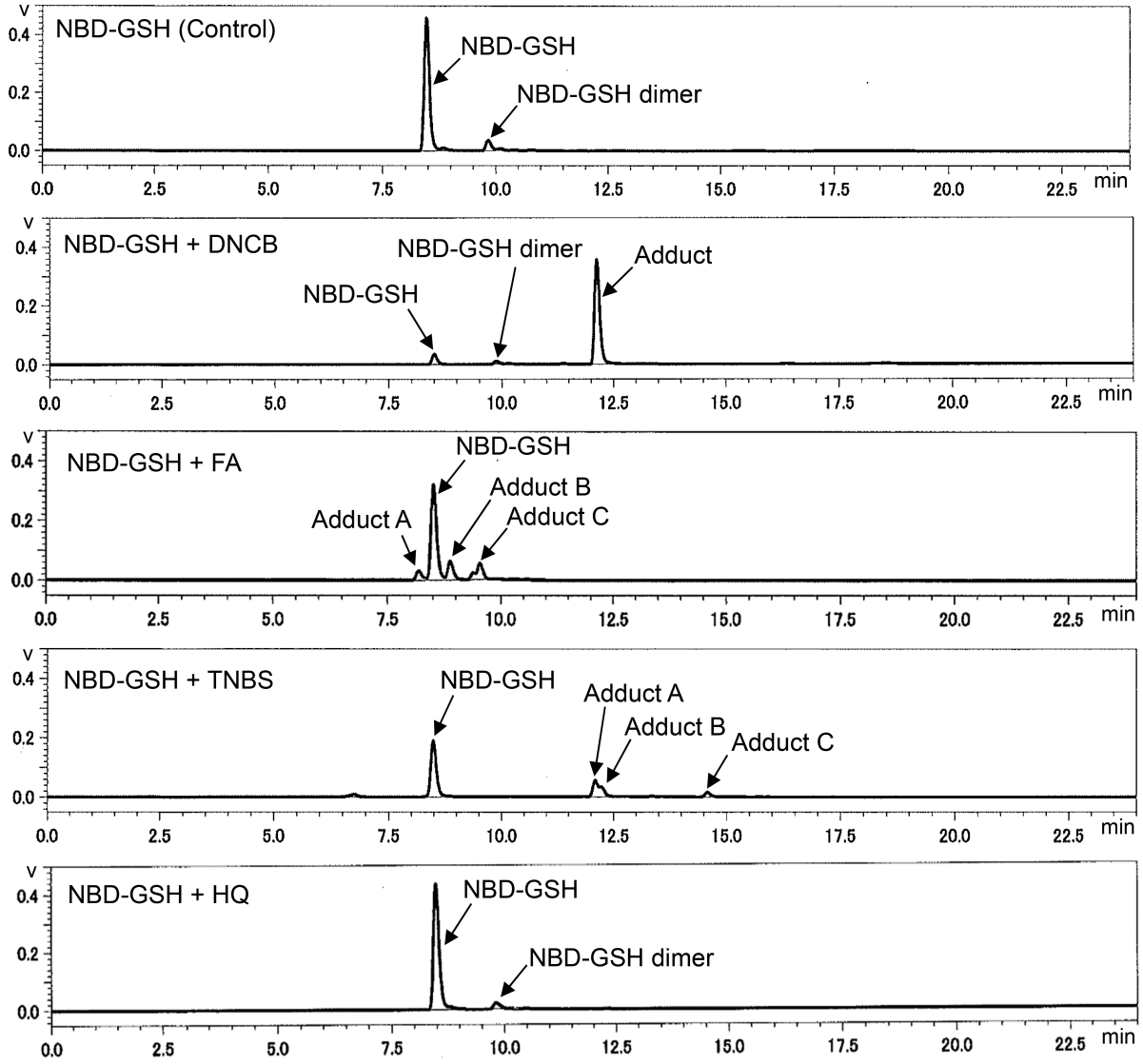

Figure 3. Chromatograms from liquid chromatography with fluorescence detection of incubated solutions of 1-chloro-2,4-dinitrobenzene (DNCB), formaldehyde (FA), trinitrobenzenesulfonic acid (TNBS), and hydroquinone (HQ) with NBD-GSH.

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Incubation of NBD-GSH with Known Skin Sensitizers and Nonsensitizers. NBD-GSH was dissolved in 0.2 mol/L phosphate buffer (pH 7.0) at a concentration of 20 μmol/mL (0.6 mg/mL). The test chemicals, 12 skin sensitizers, and 3 nonsensitizers were dissolved in acetonitrile or acetonitrile/water (1:1, v/v) at 20 μmol/mL. NBD-GSH and the chemical solutions were then mixed in a 1:1 molar ratio (NBD-GSH/chemical). After incubation for 1 h at 37 °C, the reaction mixtures were analyzed.

Limit of Detection of NBD-GSH Adducts with Skin Sensitizers. For an estimation of the limit of detection, 1-chloro-2,4-dinitrobenzene was dissolved in acetonitrile in the range from 4×10^{-5} to 4 μmol/L. These solutions were reacted with the NBD-GSH solution (4 μmol/L). Adduct peaks were detected using a fluorescence detector at 540 nm (λ_{em} = 470 nm). The limit of detection was calculated using the signal-to-noise ratio method.[29] The magnitude of the noise and the adduct peak height were measured manually on the chromatogram and a signal-to-noise ratio of 3 was used for the limit of detection.

Incubation of NBD-GSH with a Reaction Mixture. According to the procedure described by Giacomini and co-workers,[30] the oxidation of 2-phenyl-1-propanol was carried out to obtain 2-phenylpropionic acid, which is a substructure of ibuprofen. A mixture of 2-phenyl-1-propanol (1.36 g, 10 mmol), 35% aqueous H_2O_2 (0.2 mL), $Na_2WO_4 \cdot 2H_2O$ (33 mg, 0.1 mmol), tetrabutylammonium bromide (32 mg, 0.1 mmol), and 10 mol % $KHSO_4$ was stirred at 1000 rpm for 4 h at 90 °C. After the reaction was quenched with sodium thiosulfate, 40 μL of the obtained organic layer was diluted 10-fold with acetonitrile. Then 50 μL of the diluted solution was reacted for 10 min at 37 °C with 50 μL of a 4 mmol/L solution of NBD-GSH in 100 mmol/L phosphate buffer (pH 7.0)/acetonitrile (1:1). Finally, 10 μL of 400 mmol/L $NaBH_3CN$ aqueous solution was added and the mixture was reacted for 50 min at 37 °C to trap any skin-sensitizing byproducts. The reactant was analyzed by LC-MS with fluorescence detection.

RESULTS AND DISCUSSION

Synthesis and Characterization of NBD-GSH. NBD-GSH was synthesized from GSH in five steps with a total yield of 12%. It is known that thiol groups and primary amino groups in proteins react with electrophilic skin sensitizers.[31] Therefore, in the DPRA method, the cysteine peptide with a thiol group and the lysine peptide with a primary amino group were used for trapping different types of skin sensitizers.[14−16] NBD-GSH was designed to contain both a thiol group and a primary amino group. NBD was bound to the carboxyl group of GSH via an ethylene diamine linker to retain the free amino group and reduce steric hindrance of the reactive thiol and amino group caused by introduction of the NBD group. The NMR spectrum indicated that NBD was introduced selectively at the carboxyl group of the glycyl moiety (Figure S1, Supporting Information). This selectivity might be caused by steric hindrance of the butoxycarbonyl protective group toward the glutamyl moiety. NBD-GSH was dissolved in the HPLC mobile phase H_2O/CH_3CN (1:1, v/v). The maximum excitation wavelength was 467 nm and the maximum emission wavelength was 539 nm (Figure S2). These wavelengths were used to detect all adducts. The emission wavelength of NBD[32] is long enough to prevent overlap with the absorption wavelengths of a wide variety of chemicals and to give high selectivity in detection.

Trapping Known Skin Sensitizers with NBD-GSH. NBD-GSH was incubated with 12 known skin sensitizers and 3 nonsensitizers (Figure 2) for 1 h to obtain fluorescent adducts. After the reaction, the obtained reaction mixtures were analyzed with HPLC with fluorescence detection to confirm generation of the adduct peaks. All 12 skin sensitizers produced new peaks, whereas the 3 skin nonsensitizers did not give any peaks. The prohaptens hydroquinone and *p*-phenylenediamine did not form adducts with NBD-GSH within the 1 h reaction time. Adduct peaks were detected when the reaction time was extended to 24 h. It was presumed that benzoquinone and quinonediimine formed in the reaction solution by autoxidation.[33,34]

The results are summarized in Table 1. Results reported for guinea pig maximization tests, local lymph node assays, and DPRA results in previous research studies are provided for comparison.

LC-MS Structure Confirmation of the Adducts of Known Skin Sensitizers with NBD-GSH. The structures of the NBD-GSH adducts with skin sensitizers were confirmed by LC-MS. Chromatograms of the reaction mixtures of DNCB, formaldehyde, and TNBS with NBD-GSH are shown in Figure 3.

DNCB reacted completely with NBD-GSH within 1 h and a single adduct peak was observed. The adduct peak gave *m*/*z* 679 in LC-MS, and its isotope peak pattern did not indicate the presence of a chlorine atom in the molecule. Therefore, the chlorine atom of DNCB was substituted by NBD-GSH (Figure 4).

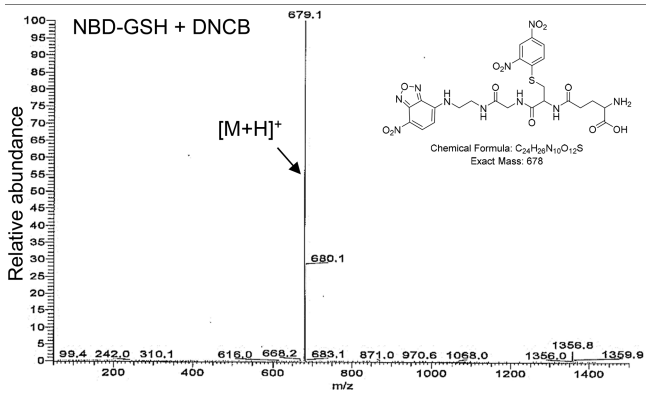

Figure 4. Mass spectrum of the NBD-GSH adduct with 1-chloro-2,4-dinitrobenzene (DNCB). Adduct structure was proposed according to the molecular weight.

Formaldehyde gave three peaks for adducts formed with NBD-GSH: one peak (*m*/*z* 543) was for an adduct formed via the thiol group and the other two peaks (*m*/*z* 525 and 537) were for adducts formed via the amino group (Figure 5).

Peaks for three adducts were observed with TNBS: Adducts A and B (*m*/*z* 724) with one TNBS and adduct C (*m*/*z* 935) with two TNBS (Figure 6). These results confirmed that NBD-GSH had two binding sites, at the thiol group and the primary amino group, as designed. The LC-MS results for the other chemicals are shown in Figures S3−S12.

Limit of Detection of the Adduct of NBD-GSH with DNCB. A DNCB concentration of 6×10^{-8} mol/L in the acetonitrile solution was required to give a NBD-GSH adduct peak 3 times the height of the noise by HPLC with fluorescence detection.

Trapping of Skin-Sensitizing Impurities Using NBD-GSH in a Reaction Mixture from Oxidization of 2-Phenyl-1-propanol. For obtainment of a phenyl propionic acid, 2-phenyl-1-propanol was oxidized by hydrogen peroxide with a tungsten catalyst. The obtained reaction mixture was reacted with NBD-GSH for 1 h to trap unknown skin-

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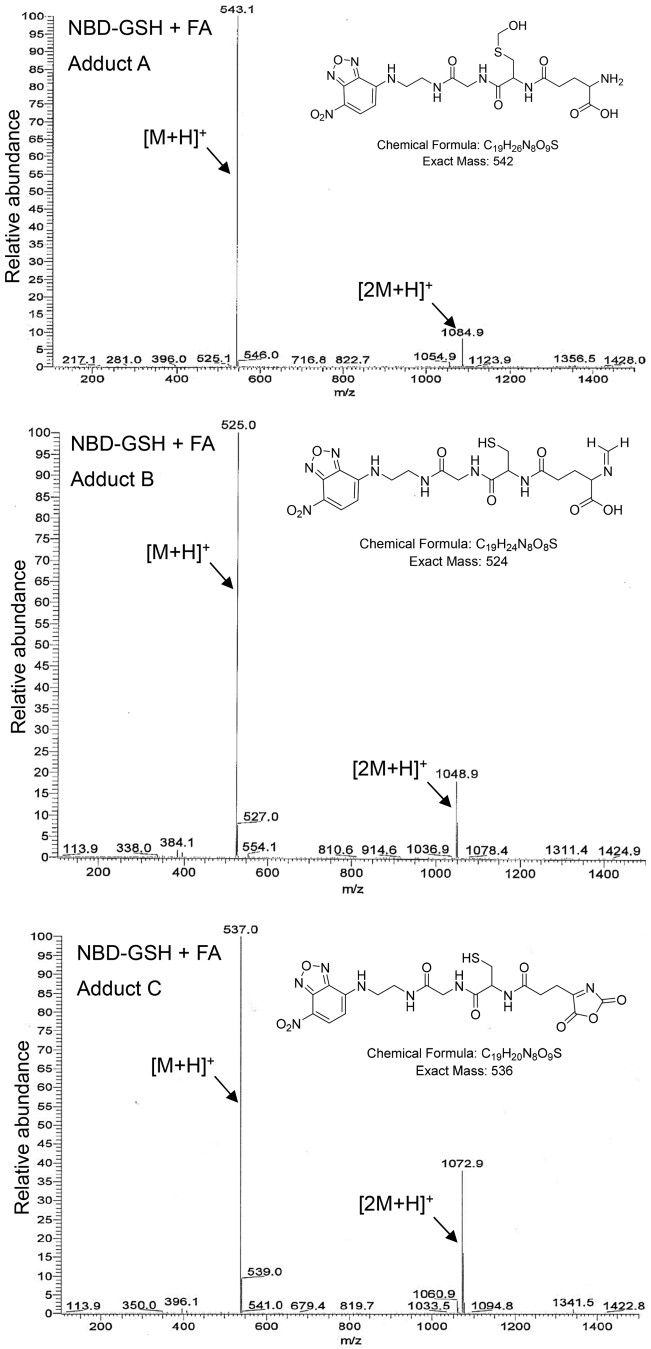

Figure 5. Mass spectra of the NBD-GSH adducts with formaldehyde (FA). Adduct structures were proposed according to the molecular weight.

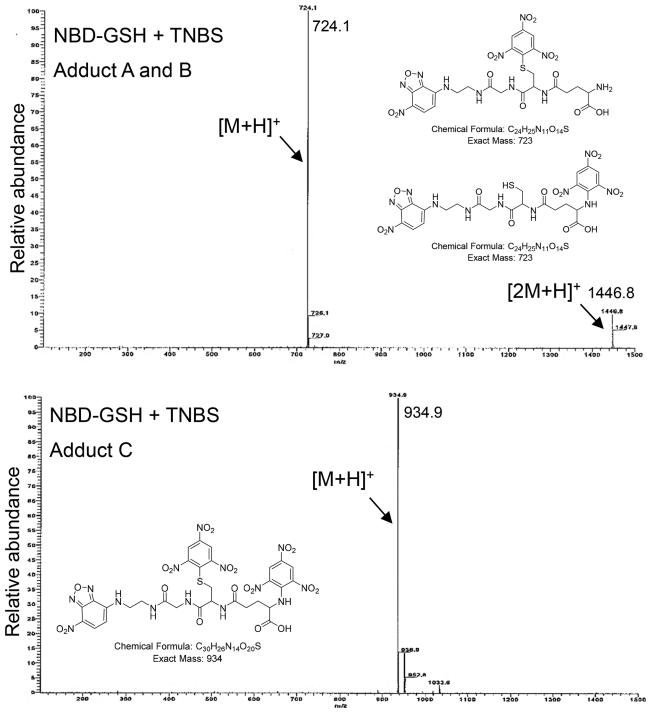

Figure 6. Mass spectra of the NBD-GSHs adducts with trinitrobenzenesulfonic acid (TNBS). Adduct structures were proposed according to the molecular weight.

sensitizing byproducts and then analyzed by LC-MS with fluorescence detection. One peak that was considered a fluorescent NBD-GSH adduct with unknown skin-sensitizing impurities was detected (Figure 7).

The mass spectrum of the adduct (m/z 631) and its elemental composition, $C_{27}H_{34}N_8O_8S$, from a high-resolution mass spectrum strongly suggested that the reduced adduct was formed by reaction of the amino group of NBD-GSH with the aldehyde byproduct 2-phenyl-1-propanal, which is known to be a skin sensitizer.[38−40] This aldehyde formed as an intermediate between the corresponding alcohol and carboxylic acid and remained in the reaction system as an impurity. Although adduct formation (m/z 629) occurred without using a reducing agent, the amount formed was very small because of the equilibrium between the amine and imine. Therefore, for breaking of the chemical equilibrium between the amine and imine and for production of the reduced adduct of NBD-GSH irreversibly, a reducing agent[41] was added following a previous report.[42] Even a small amount of an aldehyde generated as a strong skin-sensitizing impurity can give the final product strong skin-sensitizing properties; thus, the addition of a reducing agent is of great importance to allow for detection of the reduced adduct of NBD-GSH with high sensitivity and for identification of the cause of skin sensitization.

CONCLUSIONS

A method was developed for identifying small amounts of impurities with skin-sensitizing potential in reaction mixtures. Fluorescent NBD-GSH was synthesized from commercial GSH in five steps with a total yield of 12%. NBD-GSH formed adducts with 12 known skin sensitizers through its thiol group and/or amino group. These adducts were detected selectively using a fluorescence detector. The limit of detection of this method was determined as 6×10^{-8} mol/L of DNCB in the acetonitrile solution. For evaluation of the practicality of this method, a reaction mixture from alcohol oxidation of 2-phenylpropionic acid was assessed using the NBD-GSH binding assay. Under fluorescence detection, the obtained mixture produced a peak for a NBD-GSH adduct with skin-sensitizing potential impurity. Analysis of the peak by LC-MS analysis with fluorescence detection and HPLC-MS identified 2-phenyl-1-propanal as the skin-sensitizing impurity in the reaction mixture. This method eliminates the requirement for chromatographic separation and assessment of each fraction using animal or nonanimal tests to identify the main cause of

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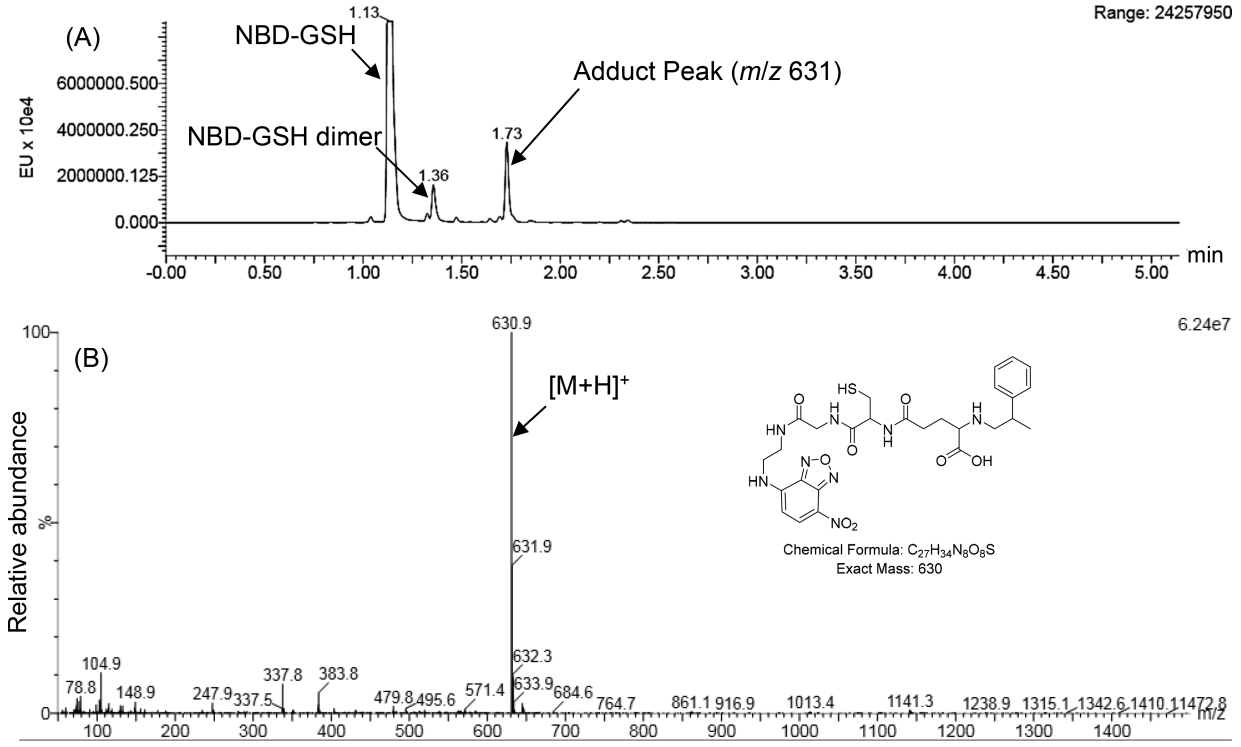

Figure 7. Trapping of skin-sensitizing impurities by NBD-GSH from the reaction mixture for oxidization of 2-phenyl-1-propanol. (A) Chromatogram from liquid chromatography with fluorescence detection of the incubated solution of the reaction mixture with NBD-GSH and $NaBH_3CN$. (B) Mass spectrum of the NBD-GSH adduct with the skin sensitizer 2-phenyl-1-propanal in the reaction mixture. The adduct structure was proposed according to the molecular weight.

the skin-sensitizing property in the mixture. Consequently, it will be useful for identifying small amounts of skin sensitizers in raw materials, intermediates, reaction mixtures, and end products in the chemical industry.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.chemrestox.0c00327.

Detailed procedure of NBD-GSH synthesis; introduction of NBD to GSH; fluorescence spectra of NBD-GSH; chromatograms from liquid chromatography with fluorescence detection; and LC mass spectra of the NBD-GSH adducts (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Takashi Tokunaga − *Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., Osaka 554-8558, Japan;* orcid.org/0000-0002-9876-0563; Email: tokunagat2@sc.sumitomo-chem.co.jp

Authors

Gaku Yamamoto − *Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., Osaka 554-8558, Japan*

Teruki Takahashi − *Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., Osaka 554-8558, Japan*

Makiko Mukumoto − *Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., Osaka 554-8558, Japan*

Masayuki Sato − *Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., Osaka 554-8558, Japan*

Masahiko Okamoto − *Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., Osaka 554-8558, Japan*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.chemrestox.0c00327

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Notes

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