Sources of Hydrogen Abstraction by Activated Neocarzinostatin Chromophore†

Der-Hang Chin and Irving H. Goldberg*

Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts 02115

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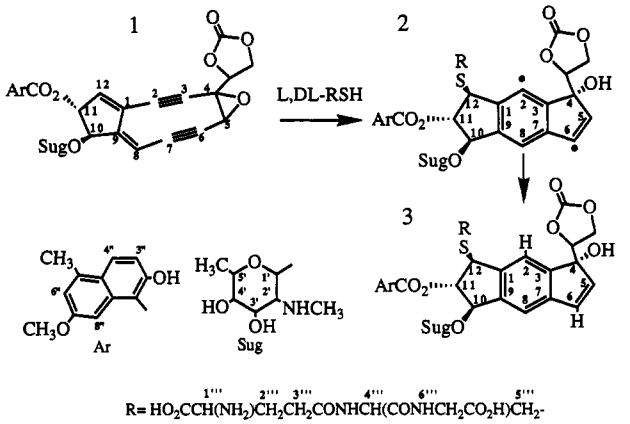
ABSTRACT: Activation of the enediyne neocarzinostatin chromophore (NCS-Chrom) by thiol addition at C-12 generates a diradical species with radical centers at C-2 and C-6, which abstract hydrogens from deoxyribose in the minor groove of DNA. Since hydrogen abstraction from DNA accounts for only part of the hydrogen incorporated at these sites, it is important to determine the other possible sources. At low concentration of thiol, a condition resembling that during NCS-Chrom-induced DNA damage, the major non-DNA hydrogen donation source was found to be the carbon-bound hydrogen of the aqueous methanol solvent, rather than the expected sulfur-bound hydrogen of the thiol. Further, experiments with the γ-L-glutamyl-DL-cysteinylglycine labeled with deuterium on the α- or β-carbons to the sulfur showed small amounts of internal transfer of hydrogen into C-2 of the drug from the naturally occurring L,L diastereomer only. Quantitation of the hydrogen transfer was accomplished by separation of the L,DL diastereomeric mixtures of the thiol–NCS-Chrom adducts. In all, these various hydrogen donation sources can account for at least 70–80% of the hydrogen incorporated at C-2 of the drug under DNA damage conditions. Selective quenching of the radical at C-2 could account for the predominance of single-stranded over double-stranded DNA lesions.

Neocarzinostatin chromophore (NCS-Chrom)[1] (**1**), the first bicyclic enediyne-containing antitumor antibiotic discovered (Goldberg, 1991), cleaves DNA by abstracting two hydrogen atoms (Chin et al., 1988) from the sugar moieties in the minor groove to form a stabilized tetrahydroindacene derivative (**3**). The activation process involves addition of a thiol at C-12 of the chromophore, which generates a diradical centered at C-2 and C-6 (**2**) (Scheme I) (Myers, 1987). When DNA containing the sequence AGC·GCT is present, the C-5′H at the T residue and the C-1′H at the C residue were found to be incomplete sources of the hydrogens abstracted by C-6 and C-2, respectively, in the formation of the bistranded lesions (Meschwitz & Goldberg, 1991; Meschwitz et al., 1992). In addition to DNA, the other sources of abstracted hydrogen can be either sulfur-bound (exchangeable) hydrogen or carbon-bound (nonexchangeable) hydrogen present in the reaction mixture. It is not clear from the literature the degree to which the various hydrogen abstraction sources contribute under conditions resembling those occurring during NCS-Chrom-induced DNA damage.

Since sulfur-bound hydrogen is considered to be a very effective hydrogen donor, it was believed that it was the main, if not only, non-DNA hydrogen source (Albers-Schonberg et al., 1980). In fact, however, the first reported molecular formula of the NCS-Chrom had to be revised downward by two hydrogens due to this incorrect assumption that reduction by methyl thiol had not occurred, since the molecular weight was the same whether or not the solvent was deuterated, showing that under the conditions used exchangeable deuterium from the solvent was not incorporated into the drug (Albers-Schonberg et al., 1980; Hensens et al., 1983). In addition, ^{1}H NMR studies of deuteriomethylthioglycolate-induced activation of NCS-Chrom in 0.1 M CD_3COOD/CH_3OD, at 2–5 equiv of thiol to the drug, showed insignificant reduction in the intensities of 2-H and 6-H, and MS analysis of the glutathione–NCS-Chrom reaction product in the presence of DNA indicated that no deuterium had been incorporated whether water or deuterium oxide was used as solvent (Chin et al., 1988; Hensens et al., 1983; Hensens & Goldberg, 1989). These results suggested that carbon-bound rather than sulfur-bound hydrogen should be considered as a source of hydrogen donation. The carbon-bound hydrogen of the thiol was proposed as a possible source (Chin et al., 1988). On the other hand, Myers et al. (1988) found that substantial deuterium was incorporated from the exchangeable hydrogen of methylthioglycolate into the C-2 and C-6 positions of the drug at high ratios of thiol (0.2 M, 300 equiv) to drug. At lower ratios of thiol to drug (Myers & Proteau, 1989) the incorporation of carbon-bound deuterium of the solvent (9:1 tetrahydrofuran-d_8/CD_3COOH) into these positions was correspondingly increased.

In an effort to quantify the contributions of the various hydrogen abstraction sources, we undertook initially to characterize the possible contribution of the carbon-bound hydrogen of the thiol. By using the synthetic diastereomeric

Scheme I



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* Author to whom correspondence should be addressed.

[1] Abbreviations: NCS, neocarzinostatin; NCS-Chrom, nonprotein chromophore of neocarzinostatin; NMR, nuclear magnetic resonance spectroscopy; HPLC, high-pressure liquid chromatography; L,DL-RSH, γ-L-glutamyl-DL-cysteinylglycine; L,L-RSH, γ-L-glutamyl-L-cysteinylglycine (glutathione); L,D-RSH, γ-L-glutamyl-D-cysteinylglycine; Tris, tris(hydroxymethyl)aminomethane.

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mixture of γ-L-glutamyl-DL-cysteinylglycine (L,DL-RSH) with deuterium replacing both hydrogens on the carbon α to the sulfur, a small percentage of deuterium incorporation from the α carbon of the adducted thiol into C-2 was found (Chin & Goldberg, 1992). Hydrogen abstraction appeared to be limited to the L,L diastereomer. This result was consistent with the findings of Wender and Tebbe (1991) of internal hydrogen transfer with a synthetic acyclic analogue of NCS-Chrom and of McAfee and Ashley (1992) showing a deuterium isotope effect on DNA double-strand damage with [2,2-$^2H_2$]-thioglycolate as the activating thiol. Nevertheless, the non-DNA origins of the remaining hydrogen at C-2 and that of all of the hydrogens at C-6 were not clear under DNA damage conditions.

In this report, we have obtained a more accurate quantitation of deuterium incorporation from the α-carbon by separating the L,DL diastereomeric mixture of the thiol–NCS-Chrom adducts. We have also made the diastereomeric mixture of L,DL-RSH with deuterium at the position β to the sulfur and examined whether any internal deuterium transfer occurred from the β-carbon of the adducted thiol into C-2. Finally, at a low concentration of thiol, it was found that the non-DNA donor of hydrogen was from the solvent, but that it was from the carbon-bound hydrogen of the methanol and not from the sulfur-bound hydrogen of the thiol. These data help to resolve previously reported ambiguities concerning the sources of non-DNA hydrogen incorporated into the radical centers of NCS-Chrom under drug–DNA reaction conditions.

EXPERIMENTAL SECTION

NCS-Chrom was repeatedly extracted from the holoprotein with 20 mM sodium citrate in methanol and stored at −70 °C as described (Chin et al., 1987). Sonicated calf thymus DNA was prepared as previously reported (Chin et al., 1984). Phage λ DNA with tritium (8.9 cpm/pmol) at the C-5′ position of thymidine was prepared as described (Chin et al., 1987). A diastereomeric mixture of L,DL-RSH was prepared by solid-phase peptide synthesis. DL-Cysteine was converted initially into *S*-trityl-DL-cysteine to protect the thiol group (Hiskey & Adams, 1965) and subsequently into *N*-Fmoc-*S*-trityl-DL-cysteine to protect the amino group (Atherton & Sheppard, 1989) for peptide synthesis. The crude product of L,DL-RSH was reduced by an equal amount of dithiothreitol in 100 mM Tris-HCl at pH 7.6 in a 60 °C bath for 1 h to yield the reduced form of L,DL-RSH. The product was then recrystallized from acetone and dried under vacuum. Amino acid analysis and ^1H NMR indicate that it was the expected product. The ability of L,DL-RSH to activate NCS-Chrom was verified by measuring the incorporation of tritium into the drug from [5′-^3H]thymidine-labeled λ-DNA at both DNA phosphorus to drug and thiol to drug ratios of 5 in a 13% methanolic aqueous solution. L,DL-RSH affords the same amount of tritium abstraction from DNA into the drug as glutathione. L,DL-RSH with deuterium replacing both hydrogens on the α-carbon or the hydrogen on the β-carbon to the sulfur was prepared by the same procedure. [α,α-$^2H_2$]-DL-Cysteine and [β-^2H]-DL-cysteine were purchased from Cambridge Isotope Laboratories. ^1H NMR analysis showed that the deuterium enrichments were more than 90%.

The thiol–drug adducts were produced by mixing the thiol with NCS-Chrom at a ratio of between 8 and 1250 (the concentration of the drug ranges from 60 to 100 μM), using sodium citrate and Tris-HCl as buffers to control the pH between 4 and 8 in 10–100% methanol in water. In the reaction involving calf thymus DNA, DNA was added to the drug at pH 4 in 10% methanol at a DNA phosphorus to drug ratio of 20. The mixture was lyophilized to dryness and redissolved in water. L,DL-RSH at a ratio of thiol to drug of 8, as well as Tris-HCl (pH 8), was added to initiate the reaction. The final concentrations were as follows: DNA, 2.5 mM; NCS-Chrom, 0.125 mM; sodium citrate, 10 mM; L,DL-RSH, 1 mM; Tris-HCl, 100 mM. The reaction mixture was subsequently lyophilized and redissolved in 1 M ammonium acetate at pH 4. After repeated precipitations of the DNA with 80% ethanol, the supernatant fluids containing the inactivated thiol–drug adduct were combined and lyophilized for purification.

Purification was by reversed-phase HPLC on a Beckman C_{18} (5 μm, 0.4 × 25 cm) column. A Beckman Model 332 liquid chromatograph system was used with gradient composed of 5 mM ammonium acetate (pH 4) and increasing concentrations of methanol at a flow rate of 1 mL/min. The final separation of the diastereomeric mixture of L,DL-RS–NCS-Chrom adduct was performed by connecting three C_{18} (5 μm, 0.4 × 25 cm) columns with an approximately 600-min gradient of 45–60% methanolic 5 mM ammonium acetate (pH 4) at a flow rate of 0.6 mL/min. The gradient is shallower, from 45 to 53%, for a better separation and steeper, from 53 to 60%, to prevent an overspreading of peaks. L,L-RS(glutathione)–NCS-Chrom adduct eluted at about 500 min and was well separated from the L,D-RS–NCS-Chrom adduct, which eluted about 10 min later. ^1H NMR spectra were obtained on an FT 500-MHz instrument by using 2–100 mM HCl in D_2O as solvent. Because the chemical shifts of the glutathione–drug adduct were very sensitive to the temperature in this solvent, samples were kept at a constant temperature (from 10 to 45 °C) during the collection of data.

Measurement of the incorporation of deuterium at C-2 and C-6 was made by a direct comparison of the labeled and nonlabeled spectra, which were accumulated under the same NMR data acquisition conditions. The labeled and unlabeled materials were also derived and purified from side-by-side drug–thiol (with or without DNA) reactions under the same conditions. Experiments using a deuterium label on the α-carbon of the thiol involved three separate sets of reactions. The peak areas of H-8, H-6″, and H-5 were chosen as a standard for normalization of both labeled and nonlabeled spectra, and the average incorporation values were derived from separate calculations based on each standard. The standard deviations by this procedure were from 0.7% up to 7.9% with an average of about 5%. When the incorporation percentage was between +5% and −5%, it was labeled as "none" in the tables.

RESULTS AND DISCUSSION

In order to obtain a well-resolved ^1H NMR spectrum for a more accurate estimation of the internal deuterium incorporation from the adducted thiol into C-2, all diastereomeric mixtures of L,DL-RS–NCS-Chrom adducts were separated by HPLC with three connected C_{18} columns and a very shallow and long gradient, as described in the Experimental Section. L,DL-RS–drug adducts, which could not be well resolved with the standard procedures of HPLC analysis, are well separated under these conditions. An example of the HPLC profile is shown in Figure 1a, which illustrates the separation of [α,α-$^2H_2$]-L,DL-RS–NCS-Chrom, a purified thiol–drug product from a reaction at pH 7.6 in a close to 100% methanol solution. Since the ^1H NMR of the material collected from the first major peak showed the characteristic spectrum of the glutathione–NCS-Chrom adduct, the first major single symmetric peak was identified as the L,L-thiol–drug adduct (Chin

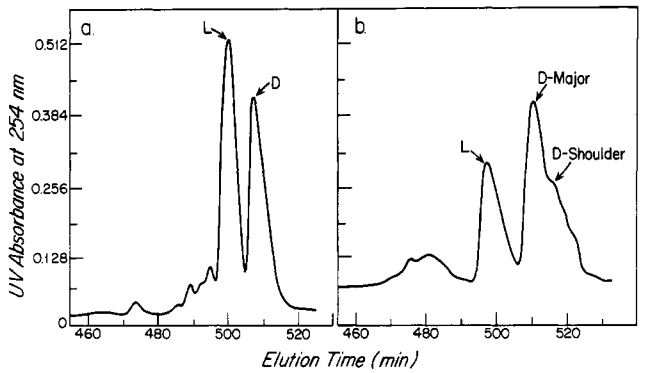

FIGURE 1: (a) HPLC profile for the isolation of [α,α-$^2H_2$]-L,DL-RS–NCS-Chrom which had been purified from a reaction at pH 7.6 in virtually 100% methanol as solvent. (b) HPLC profile for the isolation of [α,α-$^2H_2$]-L,DL-RS–NCS-Chrom which had been purified from a reaction in the presence of DNA in aqueous solution. L represents the L,L adduct, and D represents the L,D adduct.

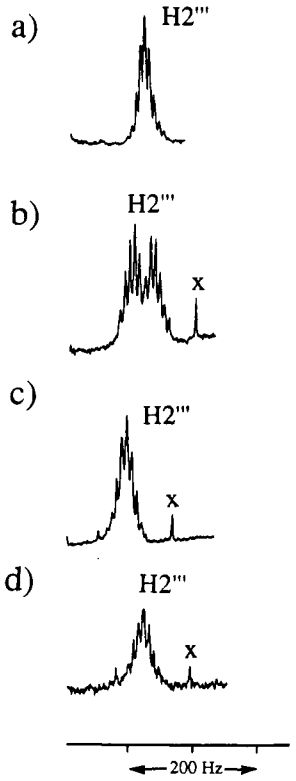

FIGURE 2: Resonance patterns of the H-2''' of the glutamyl group in ^{1}H NMR for (a) L,L-RS–NCS-Chrom; (b) L,D-RS–NCS-Chrom; (c) L,D-RS-major–NCS-Chrom without the material collected from the shoulder portion in HPLC analysis; (d) L,D-RS-shoulder–NCS-Chrom. X represents an unknown impurity.

& Goldberg, 1992). The L,D adduct, which showed a slightly asymmetric peak shape, eluted about 10 min after the L,L adduct. If a shallower gradient of the HPLC analysis was applied, shoulders could be clearly observed on the elution peak of the L,D-thiol–drug adduct, suggesting that more than one species coexisted in the L,D adduct (Figure 1b).

^{1}H NMR showed that the resonance signals of the L,D adduct from the rearranged tetrahydroindacene central core were close to those of the L,L diastereomer of the thiol–drug adduct except that for H-2. These findings indicate that the backbone of the adducted thiol is relatively rigid. Figure 2 shows the resonance pattern of the H-2''' of the glutamyl group in the adducted tripeptide. Because of the chemical shifts of the inactivated thiol–drug products were very sensitive to the temperature, the acidity of the NMR deuterium oxide solvent,

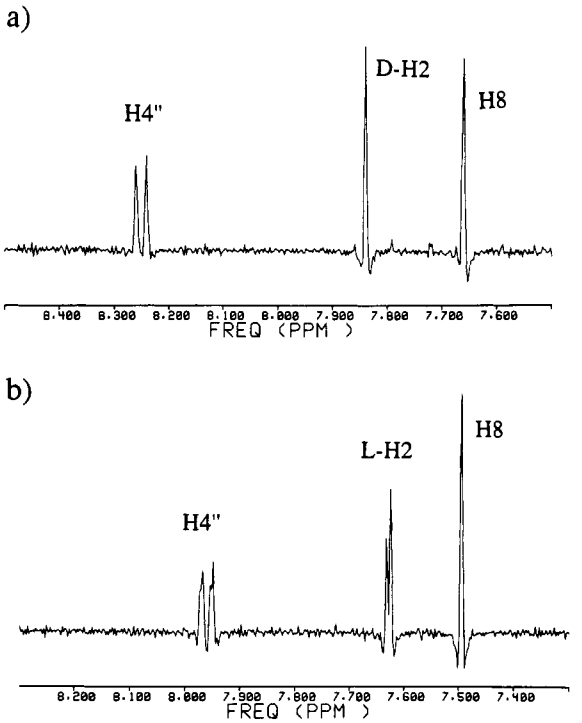

FIGURE 3: Partial ^{1}H NMR spectra of (a) L,D-RS–NCS-Chrom and (b) L,L-RS–NCS-Chrom. L represents the L,L adduct, and D represents the L,D adduct.

and the concentration of the compound, the chemical shifts of each resonance signal varied from sample to sample. One multiple resonance signal for the L,L adduct and two multiple signals for the L,D adduct were observed for both H-2''' and H-3''' of the glutamyl group in the adducted thiol. Both peaks appeared at about 1.8–2.8 ppm, with H-2''' about 0.4 ppm upfield from H-3'''. The two multiple signals from the L,D adduct were reduced to one when the L,D major and L,D shoulder components were separately collected by HPLC. Since ^{1}H NMR of the L,D shoulder component also showed the characteristic pattern of the L,D-RS–drug adduct, it is obvious that there are at least two different species formed during the reaction of L,D-RSH and NCS-Chrom.

Although only a single peak appeared on the HPLC analysis for the L,L adduct when it was isolated from the L,D adduct (Figure 1), ^{1}H NMR analysis of the collected L,L adduct indicated that the material also contained at least two species (Figure 3). Unlike the L,D adduct mixture (containing both L,D major and L,D shoulders, etc.), which had only one single resonance signal for H-2 and H-8 and one doublet signal for H-4'', the L,L form clearly showed two single resonance signals for H-2 and two doublet signals for H-4''. The ratio of the two signals varies with the reaction conditions. The regular glutathione-activated thiol–drug adduct also showed a similar pattern. These findings indicate that the mechanism of activation of NCS-Chrom may be more complicated than previously thought.

Figure 4 shows partial ^{1}H NMR spectra of the purified but unseparated L,DL-RS–NCS-Chrom adducts with and without deuterium replacing the hydrogen at the position β to the sulfur. When deuterium replaces the hydrogen at the β-position, the resonance signal of L,L-H-2 is reduced but the one corresponding to L,D-H-2 is increased. A similar effect occurred when deuterium replaced both hydrogens at the α-position (Chin & Goldberg, 1992). Integration indicates that internal quenching of the radical at C-2 by a hydrogen atom transferred from the adducted thiol not only occurred

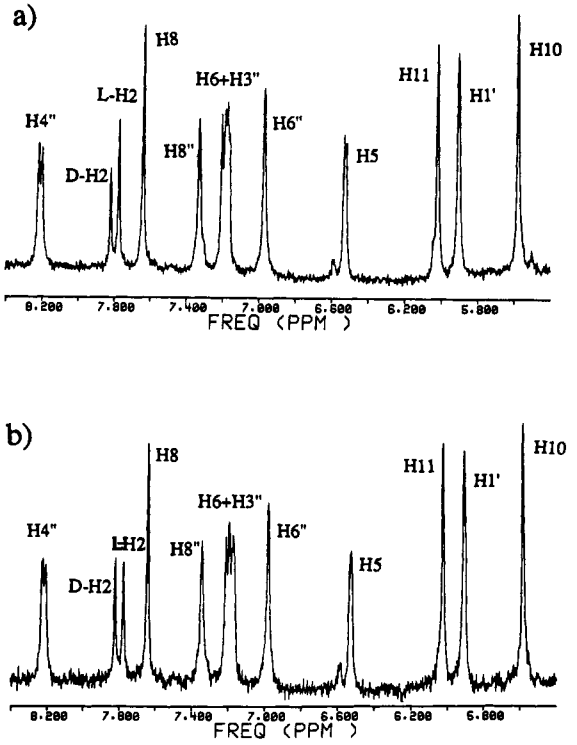

FIGURE 4: Partial ^{1}H NMR spectra of (a) L,DL-RS–NCS-Chrom and (b) [β-^{2}H]-L,DL-RS–NCS-Chrom. L represents the L,L adduct, and D represents the L,D adduct.

Table I: Deuterium Incorporation from the α- and β-Carbons of the Adducted Thiol into the Drug

	no DNA		DNA	
materials	H-2	H-6	H-2	H-6
[α,α-$^2H_2$]-L,L-RS–NSC-Chrom	15 ± 5%	none	14 ± 5%	none
[α,α-$^2H_2$]-L,D-RS–NCS-Chrom	none	none	none	none
[β-^{2}H]-L,L-RS–NCS-Chrom	10 ± 5%	none	10 ± 5%	none
[β-^{2}H]-L,D-RS–NCS-Chrom	none	none	none	none

from the α- but also from the β-position. Since the amounts of the deuterium transferred from both positions were very small, the components of the L,DL mixture of the thiol–drug adducts were isolated, and better resolved ^{1}H NMR spectra were obtained for a more accurate analysis (Table I).

^{1}H NMR spectra of both the α- and β-labeled forms of the separated L,D and L,L species of the thiol–drug adducts were basically identical, whether the purified materials were derived from the reaction with DNA in aqueous solution or without DNA in methanolic solution. Figure 5 demonstrates one set of typical ^{1}H NMR spectra of the α-labeled and nonlabeled thiol–drug adduct of the separated L,L form. Figure 6 represents one set of typical spectra for the separated L,D form. Table I summarizes the analyzed results. No deuterium incorporation was observed at the C-6 position of either the L,D or L,L adduct or at the C-2 position of the L,D adduct. Hence, the small amount of deuterium incorporated into the C-2 position of the L,L adduct was evidently not from the α- or β-carbon of the thiol free in solution.

The amount of internal quenching at C-2 is independent of the percentage of methanol in the reaction solution. Further, whether the thiol was added together with the Tris-HCl buffer into the drug-containing solution as a relatively deprotonated form or was added before the Tris-HCl buffer into the acidic drug solution as a protonated form did not significantly alter the amount of the incorporation. Since the extent of incorporation was relatively independent of the above reaction

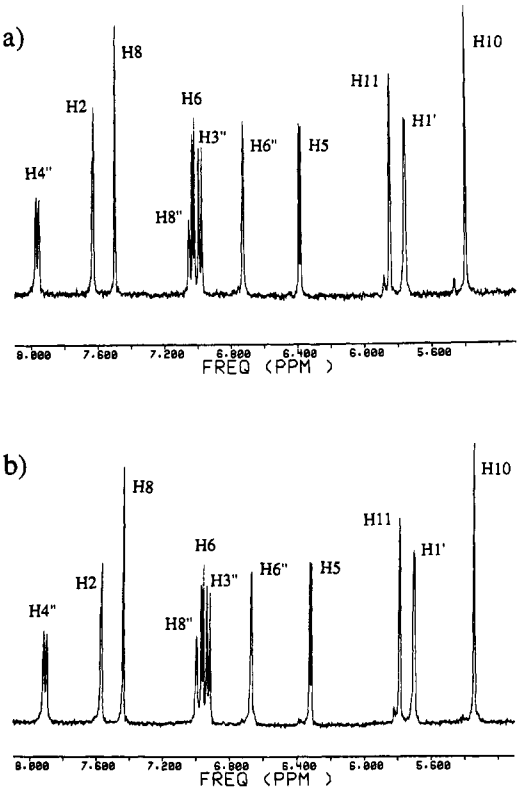

FIGURE 5: Partial ^{1}H NMR spectra of (a) L,L-RS–NCS-Chrom and (b) [α,α-$^2H_2$]-L,L-RS–NCS-Chrom. Both materials results from a reaction at pH 7.6 and virtually 100% methanol as solvent.

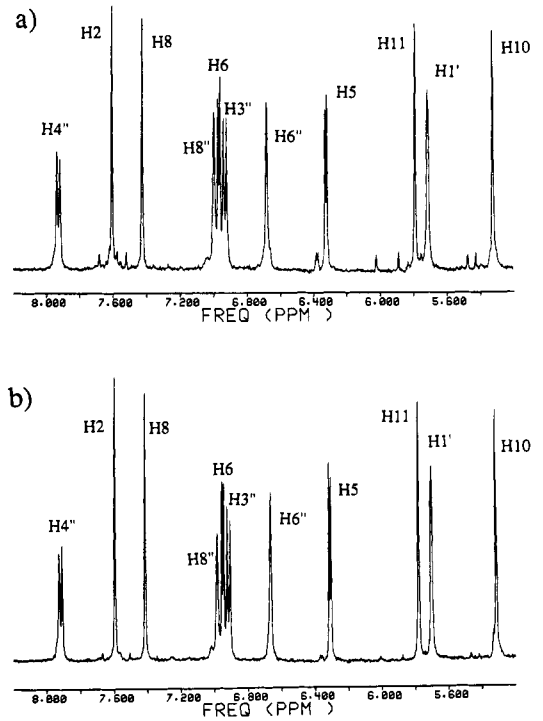

FIGURE 6: Partial ^{1}H NMR spectra of (a) L,D-RS–NCS-Chrom and (b) [α,α-$^2H_2$]-L,D-RS–NCS-Chrom. Both materials resulted from a reaction in the presence of DNA in an aqueous solution.

conditions, it appears that the hydrogen transfer occurred internally, probably after the attacking thiol had already been attached to the drug.

In contrast with experiments using the synthetic acyclic analogue of NCS-Chrom, which showed a >90% favored internal hydrogen transfer to the aryl radical over intermolecular transfer (Wender & Tebbe, 1991), the amount of

Table II: Deuterium Incorporation into NCS-Chrom for the Glutathione–Drug Reaction in Solvent Lacking Carbon-Bound Deuterium

concentrations (mM)		thiol:drug ratio	solvent	H-2	H-6
thiol	drug				
1.0	0.1	10	CH_3OD, pH 4	none	none
0.8	0.1	8	CH_3OD, pH 7.6	none	none
1.0	0.1	10	50% CH_3OD/D_2O, pH 4	none	none
125	0.1	1250	50% CH_3OD/D_2O, pH 4	30 ± 5%	50 ± 5%

Table III: Deuterium Incorporation into NCS-Chrom for the Glutathione–Drug Reaction in Solvents with Carbon-Bound Deuterium

concentration (mM)		thiol:drug ratio	solvent	H-2	H-6
thiol	drug				
1.0	0.10	10	CD_3OD, pH 4	80 ± 5%	100%
0.84	0.084	10	80% CD_3OD/CH_3OD, pH 4	16 ± 5%	32 ± 5%
1.0	0.061	16	10% CD_3OH/H_2O, pH 8	27 ± 5%	37 ± 5%

deuterium incorporated at C-2 from carbon-bound hydrogens at the α- and β-positions of the thiol in the L,DL-RS–NCS-Chrom reactions was small (Table I). In these studies the ratio of thiol to drug was 5–10 to 1. Thus, it was not clear from where the remainder of the non-DNA abstracted hydrogens at C-2 and C-6 came. It was necessary, therefore, to determine whether the rest of the remaining abstracted hydrogens were from the exchangeable sulfur-bound hydrogen. Table II shows the results of deterium incorporation for several reactions of glutathione and the drug in solvents which contained the deuterium only in the exchangeable form. No exchangeable hydrogen was incorporated into any of the C-2 or C-6 positions, except when a very high ratio of thiol to drug was used. Under normal DNA–drug reaction conditions, at low ratios of thiol to drug, sulfur-bound hydrogen apparently was not abstracted by the diradical intermediate of the drug. This finding is in contrast with the well-known behavior of mercaptans to act as radical scavengers by donation of the sulfur-bound hydrogen and is consistent with the results described earlier (Chin et al., 1988). When a very high ratio of thiol to drug was used, even up to about 100-fold of the usual ratio, the amounts of the sulfur-bound hydrogen incorporated into the drug were still far from complete. It is also worthy of note that the amount of deuterium incorporated at H-6 is about 20% higher than at H-2, most probably due to the internal hydrogen transfer from the α- and β-carbons of the adducted thiol into C-2.

Since no sulfur-bound hydrogen was observed to be incorporated into the drug under circumstances in which a low ratio of thiol to drug was used, the hydrogen which had been abstracted by the diradical intermediate of the drug over and above the internal transfer from the adducted thiol into C-2 had to come from carbon-bound hydrogen. Because there were several possible sources of carbon-bound hydrogens in the thiol–drug reaction mixture, it was essential to determine which carbon-bound hydrogen was mainly responsible for quenching the diradical intermediate of the drug. Table III demonstrates that the carbon-bound hydrogen from the methanol solvent was the actual donor for abstraction by the diradical form of the drug.

Although the carbon-bound hydrogen of methanol is generally a poor radical scavenger compared to a thiol, it can still be effectively involved in the Bergman reaction (Lockhart et al., 1981; Lockhart & Bergman, 1981). When 20% of the solvent methanol was replaced with the protio form, then the incorporation into H-6 was reduced drastically from 100% to 32% and into H-2 from 80% to 16%. When the carbon-bound deuterium from the methanol was further reduced to 50%, almost no incorporation was observed. These results are consistent with an isotope effect at both positions. The fact that the incorporation at C-2 was less than that at C-6 was, again, probably due to the effect of the internal hydrogen transfer from the adducted thiol into C-2. The finding that the carbon-bound hydrogen of methanol is the main donor for the non-DNA abstraction by the drug explains the earlier results of Tanaka et al. (1990) that, even at a low ratio of thiol to drug (3 equiv), there was significant deuterium incorporation into C-2 and C-6 of the drug when deuteriomethylthioglycolate was in fully deuterated solvent. Since CD_3COOD/CD_3OD (1.6 M) was used in their thiol–drug reaction, it seems likely that it was the carbon-bound hydrogen rather than the sulfur-bound hydrogen that had been incorporated into the drug.

Earlier it was observed that 35% deuterium was incorporated into the C-2 position but none into C-6 of the drug, when DNA was treated with the drug in a fully deuterated medium (10% CD_3OD in D_2O, thiol to drug ratio of 20) (Meschwitz et al., 1992). In Table III, our data show that, when the thiol–drug reaction was performed in 10% CD_3OH in H_2O under similar conditions but without DNA, about 30–40% of deuterium was incorporated into both the C-2 and C-6 positions. These results are consistent with each other, and they indicate that the incorporated deuterium from the drug–DNA reaction was from a carbon-bound source in the solvent, rather than from the sulfur-bound position of the thiol, and confirmed that the C-6 position is protected by DNA from deuterium incorporated from the solvent (Meschwitz et al., 1992).

Although the extent of hydrogen transfer depends on the exact drug reaction conditions, it is still possible to determine the amount of hydrogen transferred from each of the possible sources. For instance, when DNA was present in a 10% methanolic aqueous solution, about 22% deuterium was incorporated from C-1′ of DNA deoxycytidylate into C-2 (Meschwitz et al., 1992). Under similar conditions, internal transfer from both the α- and β-positions of the adducted thiol occurred to the extent of about 20%, and the carbon-bound deuterium of solvent methanol accounted for an additional 30% incorporation into C-2. These sources can, therefore, account for a total of at least 70–80% of the incorporation at C-2. These values could be even higher if not for the isotope effect.

CONCLUDING REMARKS

Detailed analysis of the sources of hydrogen abstraction by the radical centers of the activated species of NSC-Chrom reveals that, under conditions resembling those involved in DNA damage, the radical center at C-2, in particular, is susceptible to both internal and external quenching by hydrogen from the adducted thiol and from the CH of the methanol in the solvent, respectively. The ready quenching of the C-2 radical may be responsible for the finding that single-stranded DNA lesions exceed bistranded ones (Goldberg, 1991). Since single-stranded breaks involve mainly abstraction of the hydrogen at C-5′ of deoxyribose by the radical at C-6 of the chromophore (Meschwitz & Goldberg, 1991), whereas bistranded lesions in addition involve hydrogen abstraction from C-1′ (Meschwitz et al., 1992; Kappen et al.,

1990) or C-4′ (Frank et al., 1991; Kappen et al., 1991) of the deoxyribose on the complementary strand by the radical at C-2, selective quenching of the latter would result in the conversion of a bifunctional into a monofunctional DNA-damaging species.

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