COVALENT SEQUESTRATION OF THE NITROGEN MUSTARD MECHLORETHAMINE BY METALLOTHIONEIN

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ABSTRACT:
The research reported here demonstrates covalent binding to the metal-binding protein metallothionein (MT) by the therapeutic nitrogen mustard mechlorethamine. The most surprising aspect of this interaction is the selectivity of the alkylating agent for specific residues of MT. A combination of MS and proteolytic and enzymatic methods was used to deduce specific locations of mechlorethamine alkylation. These experiments indicated that alkylation occurs predominantly in the carboxyl domain of MT, with one molecule of mechlorethamine covalently cross-linking two cysteine residues. Electrospray MS revealed the retention of all seven metal ions in the cross-linked MT/mechlorethamine adducts, highlighting the uniqueness of this protein. Computerized docking experiments supported the hypothesis that selective binding precedes selective alkylation, and the structure of the drug indicates the minimal structural requirements for this binding. These results support the idea that MT overexpressed in tumor cells contributes to the inactivation of anticancer drugs.

MTs\(^1\) constitute a family of sulfhydryl-rich, metal-binding proteins that were first discovered and isolated in 1957 (Margoshes and Vallee, 1957). MT does not contain any aromatic residues, and 20 of its 61 or 62 amino acid residues are cysteines. Mammalian MTs bind up to seven bivalent cadmium and zinc ions, which are chelated through thiolate bonds to all 20 cysteine residues. These proteins can be isolated from different species and tissues in concentrations that depend on exposure to various types of dietary and physiological factors, including stress, age, and stage of development (Hamer, 1986).

In its native state, MT is folded into two globular domains. The α-domain, which includes the carboxyl terminus, contains 11 cysteines. Five cysteines act as thiolate bridges connecting two adjacent metal ions, and the remaining six are terminal ligands coordinating one metal ion each (Kagi, 1991). The β-domain, which includes the amino terminus, possesses nine cysteines; three form thiolate bridges and six serve as terminal ligands (Kagi, 1991). The polypeptide chains of each domain are tightly folded around the metal clusters, allowing each metal ion to be coordinated to four cysteine residues in a tetrahedral configuration, stabilizing the protein conformation (Otvo and Armitage, 1980).

The induction of MT synthesis occurs at the level of transcription initiation. Previous studies demonstrated that a multitude of environmental factors, including metal ions (Durham and Palmiter, 1981; Hamer, 1986), hormones (Karim and Herschman, 1979), alkylating agents (Kotsonis and Klaassen, 1979), and exposure to various types of physical and chemical stresses (Oh et al., 1978), can stimulate the biosynthesis of MT in vivo or in cultured cells.

The majority of research investigating MT has focused on its physiological functions, which after 40 years remain uncertain. The differential selectivities for metals displayed by the two clusters of MT support the view that each cluster has an independent physiological function. It has been suggested that the more thermodynamically stable α-domain sequesters xenobiotics, leaving the β-domain available for the regulation of essential metal ions, such as \(\text{Zn}^{2+}\) and \(\text{Cu}^{2+}\) (Brouwer, 1996). It has been proposed that MT can serve as a cellular pool for \(\text{Zn}^{2+}\) and donate ions to \(\text{Zn}^{2+}\)-depleted systems (Richards and Cousins, 1976; Li et al., 1980; Vallee, 1995). In addition, apo-MT can act as a scavenger and bind free \(\text{Zn}^{2+}\) ions, thus providing a feedback mechanism for its own biosynthesis (Vallee, 1995; Kelly et al., 1996).

MT has been shown to be involved in cellular defense mechanisms associated with oxidation and stress. Overexpression of MT in cells exposed to electrophilic compounds has generated interest regarding its role in the resistance of tumor cells to chemotherapeutic compounds. Increased levels of MT in the livers of rats were attributed to treatment with alkylating agents (Kotsonis and Klaassen, 1979). This initiated speculation that the sulfhydryl groups of MT could act as binding sites for electrophilic compounds and thus modulate their cytotoxicity (Cagen and Klaassen, 1980). Cells incubated with \(^{14}\text{C}\)chlorambucil contained elevated levels of MT, and 20–40% of the radiolabeled drug coeluted with MT (Endrezen et al., 1983).

Cellular resistance to antineoplastic drugs has been a concern of researchers since the initial use of these drugs in cancer chemotherapy. There is growing evidence for a role for MT in the detoxification of or resistance to electrophilic compounds, such as alkylating agents used in chemotherapy. Overexpression of MT resulting from chronic exposure to heavy metals conferred cross-resistance to cis-dichlorodiammineplatinum and the alkylating mustards melphalan, chlorambucil, 4-hydroperoxycyclophosphoramide, and mechlorethamine (Basu and Lazo, 1990). Mouse cells transfected with a bovine papil-
loma virus containing a human MT-2a gene exhibited a 10-fold increase in MT levels, compared with cells transfected with the virus alone, and a 4-fold increase in resistance to melphalan and chlorambucil (Kelley et al., 1988). Conjugation of MT with the antineoplastic agent is proposed to reduce the amount of drug available for interaction with the target DNA (Doz et al., 1993), and several researchers have demonstrated the covalent alkylation of MT by antineoplastic mustards (Yu et al., 1995; Zaia et al., 1996).

The recent work of Yu and Zaia and co-workers (Yu et al., 1995; Zaia et al., 1996) demonstrated that MT covalently and selectively sequesters the nitrogen mustards melphalan and chlorambucil. Their results indicated that selective binding precedes alkylation, because 90% of melphalan and 80% of chlorambucil react initially with the MT structure for the family of therapeutic nitrogen mustards and has the potential to test the minimal structural requirements for selective binding.

Materials and Methods

Chemicals. Rabbit liver MT-2a (lot 34H95161) and mechlorethamine were purchased from Sigma Chemical Co. (St. Louis, MO). Also purchased from Sigma were TFA, methyl-4-nitrobenzenesulfonate, bovine trypsin (L-1-tryosyl-2-phenylethylchloromethyl ketone-treated), Tris, and guanidine hydrochloride. HPLC-grade ACN was obtained from J. T. Baker Chemical Co. (Phillipsburg, NJ). Carboxypeptidase Y was obtained from Boehringer Mannheim (Indianapolis, IN).

Isolation of Mechlorethamine/MT Adducts. Rabbit liver MT-2a (5 mg) was dissolved in 0.1 M potassium phosphate buffer, pH 7.4, and then mechlorethamine (mechlorethamine/MT molar ratio, 1:1), dissolved in 0.1 M phosphate buffer, was added. The MT concentration was 140 μM, and the reaction mixture was stirred for 30 min at 37°C. MT was denatured by acidification to pH 2 using 20% (v/v) TFA in H2O, and the MT/mechlorethamine reaction products were isolated by gel filtration using a Sephadex G-25M PD-10 desalting column that had been equilibrated with 0.1% TFA in H2O. The products were isolated by gel filtration (using a Sephadex G-25M PD-10 desalting column that had been equilibrated with 0.1% TFA in H2O), freeze-dried, and lyophilized to dryness. Data were acquired in linear and reflection modes, and the matrix used was 3,5-dimethoxy-4-hydroxyphenylacetic acid (50 mM in 70:30, v/v, ACN/0.1% TFA in H2O). Samples were analyzed by placing 0.5 μl of protein (10–25 μg in 0.1% TFA in H2O) on a MALDI slide, followed by 0.5 μl of matrix. The spot was allowed to dry in ambient air and inserted into the mass spectrometer for analysis. External calibration, with a mass accuracy of 0.1% or better, was used.

ES MS data were acquired using the first mass spectrometer of a JEOL HX110/HX110 four-sector tandem instrument (JEOL, Tokyo, Japan). Data were acquired with a thermally assisted ES ion source (Analytica of Branford, Branford, CT). Dried samples were dissolved in H2O/methanol/acetic acid (49:49:2) and pumped into the source at 1 μl/min, using a syringe pump. The temperature of the interfacing capillary was heated to 120°C, and resolution was set at 500. External calibration, with a mass accuracy of 0.03%, was used. In some cases, reactions were allowed to occur in a solution that was continuously infused into the mass spectrometer and bombarded with xenon. The FAB gun was operated at 6 kW, and the accelerating voltage was 10 kV. FAB MS experiments were conducted on the first analyzer of a JEOL HX110/HX110 tandem mass spectrometer, and resolution was set at 1000. For CID in MS/MS experiments, the precursor ions were selected in the first analyzer of the JEOL instrument and collided with xenon in the collision cell (floated at 4 kV) to promote their fragmentation. The second stage of the mass spectrometer was used to detect the fragment ions generated.

Carboxypeptidase Digestion. In other experiments, cysteine-methylated MT and cysteine-methylated MT/mechlorethamine proteins were incubated with carboxypeptidase Y in 25 mM sodium citrate buffer, pH 6, for times ranging from 2 to 60 min (enzyme/protein ratio, 1:200, by weight), after which aliquots were removed, quenched with 0.1% TFA, and analyzed (Cotter, 1994). For MALDI MS analysis of carboxypeptidase products, aliquots of the reaction were deposited in wells on the MALDI stainless steel slide, and α-cyano-4-hydroxyphenylacetic acid (50 mM in 70:30, v/v, ACN/0.1% TFA in H2O) was added as the MALDI matrix. Before crystallization of the protein/matrix mixture, ammonium citrate was added, followed by several washings of the sample spots with H2O or 0.1% TFA in H2O.

Solid-Docking Simulations. Solid-docking simulations were conducted using the QUANTA4.0/CHARMM22 program, supplied by Molecular Simulations (Waltham, MA). The X-ray crystal structure of rat MT-2 (Robbins et al., 1991) provided by the Brookhaven Protein Databank was used. This rat MT isoform is considered to be isostructural with rabbit MT-2a, used in the present experiments (Vasak et al., 1987). Mechlorethamine was considered in its aziridinium form. The drug was solid-docked with MT by placing methyl-ene groups in the aziridinium ring as close as possible to each of the eight solvent-accessible sulfur atoms (Cys-6, -8, -14, -34, -38, -42, -49, and -58) (Robbins et al., 1991) of cysteine residues. Dynamic simulations were conducted (heating, 500°K for 1 psec; equilibration, 500°K for 1 psec; simulation, 500°K for 10 psec), and distance constraints were set at 3.0 Å between the closest aziridinium carbon and the cysteine sulfur. The lowest-energy conformation from the dynamic simulation was then minimized (steepest descents, 5000 steps). After dynamic simulation and minimization, the CHARMM

\(^2\) The numbering system used here for rabbit liver MT isoform 2a (the 62-amino acid protein) is that used by Fowle and Stillman (1997) and differs from that used in earlier reports from this laboratory, which followed the numbering system of Kagi and Kojima (1987).
After a 30-min reaction between equimolar amounts of MT and mechlorethamine, protein products were denatured and desalted as described in Materials and Methods and were characterized by MALDI MS (fig. 1). The most prominent peak, at 6126 Da, corresponds to the [M + H]⁺ molecular ion for unreacted apo-MT; however, drug-modified MT molecular ions were identified at 6209 and 6248 Da. The peak at 6209 Da represents the [M + H]⁺ molecular ion for apo-MT with one molecule of mechlorethamine covalently attached to two nucleophilic residues of MT. More specifically, one molecule of mechlorethamine lost both chlorine atoms and alkylated at one electrophilic site. The peak at 6248 Da was found to correspond to protonated apo-MT with one molecule of mechlorethamine covalently attached to one residue of MT. The [M + H]⁺ values obtained experimentally are in good agreement with the calculated protonated molecular masses of 6209 Da for apo-MT with mechlorethamine cross-linked to two positions and 6246 Da for apo-MT with mechlorethamine at one electrophilic site.

The covalent alkylation of MT by mechlorethamine was subsequently monitored by ES MS. The ES mass spectrum of native Cd⁴, Zn³-MT is shown in fig. 2 (top). Ions observed represent the [M+4H]⁴⁺ molecular ions of the different metal ion combinations present in the sample. Metal ion combinations observed include Cd⁶, Zn²-MT (m/z 1691), Cd⁴, Zn³-MT (m/z 1703), Cd⁴, Zn⁴-MT (m/z 1714), and Cd⁷-MT (m/z 1726). A small amount of Cd⁴, Zn⁴-MT is seen at m/z 1680. The shoulders visible on the more intense peaks are the result of the MT isoform 2a, which has a threonine substituted for alanine at position 8, resulting in a mass increase of 30 Da (for the 4+ charge state, the difference in the observed mass value is 7.5 Da). The observed masses were within 0.1% of the calculated values. Calculated and observed values for the different metallated proteins are given in table 1.

An ES mass spectrum obtained directly from the reaction of equimolar amounts of MT and mechlorethamine, at 30 min, is shown in fig. 2 (bottom). Ions corresponding to unreacted MT, i.e. Cd⁴, Zn³-MT, Cd⁴, Zn²-MT, Cd⁴, Zn⁴-MT, and Cd⁷-MT, are observed, as well as ions corresponding to MT with one molecule of mechlorethamine attached. Molecular ions appearing at m/z 1733, 1745, and 1756 are characteristic of MT with one molecule of mechlorethamine attached to one residue (table 1). All metal ions are still complexed. At longer reaction times, fully metallated cross-linked MT/mechlorethamine adducts become the most prominent species (data not shown).

The MT/mechlorethamine protein adducts were denatured, methylated at uncomplexed cysteine residues (to prevent disulfide bond formation and other chemical reactions in the apoprotein), and mapped by tryptic digestion with trypsin. The HPLC chromatograms of trypsin-digested methylated MT and methylated MT/mechlorethamine are displayed in fig. 3. Peptides from the control and alkylated proteins appeared very similar; however, close examination revealed an additional peak (fig. 3, asterisk) in the MT/mechlorethamine chromatogram, eluting at 17 min. Table 2 summarizes the mass map of the tryptic peptides from methylated MT/mechlorethamine, as obtained by FAB MS. Peptide fragments from methylated MT could be identified to account for its entire sequence. The new peak observed in the chromatogram of the product mixture was found to contain two peptide fragments, with protonated molecular masses of 1450.0 and 1909.1 Da. The protonated molecular mass of peptide 1 (1450.0 Da) was assigned to methylated MT peptides CAQGCICK (residues 45–52) and CSCCA (residues 58–62) cross-linked by one molecule of mechlorethamine. Peptide fragment 2 (1909.1 Da) was assigned as the cysteine-methylated peptides CAQGCICK (residues 45–52) and GASDKCSCCA (residues 53–62) cross-linked by one molecule of mechlorethamine. The [M+H]⁺ values obtained experimentally are in good agreement with the calculated values of 1449.7 and 1908.4 Da, respectively.

Results from the mapping study localized mechlorethamine alkylation to two peptide fragments in the α-domain of MT. Furthermore, because all lysine residues could be cleaved by trypsin, as evidenced by the FAB MS molecular mass measurements, lysines could be excluded as the major targets of mechlorethamine alkylation. Cysteines are the most probable alkylation sites; however, each of these peptides contains multiple cysteine residues. Therefore, more direct analysis of these fragments was undertaken, using CID in a MS/MS experiment. The CID mass spectrum of the mechlorethamine-cross-linked peptide product 1 is shown in fig. 4. As reported by others (Yu et al., 1995; Zaia et al., 1996), the most abundant ions in the mass
spectrum arise from fragmentation within the drug moiety. The base peak in the spectrum (m/z 804.9) results from fragmentation in the side chain of the alkylated cysteine in peptide 45–52. Other ions produced from fragmentation in the drug moiety are observed, with masses of 790.8, 819.1, 852.0, 879.0, 922.0, and 935.0 Da; they represent protonated peptides containing the CAQGCICK peptide fragment. The one exception is the fragment at m/z 582.9, in which the charge is retained on the tertiary nitrogen of mechlorethamine; it contains the peptide fragment CSCCA. These fragment ions confirm the presence of mechlorethamine and both peptides.

The extensive series of fragment ions labeled as the \( w_x \) series in fig. 4 results from cleavage between an amide nitrogen and \( \alpha \)-carbon, in conjunction with loss of the side chain group from the cleaved \( \alpha \)-carbon (Biemann, 1988). The masses of the \( w_2 \) and \( w_3 \) ions confirm methylation at Cys-51. (The \( w_2 \) ion results from cleavage of the ethyl group of the isoleucine side chain.) The \( a_1 \), \( a_2 \), and \( b_1 \) ions identify methylation on Cys-45. Mechlorethamine alkylation at Cys-49 can be

**TABLE 1**

<table>
<thead>
<tr>
<th>Protein Species</th>
<th>Native MT</th>
<th>MT Monoalkylated by Mechlorethamine</th>
</tr>
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<tr>
<td></td>
<td>Observed</td>
<td>Calculated</td>
</tr>
<tr>
<td>Cd-MT</td>
<td>1726</td>
<td>1726</td>
</tr>
<tr>
<td>Cd,Zn-MT</td>
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<td>1679</td>
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</tr>
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<td>Zn-MT</td>
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**TABLE 2**

<table>
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<tr>
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<td>2383.1</td>
<td>2382.6</td>
</tr>
<tr>
<td>24–26</td>
<td>335.7</td>
<td>334.4</td>
</tr>
<tr>
<td>27–32</td>
<td>697.9</td>
<td>697.3</td>
</tr>
<tr>
<td>33–44</td>
<td>1229.8</td>
<td>1228.4</td>
</tr>
<tr>
<td>45–52</td>
<td>867.9</td>
<td>867.3</td>
</tr>
<tr>
<td>[45–52] + [58–62] + drug</td>
<td>1450.0</td>
<td>1449.7</td>
</tr>
<tr>
<td>[45–52] + [53–62] + drug</td>
<td>1909.1</td>
<td>1908.4</td>
</tr>
<tr>
<td>53–57</td>
<td>477.7</td>
<td>477.2</td>
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<td>58–62</td>
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<td>528.4</td>
</tr>
<tr>
<td>53–62</td>
<td>987.5</td>
<td>986.1</td>
</tr>
</tbody>
</table>

* The sequence of rabbit MT-2a is MDPNCSCAAACDSCTCASC20CKACKCTSC20 KKSCSCCPP20GCACAQGCI25CGASDKCSC25CA (Kagi, 1993).

* Monoisotopic [M+H]+ (Yergey et al., 1983).

FIG. 2. ES mass spectra of native MT (top) and the products of the \( MT/\text{mechlorethamine} \) reaction, measured on-line at 30 min (bottom). See table 1 for assignments.

FIG. 3. HPLC chromatograms of trypsin-digested MT (top) and trypsin-digested \( MT/\text{mechlorethamine} \) reaction products (bottom). Asterisk, peak occurs only in the bottom map.
identified from the masses of $x_4$, $x_5$, $w_6$, and $x_6$ ions, which also reflect the presence of the second peptide (residues 58–62). Further indication that Cys-49 is alkylated can be found in the CID mass spectrum of peptide fragment 2 ($m/z$ 1908.2) (data not shown).

The second site of alkylation, in peptide CSCCA, could not be determined by MS/MS techniques. Therefore, cysteine-methylated MT and cysteine-methylated drug-modified protein were digested with carboxypeptidase Y to cleave carboxyl-terminal residues from the protein. Table 3 summarizes the molecular weights of ions observed in a series of reactions with carboxypeptidase Y that were quenched at different times; the data identify Cys-58 as the major site of cross-linking in peptide 58–62. Among the 20 cysteine residues in MT, covalent sequestration of one molecule of mechlorethamine takes place with high selectivity at Cys-49 and Cys-58.

**Discussion**

The results of this study provide additional support for the hypothesis that covalent sequestration of therapeutic agents by induced MT contributes to acquired resistance to these drugs. It is suggested that alkylation occurs initially at Cys-49, as the result of selective binding near that residue. Selective binding near Cys-49 was previously proposed for the analogous nitrogen mustards melphalan and chlorambucil, on the basis of their selective affinity labeling patterns, solid-docking simulations, and apparent first-order kinetics for the alkylation reaction (Yu et al., 1995; Zaia et al., 1996). Similar solid-docking simulations were performed around Cys-6, -8, -14, -34, -38, -42, -49, and -58 (Robbins et al., 1991). The solvent-accessible pocket around Cys-49, which is characterized by an electron-rich region, is most favorable for electrophilic interactions with the aziridine ion of mechlorethamine.

The biological activity of nitrogen mustards is based on the reactivity of the two chloroethyl side chains attached to the nitrogen atom with nucleophilic compounds. The cytotoxic effects of these drugs are believed to result from the covalent alkylation of DNA, resulting in the formation of intra- and interstrand DNA-cross-linked compounds that inhibit cell proliferation (Colvin and Chabner, 1990; Hopkins et al., 1991). Other intracellular compounds containing nucleophilic sites, such as RNA, glutathione, and proteins, may also be alkylated (Hartley et al., 1992).

![Fig. 4. Tandem mass spectrum of the 1449.4-Da tryptic peptide from permethylated, drug-alkylated MT and the interpretation of fragment ions according to the method of Biemann (1988).](imageURL)

![Fig. 5. ES mass spectra of denatured MT (0 min) and the denatured products of the MT/mechlorethamine reaction after 1, 2, 3, and 4 min.](imageURL)

See sequence in table 2. Peak A, rabbit liver MT-2a; peak A’, rabbit liver MT-2a’; peak B, monoalkylated MT-2a; peak B’, monoalkylated MT-2a’; peak C, cross-linked MT-2a.

**Table 3**

<table>
<thead>
<tr>
<th>Cleavage Product</th>
<th>Calculated For Cys-61</th>
<th>Calculated For Cys-60</th>
<th>Calculated For Cys-58</th>
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<tr>
<td></td>
<td>$m/z$</td>
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<td>$m/z$</td>
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</tr>
<tr>
<td>1-62</td>
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<td>6461.4</td>
<td></td>
</tr>
<tr>
<td>1-61</td>
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<td>6390.3</td>
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</tr>
<tr>
<td>1-60</td>
<td>6408.3$^b$</td>
<td>6273.2</td>
<td>6273.2</td>
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</tr>
<tr>
<td>1-59</td>
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<td>6291.2$^b$</td>
<td>6156.1$^b$</td>
<td>6156</td>
</tr>
<tr>
<td>1-58</td>
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<td>6204.1$^b$</td>
<td>6069.1$^b$</td>
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</tr>
<tr>
<td>1-57</td>
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<tr>
<td>1-56</td>
<td>5958.8</td>
<td>5958.8</td>
<td>5958.8</td>
<td>5960</td>
</tr>
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</table>

The sequence was as in table 2. All free sulfhydryl groups were methylated.

$^a$Average MH$^+$ values.

$^b$Possible sites of alkylation.

$^c$Values that distinguish the three possible alkylation sites.
In the reaction of methylethamine with MT, cross-linking by the drug occurs almost as fast as monoaadduct formation. Time course experiments in which the reaction was quenched and the protein mixture was denatured, desalted, and analyzed by ES MS demonstrated alkylation of MT within the first 1 min of reaction (fig. 5). A cross-linked MT/methylethamine product could be observed after only 3 min of reaction time (fig. 5, peak C). Alkylation studies with diethyldithiocarbamic acid and nitrogen mustards have shown that the disubstituted adduct of diethyldithiocarbamic acid with methylethamine amine is also formed within minutes (Cummins et al., 1991). Studies examining the reactions of nitrogen mustards with nucleic acids have demonstrated that methylethamine react faster than its aromatic analogues and forms greater amounts of cross-linked adducts (Osborne et al., 1995). These differences are paralleled in the reactivities of MT with methylethamine and its aromatic analogues.

The molecular reach of the two alkylating arms of methylethamine (fully extended) has been estimated to be between 7.5 and 8.5 Å (Rink and Hopkins, 1995; Hathout et al., 1996). This is not quite long enough to cross-link Cys-49 and Cys-58, which are separated by nearly 8.6 Å in the crystal structure of rat MT-2 used for docking studies. The discrepancy might be explained by small differences between rat and rabbit MT-2, or between solution and crystal states. It has been postulated that cross-linking of DNA by methylethamine results in bent or curved DNA, because the diethylenetetramine tether of methylethamine is smaller than the N7-N7 distance in B-DNA (Rink and Hopkins, 1995). Another speculation is that some combination of propeller twisting and kinking of DNA may occur to allow cross-linking of methylethamine (Hopkins et al., 1991).

Because both monoaalkylation and cross-linking of MT by methylethamine occur with retention of metal ions, large-scale distortion of the protein conformation is unlikely. However, the carboxyl terminus has been observed to be flexible in solution (Arseniev et al., 1988; Messerle et al., 1990). Cys-58 has also been shown to be one of the most solvent-accessible cysteines in the carboxyl-terminal domain (Robbins et al., 1991).

The implications of selective binding, in the carboxyl-terminal domain, by the family of therapeutic mustards are intriguing. The minimal structural requirement revealed by methylethamine (i.e., a quaternary ammonium/iminium center) could be characteristic of endogenous substrates, such as arginine side chains in other proteins or guanines in polynucleotides. Many drugs exhibit selective reactions, presumably preceded by selective binding to proteins (Fenselau, 1997; Skipper, 1996). In many cases, drugs resemble functional dionium/ammonium center) could be characteristic of endogenous substrates, such as arginine side chains in other proteins or guanines in polynucleotides. Many drugs exhibit selective reactions, presumably preceded by selective binding to proteins (Fenselau, 1997; Skipper, 1996). In many cases, drugs resemble functional

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