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 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 cysteine. 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 cysteines in a tetrahedral configuration, stabilizing the protein conformation (Otros 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 (Durnam 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 Zn²⁺ and Cu²⁺ (Brouwer, 1996). It has been proposed that MT can serve as a cellular pool for Zn²⁺ and donate ions to Zn²⁺-depleted systems (Richards and Cousins, 1976; Li et al., 1980; Vallee, 1995). In addition, apo-MT can act as a scavenger and bind free Zn²⁺ 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 [¹⁴C]chlorambucil contained elevated levels of MT, and 20–40% of the radiolabeled drug coeluted with MT (Endresen 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-hydroperoxycyclophosphamide, 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 MT. Mechanolamine comprises the minimal structure for the family of therapeutic nitrogen mustards and has the potential to test the minimal structural requirements for selective binding with cysteine residues in MT. Mechloroethamine comprises the minimal 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 mechloroethamine were purchased from Sigma Chemical Co. (St. Louis, MO). Also purchased from Sigma were TFA, methyl-4-nitrobenzenesulfonate, bovine trypsin (L-1-tosylamide-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 Mechloroethamine/MT Adducts. Rabbit liver MT-2a (5 mg) was dissolved in 0.1 M potassium phosphate buffer, pH 7.4, and then mechloroethamine (mechloroethamine/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/mechloroethamine reaction products were isolated by gel filtration using a Sephadex G-25M PD-10 desalting column (Pharmacia LKB Biotechnology, Piscataway, NJ) that had been equilibrated with 0.1% TFA in H₂O. Approximately 90% of the protein was recovered, freeze-dried, and lyophilized to dryness. Analysis of the reaction products was conducted using reverse-phase HPLC, MALDI MS, and ES MS.

HPLC was performed using an analytical, reverse-phase, C₁₈ HPLC column (Aquapore OD 300, 250 × 4.6 mm; Applied Biosystems, San Jose, CA), with two Shimadzu (Columbia, MD) LC-6A pumps. Absorbance was monitored at 215 nm using a Shimadzu SPD-6A UV detector. The mobile phases for all separations were as follows: solvent A, 0.1% TFA in H₂O; solvent B, 0.08% TFA in ACN. A flow rate of 1 ml/min was used. For the analysis of MT and MT/mechloroethamine reaction products, a shallow linear gradient program was used to separate the isoforms of MT. Typically, solvent B was increased from 10 to 20% during the first 10 min of the run and then from 20 to 27% in 24.6 min. Solvent B was increased to 80% of the total eluent after 3 min, to remove any late-eluting impurities, and was decreased to 10% at 40 min.

For some analyses, the protein was methylated at all free cysteines by following a published procedure (Hunziker, 1991), with some modifications incorporated by Yu et al. (1995). Dried apo-MT and apo-MT/mechloroethamine (4 mg) were dissolved in 5 ml of an argon-purged buffer solution containing 6 M guanidine hydrochloride, 0.5 M Tris-Cl, and 5 mM EDTA, pH 8.6, plus 10 mM dithiothreitol. The reaction mixtures were stirred for 60 min, under argon, at 37°C. Methyl-4-nitrobenzenesulfonate (2.5 ml, 272 mM in ACN) was added to the reaction vials, and the mixtures were incubated under argon for an additional 2 hr. The reaction was stopped by acidification to pH 2 using 20% (v/v) TFA in H₂O, and 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.

Trypsin Digestion. The cysteine-methylated MT and MT/mechloroethamine products were dissolved in 0.1 M ammonium bicarbonate, pH 8.0, at a concentration of 1 mg/ml. Trypsin (L-1-tosylamide-2-phenylethylchloromethyl ketone-treated, 1 mg/ml) in 0.1 mM CaCl₂ was added to the methylated MT samples at a 1:200 (w/v) trypsin/cysteine-methylated MT ratio. The reaction mixtures were stirred at 37°C for 60 min. The digest mixtures were analyzed on a reverse-phase C₁₈ HPLC column using the following gradient: 2 min, 10% solvent B; 20 min, 20% solvent B; 30 min, 35% solvent B; 31 min, 80% solvent B. The mobile phases used were the same as described above, and the flow rate was 1 ml/min. The tryptic peptide fragments were collected and analyzed by MS.

For FAB MS analysis, dried peptide mixtures were dissolved in 0.1% TFA in H₂O, and 1 μl was applied to the metal probe tip, followed by 1 μl of matrix solution (50:50, v/v, glycerol/thioglycerol). The sample probe was inserted into the mass spectrometer and bombarded with xenon. The FAB gun was operated at 6 kV, 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 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 continually infused into the mass spectrometer (Zaia et al., 1996).

For the 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/mechloroethamine 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, carboxypeptidase products, aliquots of the reaction were deposited in wells on the MALDI stainless steel slide, and α-cyano-4-hydroxycinnamic 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). Mechloroethamine was considered in its aziridinium form. The drug was solid-docked with MT by placing methylene 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; equilibrium, 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).
energy was recalculated for the complex and the distance vs. energy data were compared, to determine favorable sites of mechlorethamine binding.

Results

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 MT at two different sites. The peak at 6248 Da was found to correspond 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 alkylated 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]+ molecular ions of the different metal ion combinations present in the sample. Metal ion combinations observed include Cd4,Zn3-MT (m/z 1691), Cd6,Zn1-MT (m/z 1703), Cd2,Zn2-MT (m/z 1714), and Cd7-MT (m/z 1726). A small amount of Cd5,Zn1-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, Cd6,Zn1-MT, Cd2,Zn2-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 proteolytic digestions with trypsin. The HPLC chromatograms of trypsin-digested methylated MT and methylated MT/meclorethamine 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 GASDKSCCA (residues 53–62) 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 GASDKSCCA (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 $w$ 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 $w_{3a}$ ions confirm methylation at Cys-51. (The $w_{3a}$ ion results from cleavage of the ethyl group of the isoleucine side chain.) The $a_1$, $a_2$, and $b_2$ ions identify methylation on Cys-45. Mechlorethamine alkylation at Cys-49 can be

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**TABLE 1**

$m/z$ Values for the [M+4H]$^+$ molecular ions of MT and alkylated MT, obtained by ES MS

<table>
<thead>
<tr>
<th>Protein Species</th>
<th>Native MT</th>
<th>MT Monoalkylated by Mechlorethamine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Observed</td>
<td>Calculated</td>
</tr>
<tr>
<td>Cd$_2$-MT</td>
<td>1726</td>
<td>1726</td>
</tr>
<tr>
<td>Cd$_6$,Zn$_1$-MT</td>
<td>1714</td>
<td>1714</td>
</tr>
<tr>
<td>Cd$_5$,Zn$_2$-MT</td>
<td>1703</td>
<td>1702</td>
</tr>
<tr>
<td>Cd$_4$,Zn$_3$-MT</td>
<td>1691</td>
<td>1690</td>
</tr>
<tr>
<td>Cd$_3$,Zn$_4$-MT</td>
<td>1680</td>
<td>1679</td>
</tr>
<tr>
<td>Cd$_2$,Zn$_5$-MT</td>
<td>1669</td>
<td>1667</td>
</tr>
<tr>
<td>Cd$_1$,Zn$_6$-MT</td>
<td>1655</td>
<td>1655</td>
</tr>
<tr>
<td>Zn$_7$-MT</td>
<td>1644</td>
<td>1643</td>
</tr>
</tbody>
</table>

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**TABLE 2**

Mass map of tryptic peptides from the MT/mechlorethamine reaction mixture

<table>
<thead>
<tr>
<th>Peptide Fragment$^b$</th>
<th>Observed</th>
<th>Calculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–23</td>
<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>
</tr>
<tr>
<td>58–62</td>
<td>528.7</td>
<td>528.4</td>
</tr>
<tr>
<td>53–62</td>
<td>987.5</td>
<td>986.1</td>
</tr>
</tbody>
</table>

$^a$ The sequence of rabbit MT-2a is MDPNCSCAAAGDSCTCANCSCICCKACKCTSCGGKSCSCCPP9GCAGCAIQGCICCKGASDKKSCS9CA (Kagi, 1993).

$^b$ Monoisotopic [M+H]$^+$ (Yergey et al., 1983).
identified from the masses of \( x_4, x_5, w_6, \) and \( x_7 \) 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 aziridinium 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).
In the reaction of mechloethamine with MT, cross-linking by the drug occurs almost as fast as monooadduct 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 min of reaction (fig. 5). A cross-linked MT/mechloethamine product could be observed after only 3 min of reaction time (fig. 5, peak C). Alkylation studies with diethylthiocarbamic acid and nitrogen mustards have shown that the disubstituted adduct of diethylthiocarbamic acid with mechloethamine is also formed within minutes (Cummings et al., 1991). Studies examining the reactions of nitrogen mustards with nucleic acids have demonstrated that mechloethamine reacts 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 mechloethamine and its aromatic analogues.

The molecular reach of the two alkylling arms of mechloethamine (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 mechloethamine results in bent or curved DNA, because the diethylenemine tether of mechloethamine 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 MECHLOTHIONEIN (Hopkins et al., 1991).

Because both monooalkylation and cross-linking of MT by mecl roethamine 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 mecl roethamine (i.e., a quaternary ammonium/minium center) could be characteristic of en-dogenous substances, such as arginine side chains in other proteins or guanosine in polynucleotides. Many drugs exhibit selective reactions, presumably preceded by selective binding to proteins (Fenselau, 1997; Skipper, 1996). In many cases, drugs resemble functional groups of natural substrates for enzymes and receptors. Other proteins, such as albumin, are general carriers for small molecules and contain acidic, basic, and lipophilic pockets. In view of the continuing uncertainty regarding the biological functions of the ubiquitous MT family, it is difficult to decide whether selective binding of therapeutic mustards reflects a functional affinity for an endogenous substrate with a similar key chemical group or a more general binding property of the carboxyl domain.

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References


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