Crystal and Molecular Structure of a DNA Duplex Containing the Carcinogenic Lesion O^6-Methylguanine†

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Received June 12, 1990; Revised Manuscript Received August 14, 1990

ABSTRACT: The crystal and molecular structure of the first DNA duplex containing the carcinogenic lesion O^6MeG has been determined to a resolution of 1.9 Å and refined to an *R* factor of 19%. {d[CGC-(O^6Me)GCG]}$_2$ crystallizes in the left-handed Z DNA form and has crystal parameters and conformational features similar to those of the parent sequence [d(CG)$_3$]$_2$. The methyl groups on O^6 of G4 and G10 have C^5–C^6–O^6–O^6Me torsion angles of 73° and 56°, respectively, and protrude onto the major groove surface. The base-pairing conformation for the methylated G·C base pairs is of the Watson–Crick type as opposed to a wobble-type conformation that had been proposed in a B DNA fragment. As in other Z DNA structures, a spine of hydration is seen in the minor groove.

The O^6-alkylguanine lesions produced in DNA by alkylating agents such as *N*-nitroso-*N*-methylurea (NMU) are thought to be fundamental to the carcinogenicity of such agents (Singer & Grunberger, 1983; Zarbl et al., 1985). For example, the specific formation of O^6MeG in lung Clara cells by exposure to low doses of the carcinogen 4-(*N*-methyl-*N*-nitrosoamino)-1-(3-pyridyl)-1-butanone (NNK), a major nitrosamine present in tobacco, has been correlated to the subsequent incidence of malignant lung tumors (Belinsky et al., 1987). In addition, the absence of the alkyltransferase required for repair of O^6MeG has been correlated both with an increased sensitivity to the lethal and mutagenic effects of *N*-methyl-*N'*-nitrosoguanidine (MNNG) and with the nitrosourea-induced leukemic transformation of rapidly proliferating bone marrow cells (Domoradzki et al., 1984; Gerson et al., 1987).

In solution studies on duplexes containing O^6MeG residues, the O^6Me group has been shown to destabilize the duplexes markedly, with the most stable of the possible O^6MeG·N pairs being the O^6MeG·C pair, regardless of the sequence (Kuzmich et al., 1983; Gaffney et al., 1984, 1989; Pauley et al., 1988). Moreover, the molecule d[CGCGAATTC(O^6Me)GCG], which contains two O^6MeG·C pairs, was shown by ^{1}H NMR to be a right-handed helix in which the bases of the O^6MeG·C pairs maintain anti conformations (Patel et al., 1986). The NOEs observed in this study indicated only small conformational differences, relative to the unmodified parent molecule, in the area of the O^6MeG. These small differences, together with the overall similarity to the unmodified parent molecule, suggested, but could not define unambiguously, the possibility of wobble-type O^6MeG·C pairing in this case. To date there have been no examples of X-ray crystallographic analyses of oligonucleotides containing any of the possible O^6MeG·N pairings. We report the first such X-ray crystallographic analysis (Ginell et al., 1990). The structure determination of d[CGC(O^6Me)GCG] is now fully described and was undertaken as part of a program to provide definitive structural data about the nature of the base pairs in which O^6MeG is involved and the effects of this lesion on the overall structure of the DNA.

Table I: Crystal Data

compd	{d[CGC(O^6Me)GCG]}$_2$
formula	$C_{53}H_{72}N_{24}O_{36}P_5$
mol wt	1836.23
radiation	Cu Kα (λ = 1.5418 Å)
space group	$P2_12_12_1$
cell dimensions at −90 °C	a = 17.85, b = 30.87, c = 43.98 Å; $\alpha = \beta = \gamma$ = 90°
unit cell vol (Å^3)	24 234
molecules/asymmetric unit	two strands of d[CGC(O^6Me)GCG]
Z	4
data collection method	area detector
intensity type	simply summed
merging R (unweighted absolute-value R factor) (%)	6.93
no. of independent data	1597
no. of obsd data	1246
no. of obsd data 1.9–8.0 Å	1217

Experimental Procedures

Lyophilized d[CGC(O^6Me)GCG] (Kuzmich et al., 1983) was dissolved in water. Drops containing 30 mM sodium cacodylate buffer, pH 7.0, 5 mM $MgCl_2$, 0.5 mM spermine tetrahydrochloride, 10–20% 2-methyl-2,4-pentanediol (MPD), and 2 mM DNA were set up in vapor diffusion dishes containing 30% MPD. After 8–12 months three crystals grew at room temperature. No crystals appeared at 4 °C.

Following the procedure described by Hope (1988) one crystal was placed in an oil drop (50% Paratone N and 50% mineral oil) to remove precipitate, satellites, and mother liquor from the surface. The crystal was then scooped from the oil drop on the end of a glass fiber and placed on a goniometer in the path of a −90 °C cold nitrogen stream that was momentarily blocked off.

d[CGC(O^6Me)GCG] crystallizes in the orthorhombic space group $P2_12_12_1$ with cell dimensions similar to other Z DNA hexamer structures (Table I). No density (ρ_{obs}) has been determined because of the scarcity of crystals. X-ray intensity data were collected by using a Rigaku RU-200 rotating anode generator equipped with Franks double-focusing mirrors, on a Supper three-circle gonionstat with a Nicolet multiwire area detector. Data were collected for six different settings of the

† This research was supported by NIH Grants GM21589 to H.M.B. and GM31483 to R.A.J. Additional support for R.A.J. was provided by American Cancer Society Grant CA248B, an American Cancer Society Faculty Research Award, and the Busch Memorial Fund. Support to H.M.B. for the earlier part of this work conducted at the Fox Chase Cancer Center was from NIH Grants CA06927 and RR05539.
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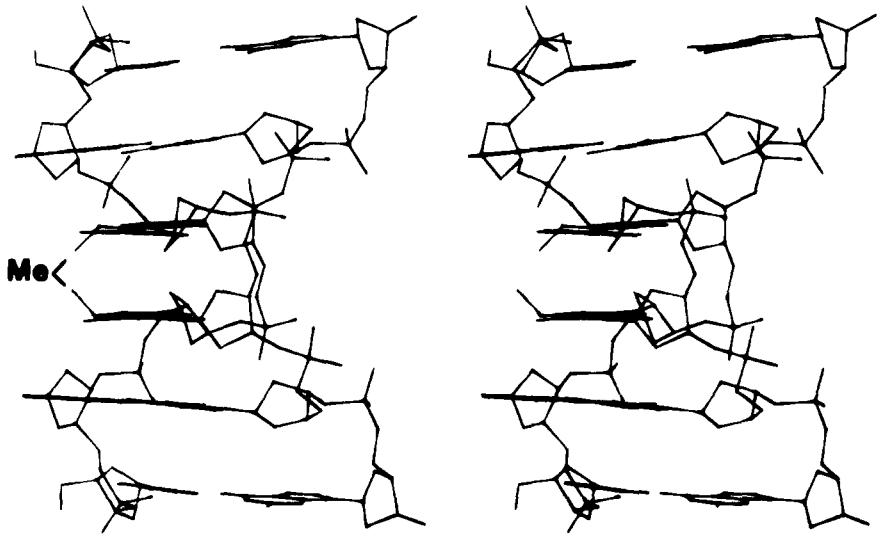

FIGURE 1: Stereoview of {d[CGC(O^{6}Me)GCG]}$_2$. Methyl groups are indicated.

detector or crystal orientations by the rotation–oscillation method using 0.5° ω oscillations per frame with a total scan angle of 100.0° ω per setting. Frame scanning, unit cell determination, data reduction, and scaling were performed by using the program package Xengen version 1.1 (Howard et al., 1987). The final merging R value is 6.9% for 5557 observations that result in 1597 independent reflections to a resolution of 1.9 Å. The number of observed reflections is 1246 as determined by consideration of the intensities of the systematically absent reflections. A total of 1217 reflections in the resolution range 1.9–8.0 Å were used for refinement.

Since the unit cell parameters for d[CGC(O^{6}Me)GCG] are nearly isomorphous with those of the Z DNA hexamers, an initial model was derived from those of d[(^{5}Me)CGTA(^{5}Me)CG] (Wang et al., 1984). Constrained–restrained least-squares refinement using the program CORELS (Sussman et al., 1977) was performed initially by using one overall group with data in the range 3–8 Å; additional cycles of refinement had the number of groups increased to 2 strands, then to 12 nucleotides, and to finally 6 base pairs and 12 phosphates–riboses. The final R factor with CORELS refinement was 0.32 with a correlation coefficient of 0.74.

Further refinement was performed by using the program NUCLSQ (Westhof et al., 1985), which is based on the Konnert-Hendrickson program PROLSQ (Hendrickson & Konnert, 1979). Initial cycles of refinement with a single overall temperature factor of 2.49 dropped the R factor to 0.29. The inclusion of individual isotropic B factors gave an R factor of 0.26. Between cycles of refinement the model was refit by using $2F_o - F_c$ Fourier maps displayed on an E&S PS390 using the program FRODO (Jones, 1978). In addition, each segment of the molecule was removed and refit by using omit $F_o - F_c$ and $2F_o - F_c$ Fourier maps. Particular attention was paid to the methylated guanines and the cytosines to which they are paired. The electron density for the O^{6}MeG·C base pairs shows unequivocally that the bases are paired in a conventional Watson–Crick arrangement (Figure 5). Difference Fourier maps in which the methyl groups were left out gave single sites for the two groups although the electron density for G4 O^{6}Me was less well defined than that for G10 O^{6}Me. As further verification that the methyl groups were still intact, one of the other crystals in the dish was shown, by reversed-phase HPLC, to be homogeneous and to comigrate with a sample of the original material (Kuzmich et al., 1983) but not with d(CGCGCG).

Sixty solvent molecules with distances 2.2–3.2 Å to the oligonucleotide or to one another were located by using $F_o - F_c$ maps. All solvent atoms were given an occupancy of 1.0 and no attempt was made to fit partially occupied solvent molecules. Sixteen solvent molecules are beyond the first hydration shell. It was not possible to model any of the solvent as Mg^{2+}, Na^+, or spermine. The addition of these solvent molecules and further refinement resulted in a final R value of 0.19 and correlation coefficient of 0.89 for the 1217 data between 1.9 and 8.0 Å. In the final stages of refinement, no restraints were placed on the sugar puckers, nor on the other torsion angles. Once the hydrogen-bonding geometry was confirmed, restraints were placed on the hydrogen bonds between base pairs. The root mean square bond and angle distances for the non-phosphate bonds are 0.014 and 0.027. The values for the PO_4 groups are 0.051 and 0.048. The average B values for the PO_4, ribose, bases, and solvent are 8.1, 7.3, 7.1, and 14.0, respectively. Coordinates and structure factors are deposited in the Protein Data Bank.

Results and Discussion

The overall conformation of the molecule (Figure 1) is similar to that of Z DNA (Wang et al., 1979; Gessner et al., 1989). As illustrated in Figure 2, when the phosphate groups are overlaid, the central base pairs overlap well but the end base pairs are somewhat displaced from one another. This may indicate that the methylation has a more long-range effect on the structure. Comparison of the conformational angles for each of the residues (Figure 3) shows remarkably good agreement with the other Z structures published to date. Only residue 2 shows any differences; in that case there is the anti correlation between the P–O$^{5'}$ and C$^{5'}$–C$^{4'}$ torsion angles. Analysis of the base morphologies shows some subtle differences among the structures (Figure 4, Table II). In particular, the base-pair parameters, buckle and propellor, for G4 deviate from the values seen in the other structures. This may be a consequence of the adjustments the bases must make in order to avoid unfavorable steric clashes between the methyl group and the bases.

The central base pairs that contain the methylated O^6 groups have normal Watson–Crick-type geometry (Figure 5); that is, the O^{6}G and N^{4}C, the N^{3}C and N^{1}G, and the O^{2}C and N^{2}G are all within hydrogen-bonding distances of one another. These results though surprising are in agreement with very recent pH-dependent UV melting studies of some other

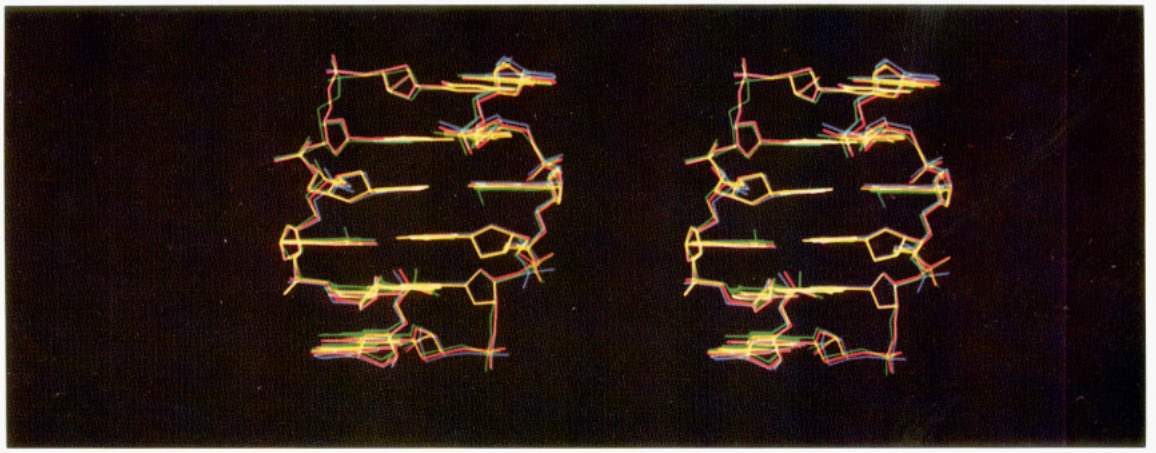

FIGURE 2: Stereoview showing a comparison of Z DNA complexed to magnesium (red bonds) and complexed to spermine (blue) and d[CGC(O⁶Me)GCG] (green bonds).

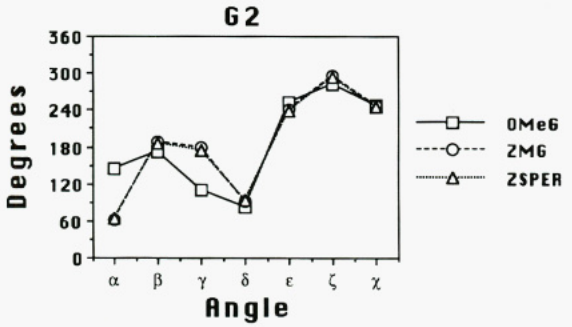

FIGURE 3: Conformation angles of residue G2 as compared with other Z DNA structures. The squares are the values in this structure. The circles are for the Z DNA complexed with magnesium and the triangles for the Z DNA complexed with spermine (Gessner et al., 1989).

Table II: Conformation Angles in {d[CGC(O⁶Me)GCG]}₂

residue	P–O$^{5'}$ α	O$^{5'}$–C$^{5'}$ β	C$^{5'}$–C$^{4'}$ γ	C$^{4'}$–C$^{3'}$ δ	C$^{3'}$–O$^{3'}$ ε	O$^{3'}$–P ζ	χ
strand 1							
C1			53	134	−70	62	26
G2	145	172	109	83	−108	−78	−111
C3	−131	−135	55	143	−95	78	26
G4	67	−179	171	98	−176	49	−116
C5	151	165	66	130	−87	58	30
G6	94	172	176	151			−99
strand 2							
C7			81	136	−103	82	25
G8	57	179	−178	110	−124	−38	−112
C9	−157	−162	59	142	−86	76	35
G10	78	169	165	109	−111	−77	−110
C11	−165	−133	79	126	−90	76	17
G12	64	178	−170	157			−100

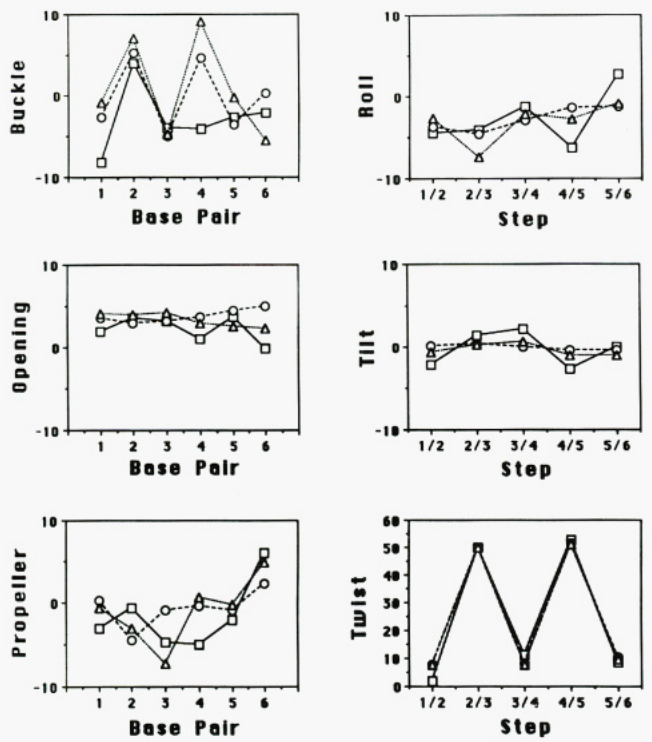

FIGURE 4: Base morphologies as compared with other Z DNA structures. The values were calculated by using a program developed by Marla Babcock (Babcock et al., 1990). The symbols in the graphs are the same as in Figure 3.

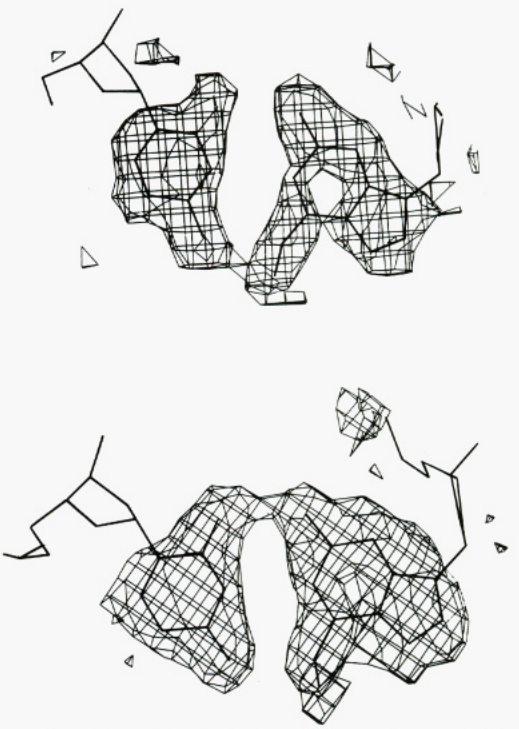

FIGURE 5: $F_o - F_c$ map in which the methylated base-paired residues have been omitted. The top is the G4·C9 base pair. The bottom is the G10·C3 base pair. In both cases, the base pairs are in a Watson–Crick orientation.

O⁶MeG sequences (T. Brown, personal communication). This implies that either the major tautomer of guanine is protonated at N^1, the guanine exists as a minor tautomer, the major tautomer of cytosine is protonated at N^3, or the cytosine exists

as a minor tautomer. It is also possible that the N^1 and N^3 are close to one another but not hydrogen bonded. Without knowing the hydrogen positions, it is not possible to determine the precise nature of these structures. However, it is clear that this structure does not contain the wobble hydrogen bonds proposed for the O^6Me guanine·cytosine base pair in B DNA. If this wobble pair were to exist in a Z-type structure, the O^6Me group would protrude even further into the major groove. Thus, unlike the bromouracil·guanine wobble pair in which the base stacking is enhanced in the Z DNA type structure, the bases in an O^6MeG·C wobble pair would be less stacked in the helix.

The conformation of the methyl group is slightly different for each chain. In the A chain the O^6Me–O^6–C^6–C^5 torsion angle has a value of 73° and in the B chain this value is 56°. These values for the conformation angles are within sterically acceptable limits. In both cases the methyl group protrudes on the major groove surface.

The hydration structure for this crystal is not as well-defined as for other Z DNA molecules. The minor groove contains a spine of water that hydrogen bonds to the O^2 of some but not all of the cytosine residues. There are also water chains that run along the phosphate backbone. The major groove heteroatoms are individually hydrated but do not participate in a water network. These results are similar to those described for the ^{5}BrC Z DNA (Chevrier et al., 1986).

Comparison of the X-ray structure of {d[CGC(O^6Me)GCG]}$_2$ with that of the parent molecule, [d(CG)$_3$]$_2$ (Wang et al., 1979; Gessner et al., 1989), indicates that the O^6MeG residues are accommodated with little distortion of the Z DNA structure. Thus, in principle, there should be no barrier to a B → Z transition in solution for this molecule. However, the CD spectra of d[CGC(O^6Me)GCG], under conditions where d(CG)$_3$ exhibits entirely Z DNA behavior, are consistent only with a partial B → Z transition (Kuzmich et al., 1983). Although the solution structure or structures of d[CGC(O^6Me)GCG] were not further characterized, the fact that the molecule now has been shown to be in a Z conformation in the crystal suggests that the CD was in fact monitoring a partial Z transition. Moreover, there is ^{1}H NMR evidence that the molecule d[CGCGAATTC(O^6Me)GCG] exists as a right-handed helix in which the bases of the O^6MeG·C pair maintain anti conformations (Patel et al., 1986). The NOEs observed in this study indicated only small conformational differences, relative to the unmodified parent molecule, in the area of the O^6MeG. These differences were interpreted in terms of a possible wobble-type O^6MeG·C pairing. Thus the O^6MeG·C pair appears to be accommodated relatively well in either a B or Z type of structure, and perhaps better in the Z form. Nevertheless, the effect of the O^6MeG on the putative B → Z transition observed with d[CGC(O^6Me)GCG] appears to be to shift the equilibrium in the direction of the B form. This is in contrast to the effect of 5-methylcytosine (^{5}MeC) residues on the B → Z transition, which markedly shift the equilibrium in the Z direction. Thus, it is clear that methylation of the bases has a profound effect on the solution behavior and that the sites of methylation confer markedly different properties to the molecules.

The explanations for many of the solution properties may lie in consideration of the complete three-dimensional structures of {d[C^5Me)CG(^{5}Me)CG(^{5}Me)CG]}$_2$ (Fuji et al., 1982), {d[CGC(O^6Me)GCG]}$_2$, and [d(CGCGCG)]$_2$ (Wang et al., 1979; Gessner et al., 1989). The ^{5}MeC Z DNA structure is thought to be stabilized by favorable hydrophobic interactions of the methyl groups and by ion binding to the exposed O^6 and N^7 groups of the guanine residues. Calculations of hydration energies have shown that the methylated sequence has only a slightly higher hydration energy than the unmethylated sequence (Ho et al., 1988). In contrast, the B form of the methylated sequence is not stabilized by ion binding and its hydration energy is significantly higher than that of the unmethylated form. It is also important to consider the solvent–solvent interactions. In the B form of both the methylated and unmethylated sequences the waters that are hydrogen bonded to the hetero base atoms would form a water network in the major groove (Berman et al., 1988); the positions of the water molecules in the methylated sequence would be more restricted. In the Z form, the water molecules in the convex major groove of the unmethylated sequence are less restricted than in the corresponding groove of B DNA; the water molecules of the C^5-methylated sequence are even more diffuse. Thus, the transition from the B to the Z forms would be accompanied by a complete release of that bound water and hence an increase in entropy; the net effect is greater in the ^{5}MeC sequence than in the unmethylated sequence. In contrast, methylation of the O^6 of the guanine in the Z form does not contribute stabilizing hydrophobic interactions and the number of ion binding sites is decreased. Furthermore, the water network in the B form would be expected to be disrupted by the presence of this methyl group. Thus, in this case, the B to Z transition would not be accompanied by a large net disruption of a water network and hence the increase in entropy due to desolvation would not be expected to be large. If the B to Z transition is entropy driven and is a function of desolvation as has been suggested by Behe and Felsenfeld (Behe et al., 1985), then the driving force is considerably diminished in the O^6-methylated compound.

This analysis shows that the methylation of the O^6 of guanine destabilizes the Watson–Crick base pairing by either decreasing the number of hydrogen bonds, forcing an unusual tautomer, or protonating the bases. In the Z form a Watson–Crick, as opposed to a wobble-type, orientation may be preferred because of the more favorable stacking, which can partially compensate for the poor hydrogen bonding. Thus, while a wobble-type orientation may be tolerated in a B DNA conformation, perhaps because the hydrogen bonding may be more favorable, such wobble pairs would inordinately destabilize the Z conformation due to the unfavorable stacking interactions. Hence the overall effect of this single alkylation is to destabilize the conformation of the DNA in any form. Moreover, in the Z conformation, the *O*^{6}-methyl group is projected into the major groove where it would interfere in Z DNA protein interactions. Such interference may play a role in the carcinogenicity of O^6-methylation.

Acknowledgments

We thank Carol Afshar for growing the crystals and Marla Babcock and A. R. Srinivasan for their help in analyzing the conformations.

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Enantioselective Oxidations of Sulfides Catalyzed by Chloroperoxidase†

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Received November 30, 1989; Revised Manuscript Received July 23, 1990

ABSTRACT: The chloroperoxidase-catalyzed and horseradish peroxidase catalyzed oxidations of sulfides by *tert*-butyl and other peroxides have been investigated. The former metal enzyme afforded the corresponding sulfoxides having *R* absolute configuration in up to 92% enantiomeric excess (ee), whereas the latter gave racemic products. The various factors that control the enantioselectivity of the oxygenation have been examined.

Chloroperoxidase (CPO) is an enzyme produced by the marine fungus *Caldariomyces fumago* (Morris & Hager, 1966). It is a glycoprotein with M_r 42 000 containing ferriprotoporphyrin IX as the prosthetic group. A great deal of work, with a variety of spectroscopic techniques, has focused on the identification of the fifth axial ligand to the heme, and there is now strong proof that this ligand is a cysteine thiolate, which is bound to a high-spin five-coordinate ferric ion in the native protein (Dawson, 1988; Bangcharoenpaurong et al., 1986). Recently, amino acid and Edman sequence analysis revealed the axial ligand to be cysteine 29 (Blanke & Hager, 1988). The close similarity in the active site structure between chloroperoxidase and cytochrome P-450 corresponds to similar enzymatic activity in a number of instances, e.g., the N-demethylation of certain organic substrates (Kedderis et al., 1980; Padbury & Sligar, 1985) and the epoxidation of cyclohexene (McCarthy & White, 1983) and styrene (Ortiz de Montellano et al., 1987), but chloroperoxidase exhibits a broader spectrum of chemical reactivities, including the reactions typical of peroxidases (Thomas et al., 1970), the use of halide ions to halogenate a variety of organic acceptor molecules (Hager et al., 1966; Libby et al., 1982), and the catalase activity in the disproportionation of hydrogen peroxide (Frew & Jones, 1984). Therefore, chloroperoxidase shares similar properties with classical heme peroxidases, which catalyze the H_2O_2-dependent one-electron oxidation of organic and inorganic substrates (Dunford, 1982), and with P-450 monooxygenases, which transfer an oxygen atom from oxygen donors to their substrates (Ortiz de Montellano, 1986).

While there are a few reactions promoted by peroxidases

† Supported by the Italian MPI.

0006-2960/90/0429-10465$02.50/0