Moreover, erythrocyte ankyrin binds the Na^+-K^+-ATPase from epithelial cells in vitro, suggesting the presence in ankyrin of conserved binding domains for two distinct integral membrane proteins.

These data present the first evidence for the interaction of the sodium channel with another cellular protein. It is likely that this finding will have implications for other cell types due to the presence of p33 in tissues not containing sodium channels. Further studies of the interaction of the sodium channel with p33 may enable us to obtain a better understanding of the mechanisms by which plasma membranes become functionally specialized during differentiation.

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Sequence Specificity of DNA Cleavage by Bis(1,10-phenanthroline)copper(I)†

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ABSTRACT: The bis(1,10-phenanthroline)copper(I) complex is a relatively simple molecule previously shown to cause DNA cleavage with a strong preference for gene control regions such as the Pribnow box. Sequence level mapping of sites of $[(Phen)_2Cu^I]^+$ cleavage in >2000 bases in histone genes and the plasmid pUC9 showed that the specificity for control regions is related to a predominant preference for minor groove binding at TAT triplets, which were cleaved most strongly at the adenosine sugar ring. The related sequences TGT, TAAT, TAGPy, and CAGT (Py = pyrimidine) were moderately preferred, while CAT and TAC triplets, PyPuPuPu quartets, PuPuPuPy quartets, and CG-rich PyPuPuPy quartets were cleaved with low to average frequency. Polypurine and polypyrimidine sequences were cleaved with low frequency. The sequence preferences of $[(Phen)_2Cu^I]^+$ can be ascribed predominantly to (i) a requirement for binding in the minor groove at a pyrimidine 3′ → 5′ step and (ii) stereoelectronic effects of the 2-amino group of guanine in the minor groove, which inhibit binding. Although the reagent appears primarily to recognize sequence features at the triplet or quartet level, lower than expected cleavage was observed for two TAT sequences adjacent to several other preferred sequences and higher than expected cleavage was observed at CAAGC sequences, suggesting that longer range sequence-dependent DNA conformational effects influence specificity in certain cases.

The bis(1,10-phenanthroline)copper(I) complex $[[(Phen)_2Cu^I]^+]$ and other phenanthroline complexes have attracted considerable interest as probes of local DNA conformation and as reagents for "footprinting" binding sites of proteins and other ligands to DNA (Barton, 1986; Sigman, 1986). $[(Phen)_2Cu^I]^+$ appears to recognize DNA with con-

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siderable sequence specificity. For example, Cartwright and Elgin (1982) and Jesse et al. (1982) showed by low-resolution mapping techniques that strongly preferred sites of $[(Phen)_2Cu^I]^+$ binding occurred commonly in 5′ flanking regions, but rarely in coding regions, of several *Drosophila* heat shock and histone genes, respectively. More recently, Sigman and co-workers have shown that the Pribnow box in the *lac* promotor is strongly preferred by $[(Phen)_2Cu^I]^+$ and that point mutations which alter the exact sites of cleavage also affect operator binding (Sigman et al., 1985; Spassky & Sigman, 1985). Knowledge of the DNA binding specificity of $[(Phen)_2Cu^I]^+$ and the structural basis for this specificity may provide additional insights into the sequence dependence of local DNA conformation and guidelines for designing drugs targeted to specific DNA sequences.

DNA binding sites of $[(Phen)_2Cu^I]^+$ have been mapped by techniques analogous to those used in DNA sequencing (Cartwright & Elgin, 1982; Drew & Travers, 1984; Sigman et al., 1985; Spassky & Sigman, 1985). The complex generates hydroxyl radicals [via the Cu(I) $\rightleftarrows$ Cu(II) redox cycle] that subsequently react with DNA, causing strand breakage in the local region of binding (Sigman, 1986). Abstraction of a proton by a hydroxyl radical, either from C1′ or C4′ of a deoxyribose, is believed to initiate the series of reactions leading to cleavage and production of 3′- and 5′-phosphomonoesters (Pope et al., 1982; Kuwabara et al., 1986). Although there are a few specific examples of sequences preferred by $[(Phen)_2Cu^I]^+$ (Cartwright & Elgin, 1982; Drew & Travers, 1984; Sigman et al., 1985; Spassky & Sigman, 1985; Suggs & Warner, 1986), the available data are not statistically sufficient to describe the sequence specificity with confidence or to understand the relationship between specificity and DNA conformation.

We report here the cutting preferences of $[(Phen)_2Cu^I]^+$, at the DNA sequence level, on >2000 bases in sequences of sea urchin and mouse histone genes and of the plasmid pUC9, which contains an ampicillin resistance gene and the *lac* promotor. Preferred cleavages occur at functionally significant sequences in these genes and are related to a predominant preference of $[(Phen)_2Cu^I]^+$ for cleavage at TAT triplets. Moderately to strongly preferred cleavages occur at TGT triplets and TAAT, TAGPy, and CAGT quartets. Longer range sequence effects play a role in determining cleavage frequencies at certain other sites.

Materials and Methods

The sea urchin *Strongylocentrotus purpuratus* early H2a and H3 genes were originally contained in plasmid pSp117 obtained from L. Kedes (Stanford) and were subsequently recloned into pUC9. A mouse H3.2 gene cloned into pUC18 (Sittman et al., 1983) was obtained from W. Marzluff (Florida State University). Plasmids were isolated by standard procedures using base/acid extraction and deproteinized by treatment with sodium dodecyl sulfate (SDS) and proteinase K, followed by phenol/chloroform extraction. Plasmids were cleaved with restriction endonucleases to produce 5′ overhanging ends and then 3′ end labeled by reverse transcriptase with [α-^{35}S]dATPαS or dCTP. Specific fragments were isolated from low melting point agarose gels after treatment with a second restriction endonuclease to remove one labeled end.

$[(Phen)_2Cu^I]^+$ reaction conditions were adopted from those of Sigman et al. (1985) and designed such that one to two nucleotides were cleaved per DNA molecule. A 100-μL solution was typically 100 μM 1,10-phenanthroline, 5 μM $CuSO_4$, 2.3 mM mercaptopropionic acid, 100 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), pH 8.0, 10 mM $MgCl_2$, and 100 mM KCl and contained 30 nmol of DNA. The reactions were initiated by addition of the mercaptopropionic acid, which reduces Cu to the +1 state, and terminated by addition of 2,9-dimethyl-1,10-phenanthroline. The extent of cleavage was controlled by adjusting the reaction duration, typically 2–64 min.

Positions of cleavage were determined by electrophoresis of the reacted DNA on 8% polyacrylamide DNA sequencing gels, followed by autoradiography. Markers consisted of the same DNA fragment subjected to chemical sequencing reactions and otherwise treated identically to samples reacted with $[(Phen)_2Cu^I]^+$. Relative intensities of bands were determined from densitometer tracings of autoradiograms using a Joyce Loebl Mark III scanning densitometer.

Table I: Relative Cleavage Frequencies at PyPuPy Triplets[a]

first base (5′)	second and third bases			
	AT	GT	AC	GC
T	7.3 ± 0.9 (N = 26)	3.7 ± 1.4 (N = 29)	3.1 ± 1.7 (N = 17)	1.8 ± 1.7 (N = 32)
C	1.5 ± 1.5 (N = 17)	1.1 ± 1.0 (N = 14)	0.4 ± 0.6 (N = 24)	0.3 ± 0.7 (N = 18)

[a] Intensities of bands corresponding to cleavages at these sites were scored as follows: low = 0, average = 2, above average = 4, moderately strong = 6, and strong = 8. Average scores are reported with standard deviations. N = number of occurrences.

Results

$[(Phen)_2Cu^I]^+$ caused cleavage at all nucleotides, but distinct variations in cleavage frequency were readily evident (Figures 1–3). Under conditions of limited reaction the relative intensities of neighboring bands on a gel are proportional to relative rates of cleavage at the corresponding nucleotides. On a given gel scan the ratio of relative areas of the most and least intense peaks frequently were >50, corresponding to a difference in apparent activation energy of >2.4 kcal/mol. Since $[(Phen)_2Cu^I]^+$ cleaved at all bases, a precise numerical analysis (in progress) is required to understand all aspects of $[(Phen)_2Cu^I]^+$ preferences. A semiquantitative analysis of the data was sufficient, however, to establish the major preferences. Electropherograms first were inspected visually to identify preferred and avoided sequences. Occurrences of sequences that were obviously preferred, and sequences related by a single base change, were then scored on a five-level scale (Tables I and II).

Triplet Cleavage Propensities: Predominant Cleavage at TAT Triplets. The sequence TAT was preferred over all other three- or four-base sequences, with primary cleavage occurring at the middle adenosine (Table I, Figures 1–3). Cleavages at the flanking T's were unequal, with the 3′ T moderately to strongly preferred over the 5′ T. For example, the sequence TTATC was cleaved intensely at the A, moderately at the 3′ T, and only slightly at other positions (Figure 1a). Within the sequences examined there were 26 occurrences of the TAT triplet. In all cases except two (see below) the central A of TAT was cleaved with higher frequency than any other nucleotide displayed on the lane. (Typically >60 nucleotides were distinguishable at the sequence level in each lane.)

By contrast, cleavages at triplets or longer strips of pyrimidines were strongly disfavored, and such sequences usually occurred at minima in gel scans (Figure 1a,c). Low-cleavage frequencies at such regions were noted in the *Escherichia coli tyr*T promotor by Drew and Travers (1984) and attributed to unusually wide or narrow minor groove widths proposed for runs of G/C or A/T, respectively, lacking Py/Pu steps.

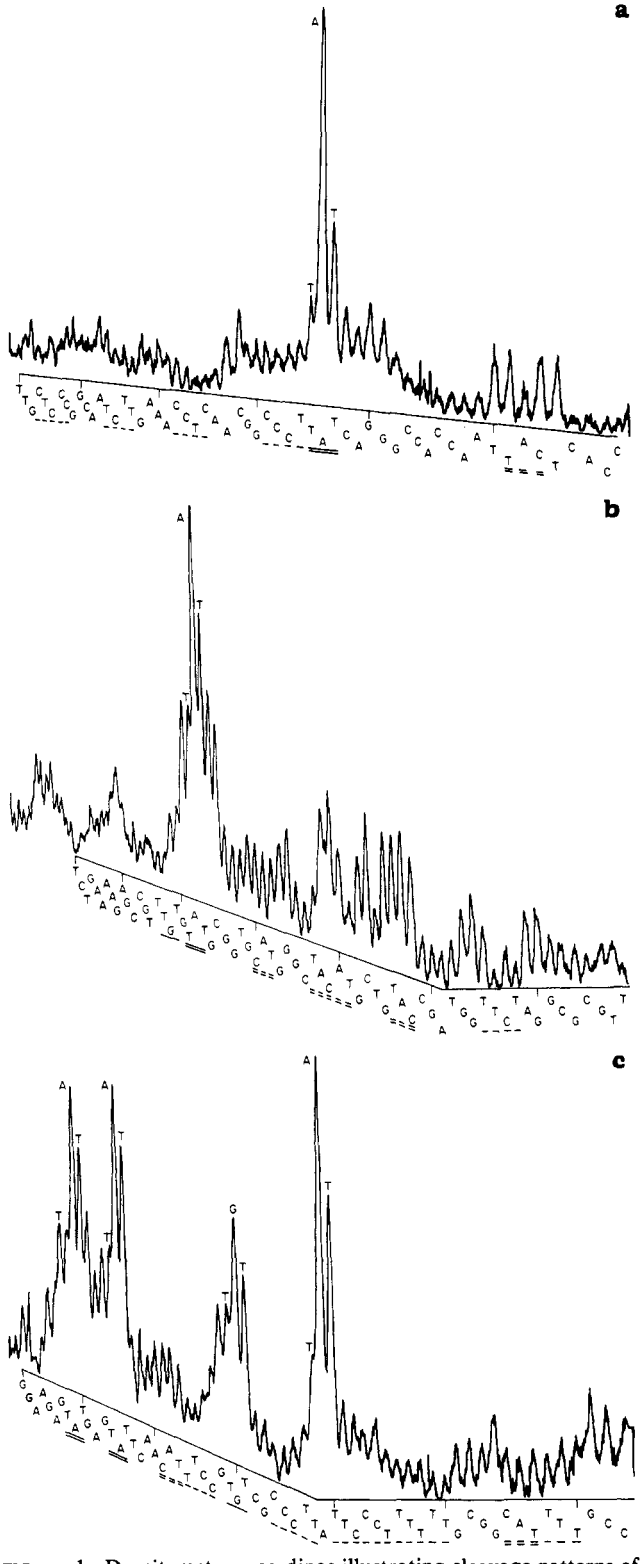

FIGURE 1: Densitometer recordings illustrating cleavage patterns of DNA reacted with $[(Phen)_2Cu^I]^+$. All sequences read 5′ → 3′ from left to right. Strongly preferred TAT sequences are underlined with a double solid line, moderately preferred TGT sequences are underlined with a single solid line, and other sequences differing from TAT by one base change (CAT, TAC) are underlined with a double dashed line; strongly disfavored $(Py)_n$ sequences are underlined with a single dashed line. (a) The 3′ transcribed but nontranslated region of the *S. purpuratus* early histone H2A gene (from an *Eco*RI/*Bst*EII digest); (b) same as in (a) but for the early histone H3 gene; (c) 5′ region of the ampicillin resistance gene (*Taq*I/*Pvu*I digest).

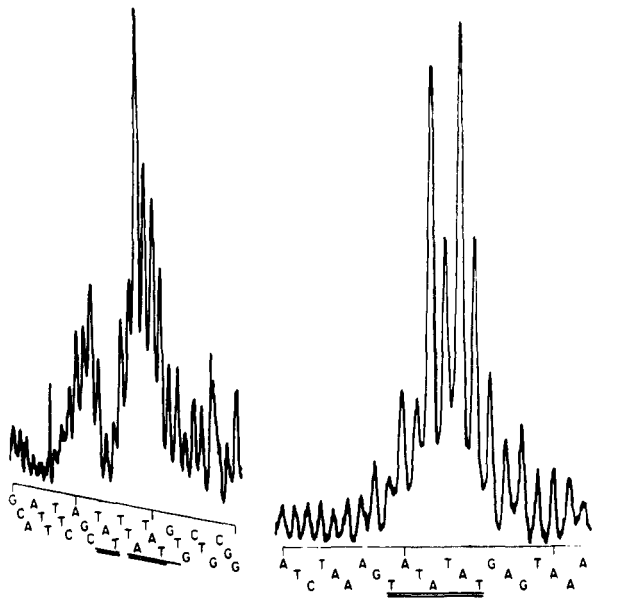

FIGURE 2: Anomalous $[(Phen)_2Cu^I]^+$ cleavage of the 5′-TA in TATATPuT sequences. See Figure 1 for conventions. (Right) 3′ flanking region of the ampicillin resistance gene (*Hin*fI/*Hae*III digest); (left) 5′ promoter region of the *S. purpuratus* early histone H3 gene (*Eco*RI/*Bst*EII digest).

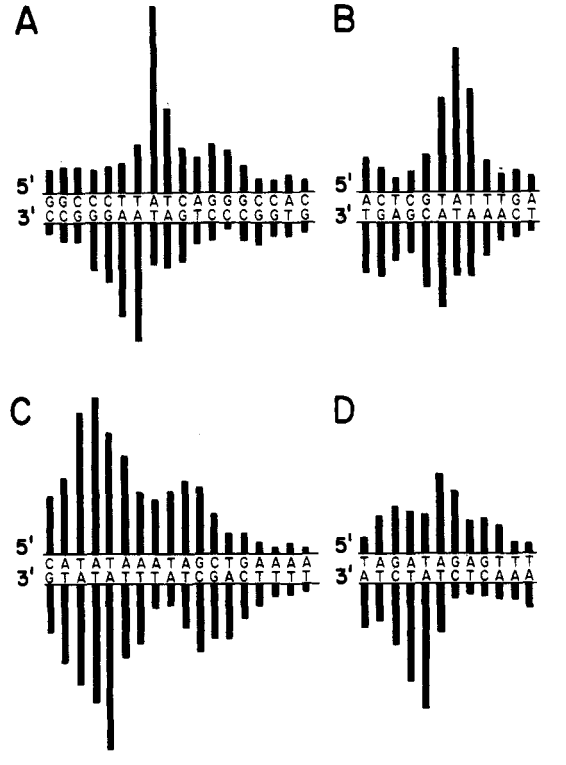

FIGURE 3: Cleavage patterns on strands opposite TAT triplets highly preferred by $[(Phen)_2Cu^I]^+$. Histograms indicate peak heights on densitometer scans. Note that the strongest cleavage sites on the strand opposite TAT are staggered to the 3′ side. (A) Hairpin loop sequence corresponding to the 3′ end of *S. purpuratus* H2a mRNA. (B) Sequence from the transcribed, nontranslated 5′ flanking region of the *S. purpuratus* H3 gene. (C) Promotor region (TATAA box) of the *S. purpuratus* H3 gene (same as in Figure 2, left). Note very high cleavage of both strands at the 5′-ATATA-3′ sequence, anomalous cleavage at the 5′-TAT sequence on the lower strand, and low cleavage at the terminal A_4·T_4 tract. (D) Sequence surrounding TAG corresponding to the *S. purpuratus* H2a stop codon (TAG).

The triplet TAT was necessary and sufficient to establish dominant preference in all sequences examined. Cleavages at triplets related by a single base change occurred with reduced (TGT, TAC) or low (CAT) frequency. For example, Figure 1a contains the sequences TTACT and TTATC, which differ by the exchange in position of a T and C. The 3′ C in

Table II: Relative Cleavage Frequencies at Quartet Sequences[a]

first two bases (5′)	third and fourth bases							
	AT	GT	AC	GC	AA	GA	AG	GG
TA	5.0 ± 1.6 (N = 5)	6.0 ± 0.0 (N = 3)	− (N = 0)	3.6 ± 2.0 (N = 5)	1.9 ± 0.8 (N = 10)	2.3 ± 1.3 (N = 6)	− (N = 0)	2.8 ± 0.8 (N = 4)
CA	0.8 ± 1.0 (N = 9)	4.4 ± 1.6 (N = 13)	1.2 ± 1.0 (N = 11)	3.4 ± 1.0 (N = 8)	0.5 ± 0.8 (N = 6)	2.5 ± 1.1 (N = 4)	2.1 ± 0.1 (N = 10)[b]	0.8 ± 1.1 (N = 6)
TG	3.3 ± 1.1 (N = 6)	3.2 ± 1.6 (N = 9)	1.5 ± 1.1 (N = 6)	2.4 ± 1.8 (N = 11)	1.4 ± 1.4 (N = 11)	0.8 ± 0.9 (N = 6)	2.1 ± 0.8 (N = 7)	0.8 ± 1.0 (N = 5)
CG	0.3 ± 0.8 (N = 6)	0.5 ± 0.9 (N = 4)	0.3 ± 0.5 (N = 3)	0.3 ± 0.4 (N = 4)	0.0 ± 0.0 (N = 4)	1.0 ± 1.0 (N = 2)	0.4 ± 0.5 (N = 5)	1.0 ± 0.8 (N = 10)
AA	1.1 ± 1.1 (N = 11)	2.4 ± 0.9 (N = 7)	0.1 ± 0.4 (N = 7)	2.1 ± 1.2 (N = 6)[b]				
GA	2.0 ± 0.0 (N = 2)	2.7 ± 0.5 (N = 6)	0.3 ± 0.5 (N = 3)	1.0 ± 1.0 (N = 8)				
AG	1.8 ± 1.0 (N = 9)	2.0 ± 1.1 (N = 7)	2.0 ± 1.3 (N = 5)	1.3 ± 1.1 (N = 6)				
GG	1.0 ± 1.2 (N = 7)	0.6 ± 0.8 (N = 5)	− (N = 0)	1.1 ± 0.9 (N = 9)				

[a] Band intensities corresponding to relative cleavage frequencies were scored as described in Table I. [b] Sequences composing the preferred CAAGC pentamer were excluded. The three CAAGC sequences in the data set were scored at 6 on this scale.

TAC virtually abolished cleavage at the central A. The sequence in Figure 1b contains two CAT triplets, neither of which was strongly cleaved. Panels b and c of Figure 1 contain two TGT triplets that were preferentially recognized by $[(Phen)_2Cu^I]^+$ but to lesser extents than nearby TAT's. Likewise, the terminal A in TATAT was more strongly preferred than the terminal G in TATGT (Figure 2, left). A dramatic example of the effect of a single base change is demonstrated in Figure 1b for the sequences GTGTATG and GTGCATG. The T → C transition transformed a hypersensitive region into one that was preferred only slightly above average. A similar effect was observed for GTATT and GCATT in Figure 1c.

Examination of the influence of the two nucleotides to the 5′ and 3′ sides of TAT on the cleavage rate at the central A indicated that highly preferred cutting at the central A of TAT always occurred unless it was proximal to other preferred sites. There were two examples of this exception in the data set. The sequence 5′-GTATATATG-3′ occurs in Figure 2 (right). Cleavages at the central and 3′ A's were intense and nearly equal, and cleavages at the T's 3′ flanking these A's were also similar. By contrast, cleavages at both the A and 3′ flanking T of the 5′-terminal TAT were strongly suppressed. A similar phenomenon is evident in Figure 2 (left). In this case cleavage was strongly suppressed at a TAT triplet that is flanked on the 5′ side by a moderately favored PyAGPy sequence and on the 3′ side by a TATAT sequence (see also Figure 3C, which shows the cleavage pattern on both strands).

More quantitative examination of the cleavage frequencies at PyPuPy sequences showed that the order of preference was TAT ≫ TGT ≥ TAC ≫ CAT, CAC > CGT, CGC (Table I). Primary cleavage of the TGT triplet occurred at the central G in nearly all cases, but the position of maximal cleavage of TAC was more variable. The effects of flanking sequences on the rates of cleavage of TGT and TAC also appeared more significant than for TAT, as indicated by the higher standard deviations of the cleavage scores (Table I).

Diffusion of the Hydroxyl Radical: Cleavages on the Strand Opposing TAT. DNA cleavage is due to reaction with a diffusible hydroxyl radical, rather than directly with $[(Phen)_2Cu^I]^+$. This feature of the mechanism complicates interpretation of band intensities in terms of $[(Phen)_2Cu^I]^+$ binding but provides information about the geometry of the $[(Phen)_2Cu^I]^+$–DNA complex because the reaction rate at a particular nucleotide should be related to its distance from the copper in the primary binding site.

We have mapped cleavage sites on both strands of sequences containing over 500 bases. Moderately strong cleavages were observed on all strands opposite TAT and TGT sequences but were clearly asymmetric (Figure 3). In the cases examined the strands complementary to preferred TAT and TGT sequences were cleaved significantly at positions displaced one and two nucleotides to the 3′ side of the A of TAT on the opposing strand, with cleavage at position 1 (the 3′ A of ATA) usually slightly stronger than at position 2. (It should be noted that this opposite strand cutting explains partly, but not entirely, the low cutting frequency at the 5′-terminal TA in TATATAT of Figure 2.) These observations confirm that $[(Phen)_2Cu^I]^+$ binds in the minor groove of TAT and TGT sequences and strongly support previous suggestions, based on more limited sequence data, that $[(Phen)_2Cu^I]^+$ binds generally in the minor DNA groove (Drew & Travers, 1984; Kuwabara et al., 1986). As a consequence of the structure of B-form DNA, the nucleotides on the opposing strand that are physically closest to the primary binding site are staggered to the 3′ side for binding in the minor groove or staggered to the 5′ side for binding in the major groove. Minor groove binding by $[(Phen)_2Cu^I]^+$ is also indicated by its ability to "footprint" a minor groove binding drug, netropsin, and its inability to footprint the major groove binding restriction endonuclease, *Eco*RI (Kuwabara et al., 1986). By contrast, cleavages opposite PyAGPy sequences appeared relatively weaker than those opposite TPuT sequences and were not as consistently staggered.

Diffusion also affects the cleavage pattern observed on the same strand as the favored nucleotide and is not, a priori, distinguishable from secondary binding sites. We observed, however, that in a favored TAT sequence the 5′ T almost invariably was cleaved to a lesser extent than the 3′ T, and low cleavage occurred at nearest-neighbor pyrimidines in PyTATPy sequences (Figure 1a,c). Low cleavage at these sites suggests that diffusion of the hydroxyl radical is largely limited to nucleotide sugars immediately neighboring the $[(Phen)_2Cu^I]^+$. Cleavages at the T's in a TAT triplet seemed to be enhanced by flanking G's, however, as illustrated by the direct repeat sequence GAGTATGAGTAT in Figure 1c, the sequence TGTGTATGC in Figure 1b, and the sequence CGTATTT in Figure 3. More quantitative analyses are required to determine if the flanking G effect is due to secondary binding or diffusion.

Preferred PyPuPuPy Quartet Sequences. Inspection of electropherograms suggested that TAAT, TAGPy, and CAGT

sequences were at least moderately preferred by $[(Phen)_2Cu^I]^+$. Scoring of PyPuPuPy sequences as described above showed that TAGT and TAAT were highly preferred, though less than TAT. Somewhat curiously, predominant cleavage occurred at the 5′ A of TAAT, but at the G of TAGT. The order of preference for PyPuPuPy sequences was TAAT $\simeq$ TAGT > CAGT > TAGC, CAGC, TGAT > TGGT > TGAC, CAAC, CAAT > CGGT, CGAT, CGAC, CGGC (Table II). All quartets with PuPuPuPy and PyPuPuPu sequences were also scored to investigate the requirement for the initial and final pyrimidines in determining the above preferences. None of these sequences was cleaved with significantly above average frequency (Table II); hence the appropriate PyPuPuPy quartet sequence is required to establish high preference.

A Preferred Pentamer Sequence: CAAGC. A curious exception was found for the sequences CAAG and AAGC when these sequences overlapped. The sequence CAAGC occurred 3 times in the data set and was most strongly cleaved in each case at the guanosine. By contrast, the other 10 CAAG and 6 AAGC sequences in the data set were cleaved with average frequency (Table II).

Discussion

The observation that $[(Phen)_2Cu^I]^+$ cleavage specificity is determined at the triplet or quartet level indicates that the reagent recognizes local variations in geometry of essentially B-form DNA. The sequence preferences described above can be ascribed mainly to (i) a requirement for $[(Phen)_2Cu^I]^+$ binding in the minor groove side of a pyrimidine 3′ → 5′ purine step and (ii) stereoelectronic effects of the 2-amino group of guanine in the minor groove, which inhibit binding at pyrimidine–purine steps; consequently, the strongest determinant of $[(Phen)_2Cu^I]^+$ preference is a T 3′ → 5′ A step. The reactivities of preferred sequences relative to those differing by a single base in the first two positions are TAT ≫ CAT, TAT > TGT, TAGT > CAGT, and TAAT $\simeq$ TAGT ≫ TGAT; likewise TGT ≫ CGT and TAAT ≫ CAAT. Triplets or quartets beginning with a C 3′ → 5′ G step were uniformly low in reactivity; hence substitution of a G·C pair for an A·T pair in the first or second position always decreases reactivity, presumably due to interference of the guanine 2-amino group. The TA step is not sufficient to determine optimal cleavage, however, because effects of succeeding bases were clearly evident. Examples are the preferences TAT > TAC, TAT > TAAT $\simeq$ TAGT and TAGT > TAGC. Third and fourth base effects were also evident in the preferences TGT > TGC, TGAT > TGGT > TGGC > TGAC and TAGPy > TAGPu.

Our data set is not sufficient to assess sequence effects beyond the quartet level with statistical significance, but some cases of longer range effects were clearly evident, such as the suppression of reaction at the A of two TAT triplets located adjacent to other preferred cleavage sites (Figures 2 and 3C) and the moderately high reactivity of CAAGC. In general, the relative reactivities of moderately preferred triplets and quartets appeared somewhat sensitive to flanking sequences as indicated by the high standard deviations in reaction preferences (Tables I and II).

The sequence specificity of $[(Phen)_2Cu^I]^+$ cleavage is rather remarkable because the unsubstituted aromatic phenanthroline ring system precludes hydrogen-bonding interactions with bases as determinants of specificity. The mode of DNA binding by $[(Phen)_2Cu^I]^+$ is not known. Other reagents with strong AT preferences such as netropsin and distamycin are typically groove binders (Feigon et al., 1984; Kopka et al., 1985; Goodsell & Dickerson, 1986), whereas intercalators tend to favor GC-rich regions unless they possess substituents that can specifically hydrogen bond with A·T pairs (Feigon et al., 1984; Wilson et al., 1985).

The $[(Phen)_2Cu^I]^+$ preference for TAT may be related, in part, to sequence-dependent variations in the B conformation discovered in X-ray crystallographic studies of oligonucleotide minihelices (Dickerson & Drew, 1981; Dickerson, 1983). Stacking interactions are favored when the bases in a base pair are "propeller twisted". Propeller twist causes steric interference between purines on opposite strands staggered by one base (Calladine, 1982; Dickerson, 1983). For pyrimidine 3′ → 5′ purine steps, interference between successive bases occurs in the minor groove and can be alleviated by a combination of rolling the base pairs relative to each other and local helix unwinding. Predictions of roll and twist angles of the sequence CTTATCC according to the method of Tung and Harvey (1986) describe the helix as severely underwound at the central TA step (twist angle, $t = 26°$) and overwound at adjacent steps ($t_{TT} = 39°$; $t_{AT} = 38°$). The predicted change in roll angle for the TA step is positive and relatively large ($\Delta\theta_R = 3.7°$), while that at the AT step is negative ($\Delta\theta_R = -4.0°$). These changes in roll and twist open the minor DNA groove at the TA step and could facilitate penetration of the helix by a phenanthroline ring in either a partial intercalation or groove binding mode.

$[(Phen)_2Cu^I]^+$ is precluded by its tetrahedral geometry from full intercalation, but a model in which one phenanthroline partially intercalates at the TA step is plausible. The helix presumably is already underwound at this step. Modeling suggested that favorable stacking interactions could occur between the central ring and one outer ring of phenanthroline and the adenines above and below the partial intercalation site in a geometry consistent with preferred cleavage at the A in TAT, as well as the relative rates of cleavage at neighboring sites on both strands. An exclusively groove binding model of $[(Phen)_2Cu^I]^+$ binding at TAT must also be seriously considered. A physically reasonable model can be built, which places the copper near adenosine in TAT. Any acceptable model for $[(Phen)_2Cu^I]^+$ binding to preferred sequences must be consistent with the third and fourth base preferences observed. Since $[(Phen)_2Cu^I]^+$ is large enough to span nearly four base pairs in B-form DNA, simple steric considerations of the ligand and groove geometry, particularly placement of the guanine 2-amino groups, may suffice to explain the specificity. It is reasonable to suppose, however, that steric effects analogous to those expressed in Calladine's rules cause sequence-dependent variations in conformations about an intercalation or groove binding site, and thereby additionally influence specificity.

This compilation of a statistically significant data set for $[(Phen)_2Cu^I]^+$-induced DNA cleavage represents an initial effort toward understanding the recognition of DNA by $[(Phen)_2Cu^I]^+$. The fact that $[(Phen)_2Cu^I]^+$-sensitive sites are often protein binding sites (e.g., the Pribnow box) and, for the histone genes, correlate with critical regulatory sequences (Cartwright & Elgin, 1982; Jesse et al., 1982) implies that an understanding of sequence determinants of $[(Phen)_2Cu^I]^+$ specificity might facilitate an understanding of protein–DNA interactions. For example, mutations that increase the $[(Phen)_2Cu^I]^+$ sensitivity of the *lac* promotor are related to an increase in promotor efficiency (Sigman et al., 1985; Spassky & Sigman, 1985). The observed differences in $[(Phen)_2Cu^I]^+$ sensitivity between wild-type, Ps, and UV-5 mutants can now be explained largely in terms of the introduction of new preferred TAT or TAAT sites on one or both strands by successive single base changes in the −13 to −6 region of the *lac* gene:

5′-GTATGTTG-3′ (wt) → 5′-GTATATTG-3′ (Ps) → 5′-GTATAATG-3′ (UV-5)
3′-CATACAAC-5′ 3′-CATATAAC-5′ 3′-CATATTAC-5′

Another interesting observation from our data set in this regard is that adjacent sequences occasionally act to abolish what should be a preferred binding site. One such region (Figure 2, right) may assume the Z conformation in supercoiled plasmids (Barton & Raphael, 1985). In general, however, the interactions of $[(Phen)_2Cu^I]^+$ can best be rationalized in terms of present knowledge of effects of sequence on the local conformation of B-form DNA.

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The Receptor Site for the Bee Venom Mast Cell Degranulating Peptide. Affinity Labeling and Evidence for a Common Molecular Target for Mast Cell Degranulating Peptide and Dendrotoxin I, a Snake Toxin Active on K^+ Channels†

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ABSTRACT: The mast cell degranulating peptide (MCD) and dendrotoxin I (DTX_I) are two toxins, one extracted from bee venom, the other one from snake venom, that are thought to act on voltage-sensitive K^+ channels. Binding sites for the two toxins have been solubilized. The solubilized sites were stable and retained their high affinity for ^{125}I-DTX_I and ^{125}I-MCD ($K_d \simeq 100$ pM). Interactions were found between MCD and DTX_I binding sites in the solubilized state, establishing that the two different toxins act on the same protein complex. This conclusion was strengthened by the observations (i) that conditions of solubilization that eliminated ^{125}I-MCD binding activity also eliminated ^{125}I-DTX binding activity while both types of activities were preserved in the presence of K^+ or Rb^+ and (ii) that binding components for the two types of toxins had similar sedimentation coefficients and copurified in partial purifications. A component of the receptor protein for ^{125}I-MCD has been identified; it has a M_r of 77 000 ± 2000. This polypeptide was similar to or identical in molecular weight with that which serves as a receptor for DTX_I (M_r 76 000 ± 2000).

The mast cell degranulating peptide (MCD)[1] is a basic toxin of 22 amino acids (Habermann, 1972). Aside from its mast cell degranulating activity, the peptide also acts in the central nervous system. Depending on the concentrations injected, MCD induces hippocampal θ rhythms associated with arousal

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[1] Abbreviations: MCD, mast cell degranulating peptide; DTX_I, dendrotoxin I; DMS, dimethyl suberimidate; SDS, sodium dodecyl sulfate; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; DEAE, diethylaminoethyl; WGA, wheat germ agglutinin.

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