Published in final edited form as:

Biochemistry. 2009 December 1; 48(47): 11312–11318. doi:10.1021/bi901154t.

The pH-dependent configurations of a 5-Chlorouracil-Guanine Base Pair†

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Abstract

Hypochlorous acid (HOCl) from activated neutrophils at sites of inflammation can react with and damage biological molecules including nucleic acids. The reaction of HOCl with cytosine analogs can generate multiple products including 5-chlorouracil (CIU). In this paper, we have constructed oligonucleotides containing CIU paired opposite guanine (CIU-G). Melting studies indicate that oligonucleotide duplexes containing the CIU-G mispair are substantially less stable than those containing a CIU-A base pair. The melting temperature of the CIU-G mispair is not experimentally distinguishable from G-T. NMR studies indicate that the CIU-G base pair adopts a wobble geometry at neutral pH, similar to a T-G mispair. The exchangeable protons of the CIU-G mispair broaden rapidly with increasing temperature, indicating that the CIU-G mispair is less stable and opens more easily than the surrounding adjacent base pairs. Unlike the CIU-A base pair studied previously (*Biochemistry* 48, 7539–46, 2009), the CIU-G mispair undergoes a pH-dependent structural change, assuming an ionized base pair configuration that approximates a Watson-Crick base pair at higher pH. Ionization of CIU in a DNA template could promote mispair formation and mutation, in accord with previous studies on other 5-halouracil analogs. The electron-withdrawing 5-chloro substituent facilitates ionization of the CIU N3 proton, promoting mispair formation, but it also renders the glycosidic bond susceptible to base cleavage by DNA repair glycosylases.

Reactive molecules generated by cells of the innate immune system, including neutrophils and eosinophils, are known to react with lipids, proteins and nucleic acids (1–5). While highly reactive molecules, including hypochlorous acid (HOCl), have potent antimicrobial activity, these reactive species can also cause collateral damage to host molecules. Emerging evidence indicates that HOCl-mediated damage is frequently found at sites of inflammation suggesting a potential connection between inflammation and the development of human disease, including cancer (6).

The reaction of HOCl with cytosine generates an array of products (7), including 5-chlorocytosine (CIC) and 5-chlorouracil (CIU). If the initial reaction occurred with a cytosine residue in a G-C base pair, the result could be a CIU-G mispair (Figure 1a). Base pairs in DNA containing CIU can also result from the incorporation of CIU-containing nucleotides generated by the reaction of HOCl with nucleotide pools (8–11).

†This work is supported by grants from the National Institute of Health.

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SUPPORTING INFORMATION AVAILABLE Additional experimental data includes two tables of NMR chemical shifts, the mass spectral characterization of the CIU-G oligonucleotide, and 10 figures showing 1D and 2D NMR spectra. This material is available free of charge via the Internet at <http://pubs.acs.org>.

In a recent study, we examined the configuration and stability of a base pair containing CIU paired with adenine in a model synthetic oligonucleotide duplex (12). The configuration of the CIU-A base pair was similar to that of a normal T-A base pair; however, it was slightly more thermally and thermodynamically stable. The linewidth of the CIU imino proton did not increase with increasing solution pH. Although the CIU imino proton is more acidic than the corresponding thymine (T) imino proton, the absence of pH-dependent line-broadening at pH values well above the pK_a of CIdU (5-chloro-2'-deoxyuridine) was attributed to the formation of a stronger hydrogen bond with the opposing adenine residue and an indicator that the N3 proton was needed for the formation of the CIU-A base pair. The inability of a repair glycosylase to remove CIU when paired with A was attributed to the apparent stability of the CIU-A base pair, suggesting that CIU when paired with A might be a persistent lesion in DNA.

Previous studies have examined oligonucleotide duplexes containing analogous 5-fluorouracil (FU) and 5-bromouracil (BrU) mispairs with guanine. The FU-G (13) and BrU-G (14) mispairs have been shown to be in a wobble geometry at neutral pH both by NMR and, in the case of BrU-G, by crystallography in a duplex that assumed a Z-DNA structure (15). With increasing pH, the FU-G and BrU-G mispairs were shown to undergo a pH-dependent transition with formation of an ionized base pair in a geometry that approximated a Watson-Crick base pair (13,14). The capacity of the halouracil-G mispairs to undergo a pH-dependent transition was attributed to the electron-withdrawing property of the 5-halo substituent, which is opposite to that of the thymine 5-methyl group. Subsequent studies from the Goodman laboratory (16) demonstrated that the mispairing of the halouracil could be increased by increasing solution pH, consistent with the ionized base pair model (17,18).

The pK_a of the 2'-deoxynucleoside analogs of FU, CIU, BrU and thymine are 7.7, 7.9, 8.0 and 9.7, respectively (19,20). The 5-chloro substituent, like the analogous fluoro and bromo substituents, facilitates ionization of the pyrimidine ring N3 proton and destabilizes the glycosidic bond, promoting glycosylase repair (21–26). Although FU and BrU are similar with respect to the inductive properties of the 5-substituent, the biological properties are distinct. Derivatives of FU are highly toxic (27,28). BrU is mutagenic although mammalian cell grown in culture can tolerate substantial substitution of T with BrU (29,30).

The distinct properties of FU and BrU are likely due in part to the size of the 5-substituent. The smaller size of fluorine allows 5-fluoro-2'-deoxyuridine-5'-monophosphate to mimic 2'-deoxyuridine-5'-monophosphate and poison thymidylate synthase. When in DNA, FU is also readily removed by all members of the uracilglycosylase family of repair enzymes (21–26). In contrast, BrU can replace T in facilitating the binding of sequence-specific DNA-protein interactions (31,32). BrU residues are repaired only by a subset of uracil glycosylases and usually only when mispaired with G (21–26).

The size of the 5-substituent of CIU is intermediate in size between fluorine and bromine, predicting that in some cases CIU would mimic FU, but in other cases be more similar to BrU. Among the 5-halouracils, CIU would be the most likely formed under endogenous conditions. The incorporation of CIU into DNA is known to cause chromosomal aberrations, cellular senescence and toxicity by as yet incompletely understood mechanisms (33–36). Recent studies indicating the formation of CIU as a by-product of inflammation damage (1–6) bring new significance to understanding the properties of CIU. In this paper, we describe the results of studies with synthetic oligonucleotides in which CIU has been placed opposite G. We report the impact of this substitution on oligonucleotide stability and conformation.

Materials and methods

Oligonucleotide synthesis

Commercially available 2'-deoxyuridine was converted to CldU using the method of Kumar et al. (37). The corresponding phosphoramidite of CldU was prepared by standard methods, as previously described (36). All other phosphoramidites were obtained from Glen Research (Sterling, VA). Oligonucleotide synthesis was conducted with an Expedite synthesizer from Applied Biosystems (Foster City, CA). Oligonucleotides containing CldU were deprotected with concentrated aqueous ammonia at room temperature for 24 h.

The sequence of the 12-mer oligonucleotide examined here is shown in Figure 1b. Oligonucleotides were purified by two rounds of HPLC, first with the dimethoxytrityl (DMT) group on, and the second with the DMT group off. Oligonucleotides were examined by MALDI-TOF-MS (38) and the free base composition was verified by GC/MS following acid hydrolysis (39).

Oligonucleotide UV Melting studies

The melting temperature (T_m) and thermodynamic values of the oligonucleotide was obtained as previously described (40–42) using a Varian Bio 300 Cary UV-vis spectrophotometer (Palo Alto, CA). The self complementary oligonucleotide at various concentrations (2 μ M to 60 μ M) was dissolved in a buffer containing 100 mM NaCl, 0.1 mM EDTA, and 10 mM sodium phosphate at pH 7. The sample was then treated with a thermal cycle from 10 °C to 90 °C at an interval of 0.5 °C. The thermodynamic values are obtained from the average of five such temperature cycles. T_m values are reported at 28 μ M total strand concentration (Table 1). Melting temperatures were also obtained from pH 7 to pH 11.

Nuclear Magnetic Resonance Spectroscopy (NMR)

NMR spectra were obtained with a 500 MHz Bruker NMR system (Billerica, MA.). The proton NMR spectra of the oligonucleotide were taken in a solution containing 10% D₂O, 100 mM NaCl, 10 mM sodium phosphate and 0.2 mM EDTA at pH 7.0 and compared with results from similar sequences published by other laboratories (43–45). The oligonucleotide (200 A₂₆₀ OD units, 2.5 mM) was annealed at 86 °C for 2 min and slowly cooled prior to acquisition of NMR spectra. Each spectrum was calibrated using DSS (4,4-dimethyl-4-silapentane-1-sulphonate) as an internal standard reference. Proton NMR spectra of oligonucleotides in 90% H₂O were acquired with a water suppression double gradient echo WATERGATE W5 pulse program (46). The temperature of the sample was controlled by a variable temperature monitor (Eurotherm, BVT 3000) from Bruker.

A typical spectrum was acquired with a binomial water suppression delay (d19) of 160 μ sec which optimized water suppression. Since the delay (d19) of 160 μ sec affected some of the imino resonances near 11 ppm, it was changed to 40 μ sec when we examined the imino protons. The null point repetition was every 6.25 ppm from the suppression point when the d19 was 160 μ sec. When the delay (d19) was 40 μ sec, the null point repetition was far from any of the imino proton resonances. The line width studies were performed using the d19 delay of 40 μ sec. The 2D NOE spectra were acquired using a mixing time of 300 ms unless otherwise indicated.

Results

Commercially available 2'-deoxyuridine was converted to 5-chloro-2'-deoxyuridine (37) and then converted to the corresponding 5'-dimethoxytrityl-3'-cyanoethyl-

diisopropylaminophosphoramidite (36). The phosphoramidite analog of CIU was incorporated into a self-complementary synthetic oligonucleotide using standard solid phase methods. In the sequence prepared for this study, the CIU residue was paired opposite guanine (Figure 1b).

Oligonucleotides containing CIU were synthesized, deprotected and purified using standard conditions, except that deprotection was conducted in concentrated aqueous ammonia at room temperature for 24 h. Due to the high natural abundance of both ^{35}Cl and ^{37}Cl isotopes, the mass spectrum of chlorine-containing molecules has a significant $M+2$ line for the parent and M -methyl ions. Intact oligonucleotides were also examined by MALDI-TOF-MS. The isotope envelope of larger molecules can also be simulated (38); the presence of one chlorine atom in the oligonucleotide profoundly impacts the shape of the isotope envelope. The simulated ion cluster of an oligonucleotide of composition $\text{C}_{116}\text{H}_{145}\text{N}_{45}\text{O}_{71}\text{P}_{11}\text{Cl}$ matches the experimental spectrum (Figure S1a). The base composition of each oligonucleotide was verified by GC/MS following acid hydrolysis (Figure S1b).

The thermal stability of the oligonucleotide duplex (40–42) containing CIU was examined and compared with that of an oligonucleotide containing T. The melting temperature at pH 7 of the CIU-G-containing oligonucleotide was observed to be $41.8 \pm 0.2^\circ\text{C}$, while that of the oligonucleotide containing T-G was $42.2 \pm 0.2^\circ\text{C}$. Thermodynamic parameters obtained are shown in Table 1. The thermal melting profiles of the T and CIU-containing oligonucleotides are shown in Figure 2.

Proton NMR spectra of the non-exchangeable protons of each duplex were obtained and shown in Figure 3. Proton resonances were assigned by standard 2D methods (47) and assignments are provided in Table S1. The corresponding 2D spectra for the duplexes containing the T-G and CIU-G mispairs are shown as Figures S2 and S3. The observed proton connectivities indicate that both the T-G and CIU-G duplexes are predominantly in a B-form geometry and all bases are intrahelical. The H6 proton for T3 of the T-G oligonucleotide is observed at 7.18 ppm, whereas the corresponding H6 proton of the CIU residue of the CIU-G duplex is observed at 7.70 ppm.

Proton spectra of the exchangeable proton resonances were obtained in 90% H_2O , 10% D_2O , and the spectra of the T-G and CIU-G duplexes are shown in Figure 4. Proton resonances were assigned by standard 2D methods, as described above. The 2D NOESY spectra of the exchangeable proton region for the T-G and CIU-G oligonucleotides are shown as Figures S4 and S5, respectively. The NOEs observed in Figures S4 and S5 between T3 or CIU3, respectively, and G10 imino protons are consistent with these mispairs being in a wobble base pair geometry. The resonance assignments are provided in Table S3. The N3 imino proton of T3 of the T-G oligonucleotide was observed at 11.75 ppm and for CIU3 of the CIU-G oligonucleotide at 12.25 ppm. Other than chemical shift differences between protons in the T-G and CIU-G mispairs, all chemical shifts in the other base pairs were the same (± 0.02 ppm).

The imino protons of the CIU-G mispair broaden more rapidly with increasing temperature than the corresponding imino protons of the T-G mispair as shown in Figure 5. The 1D spectra for the data plotted in Figure 5 are shown as Figures S6 and S7. The magnitude of the imino proton-water crosspeaks in the 2D NOESY spectra for the CIU-G mispair are substantially greater than those for the T-G mispair (Figure S8). Potential base pair configurations are shown in Figure 6 and are discussed below.

With increasing solution pH, the imino protons of the mispaired T-G and CIU-G bases broaden rapidly and are lost from the spectrum at pH 9. (Figure S9). The chemical shifts of

nonexchangeable protons of bases above and below the mismatched CIU-G base pair move as a function of solution pH (Figure 7 and Figure S11). The apparent midpoint of the pH-dependent transition for the CIU-G mispair is approximately 8.75. As the pH increases, the G4 imino proton resonance shifts upfield from 12.95 to 12.45 ppm as the CIU-G base pair approaches pseudo Watson-Crick geometry. A cross peak is observed in the 2D NOESY at 12.45 and 12.95 for the G4 imino proton due to chemical exchange (Figure S10). No pH dependent transition is observed for the T-G mispair prior to alkaline denaturation of the helix. The observed melting temperatures of the CIU-A and CIU-G duplexes as a function of solution pH are shown in Figure 8. No apparent change in helix thermal stability is observed in the region of the apparent pK_a of the CIU-G mispair.

Discussion

DNA can be damaged by reactive molecules derived from activated neutrophils and eosinophils at sites of inflammation forming CIU and BrU, respectively (1–5,30). Oxidized and halogenated pyrimidines, including CIU are among the multiple damage products (Figure 1a). DNA damage products derived from reactive oxygen and halogen species might provide a mechanistic link between inflammation and human diseases including cancer (6).

The 5-halogenated analogs of 2'-deoxyuridine can be metabolized to the triphosphate derivatives and incorporated into polynucleotides by polymerases. The FU analogs also serve as potent chemotherapy agents, in part due to their capacity to inhibit the synthesis of the normal DNA precursor, TTP (27,28). While the halouracils are best known as synthetic analogs (31,32) useful in biochemical and pharmacological studies, CIU derivatives, and to a lesser extent BrU derivatives could be formed endogenously in the tissue of higher organisms. Derivatives of CIU can be incorporated into the DNA of replicating cells (8–11). Furthermore, CIU has been shown to induce cellular toxicity, cause senescence, and increase the frequency of sister-chromatid exchanges (33–36). It has been proposed that the toxicity might, in part, be attributed to interference with nucleotide metabolism, which promotes dUTP misincorporation and repair by a mechanism similar to that proposed for FU (36). However, the mechanism of sister chromatid exchange remains to be resolved.

Recently, we have incorporated CIU into synthetic oligonucleotides to determine its base-pairing configuration when paired opposite adenine and to examine the impact of CIU-A substitution on the stability of an oligonucleotide duplex (12). We observed that oligonucleotides containing CIU-A were slightly more stable than the control oligonucleotides containing a T-A base pair. The CIU-A base pair is predominantly in a geometry that approximates a Watson-Crick base pair and the duplex containing the CIU-A base pairs was B-form.

In this study, we examined the stability and base pair configuration of a duplex containing a CIU-G mispair (Figure 2). The data obtained was compared with a corresponding duplex containing a T-G mispair. The CIU-G duplex has the same thermal stability, within experimental error, as the corresponding T-G duplex. Both mispairs are substantially less stable than the CIU-A duplex (12). Melting temperatures and thermodynamic parameters are recorded in Table 1 and representative melting curves are shown in Figure 2.

Proton NMR spectra were recorded for the CIU-G and T-G containing duplexes under the same experimental conditions of temperature and neutral pH. The downfield shift of the CIU3 H6 proton relative to the T3 H6 proton by 0.53 ppm is attributed to the opposing inductive properties of the 5-methyl and 5-chloro substituents. A shift of similar magnitude (0.52 ppm) was observed for the corresponding T-A and CIU-A duplexes (12). The nonexchangeable proton connectivities indicate that all bases are intrahelical. The observed

chemical shifts of the H6 protons of T3 and CIU3 indicate that the pyrimidines of both the T-G and CIU-G mispairs are predominantly in their neutral, keto tautomeric forms at neutral pH (48).

Exchangeable proton resonances were observed for each base pair in both the T-G and CIU-G duplexes (Figure 4). Two imino proton resonances are observed for each mispair, shifted upfield from the normal imino proton region as is characteristic of wobble mispairs (49–51). The exchangeable and nonexchangeable proton NMR data indicate that the bases of both the CIU-G mispair and the T-G mispair are paired with one another in a wobble configuration as shown in Figure 6a.

With increasing temperature, the exchangeable resonances of the CIU-G mispair broaden rapidly and are lost from the spectrum prior to thermal denaturation of the duplex (Figure 5b and Figures S6 and S7). The rapid broadening of the CIU-G imino protons distinguished the CIU-G and T-G mispairs in that the imino protons of the T-G mispair broaden only as the remainder of the helix is thermally denatured (Figure 5a). The magnitude of the crosspeaks in our NOESY spectra between the CIU-G imino proton resonances and water are 3–4 times larger than those of the T-G mispair at equal mixing time suggesting that the enhanced broadening of the CIU-G imino protons is by exchange with water (Figure S8). The CIU base of the CIU-G mispair (Figure 6A) could move, allowing the CIU imino proton to be exposed to solvent (Figure 6B). The acidic CIU N3 proton could then exchange with water by ionization of the CIU residue (Figure 6C). The ionized CIU residue could then regain a proton and return to the neutral wobble (Figure 6B to 6A) or remain ionized and form two hydrogen bonds with G (Figure 6D).

A distinguishing feature of CIU, relative to T, is that the 5-chloro substituent of CIU is electron-withdrawing whereas the T methyl group is electron donating. The electronic-inductive property of a 5-substituted uracil analog significantly impacts the pK_a of the N3 proton. The pK_a of CldU is reported to be 7.9 whereas the pK_a of the corresponding thymidine N3 proton is 9.7 (19,21). Previously, both the FU-G (13) and BrU-G (14) mispairs were shown to undergo a pH-dependent structural transition. The pK_a of FdU is reported to be 7.7 (19), whereas the pK_a when paired with G is 8.3 (13). Similarly, the pK_a of BrdU is 8.0 (19) and the pK_a of BrU when mispaired with G is 8.6 (14). We therefore examined the CIU-G duplex as a function of solution pH. As shown in Figure 7 and Figure S11, multiple protons within the duplex undergo pH-dependent changes in chemical shift. The H6 proton of the CIU residue begins to shift upfield, broaden, and is lost from the spectrum by pH 7.9 as it moves beneath other nonexchangeable resonances (Figure S11).

The pK_a of CldU is 7.9 (19) whereas the pK_a in the CIU-G mispair observed here shifted to 8.7. The increase in the apparent pK_a for CIU when paired opposite G is similar in magnitude for the shifts in pK_a seen for FU-G and BrU-G discussed above, and would correspond to a difference of approximately 1.0 kcal/mol at pH 7.4 and 25 °C (18). The thermal stability of the CIU-A and CIU-G duplexes was examined as a function of solution pH (Figure 8). The observed T_m for each duplex drops with increasing solution pH, as would be expected for alkaline-induced denaturation. No unusual pH-dependent change in duplex stability is observed in the region of pH 8.7 for the CIU-G duplex, indicating that the ionized configuration of the CIU-G duplex (pK_a 8.7) does not destabilize the duplex.

Upon the basis of the pH-dependent change from neutral to wobble to ionized pseudo-Watson-Crick configuration, we would anticipate that the base-pairing properties of CIU when in DNA would be pH dependent, as has been demonstrated for the FU and BrU bases (16,18,20). As the pH increases, we would anticipate more mispair formation. Several recent studies have indicated that base pair geometry is one of the primary properties that govern

base selection by DNA polymerase (52–54). Increased miscoding with increasing pH has been observed for the FU and BrU bases, as well as the thymine oxidation damage product, 5-formyluracil (55). The increased miscoding potential is attributed to an electron-withdrawing substituent in the 5-position (20).

The capacity of a base analog to induce mutation in a replicating cell would increase with its capacity to miscode, but decrease with increasing efficiency of repair. In *in vitro* systems, both FU and BrU have similar miscoding potential; however, BrU is a known mutagenic base analog whereas FU is considered to be only weakly or non-mutagenic. Unlike BrU, FU is repaired by several members of the uracil glycosylase family (21–26). The efficiency of FU repair is attributed to the small size of the 5-substituent and the electron-withdrawing property of the 5-substituent (21–26). Although both bromine and fluorine substituents are electron-withdrawing and induce similar changes in the pK_a values for the corresponding 5-substituted uracil derivatives, the larger size of the Br substituent prevents its removal by the major uracil glycosylase, UNG.

The repair of CIU is more similar to BrU than to FU. The CIU analog is repaired by SMUG1 and TDG, but not UNG. The efficiency of CIU repair is substantially greater when the CIU is mispaired with G than when paired with A (12). The difference in the repair of CIU-A versus CIU-G has been attributed to the reduced stability of the CIU-G mispair. The reduced stability of damaged or mispaired bases appears to be a common recognition mechanism for glycosylases (21–26).

In accord with our previous results with the CIU-A base pair (12), CIU incorporated as a dNTP analog would be expected to pair predominantly with template A. As a stable base resistant to glycosylase removal (12), CIU-A could be a persistent DNA lesion. When templates containing CIU are replicated, it would potentially miscode with G at a higher frequency than T due to its increased propensity to form an ionized base pair in pseudo-Watson-Crick geometry, in accord with previous studies with BrU, FU and 5-formyluracil (16,55). Once formed, however, the CIU of a CIU-G mispair would be a target for repair by members of the uracil glycosylase family (12). The CIU of the mispair would be targeted for glycosylase removal, leaving the incorrect G in the opposing strand to serve as the template for repair synthesis. Upon the basis of these properties, the formation of CIU in the nucleotide pool could prove highly mutagenic.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

HOCl	hypochlorous acid
CIC	5- chlorocytosine
CIU	5-chlorouracil
CldU	5-chloro-2'-deoxyuridine
DMT	dimethoxytrityl
GC/MS	gas chromatography/mass spectrometry
MALDI-TOF-MS	matrix-assisted laser desorption ionization-time of flight mass spectrometry

DSS	4,4-dimethyl-4-silapentane-1-sulphonate
T_m	melting temperature
EDTA	ethylenediaminetetraacetic acid
NOE	nuclear overhauser effect
FU	5-fluorouracil
BrU	5-bromouracil
TTP	thymidine triphosphate
dUTP	2'- deoxyuridine-5'-triphosphate
UNG	uracil – DNA glycosylase
SMUG1	single strand selective monofunctional uracil – DNA glycosylase
TDG	thymine – DNA glycosylase
T	Thymine

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Figure 1.

(a) Pathway for HOCl mediated conversion of C-G to CIU-G mispair, A. Normal C-G base pair. B. Dihydrocytosine intermediate formed from the addition of HOCl to cytosine. C. Conversion of dihydrocytosine to dihydrouracil intermediate. D. The CIU-G mispair formed by dehydration. (b) The 12mer oligonucleotide used for the study, X= 5-chlorouracil.

Figure 2. Oligonucleotide melting profiles examined by temperature dependent absorbance at 260 nm. UV melting profiles (normalized) of 12mer oligonucleotide at 28 μ M concentration in a buffer containing 100 mM sodium chloride, 10 mM sodium phosphate, 0.1 mM EDTA at pH 7.0. T-G (solid line), CIU-G (dashed line) and CIU-A (dotted line, from reference 12).

Figure 3.

¹H NMR spectra of the T-G (top) and CIU-G (bottom) oligonucleotide in the aromatic region. The H6/H8 and H2 assignments are written in the top spectrum. CIU3 of CIU-G oligo is indicated by the arrow mark in the bottom panel. The spectra were taken at 27 °C with 256 scans using a water suppression WATERGATE program with binomial water suppression delay (d19) of 160 μ sec. The oligonucleotide was dissolved in a buffer containing 10% D₂O, 100 mM sodium chloride, 10 mM sodium phosphate, 0.2 mM EDTA at pH 7.

Figure 4.

¹H NMR spectrum of the T-G (top) and CIU-G (bottom) oligonucleotides in the imino region. The spectra were taken at 5 °C with 256 scans using a water suppression WATERGATE program with binomial water suppression delay (d19) of 40 μsec. The oligonucleotide was dissolved in a buffer containing 10% D₂O, 100 mM sodium chloride, 10 mM sodium phosphate, 0.2 mM EDTA at pH 6.

Figure 5.

Line widths of imino protons as a function of temperature at pH 6. (a) T-G oligo (b) CIU-G oligo. The spectra were taken with 256 scans using a water suppression WATERGATE program with binomial water suppression delay (d19) of 40 μ sec. The oligonucleotide was dissolved in a buffer containing 10% D₂O, 100 mM sodium chloride, 10 mM sodium phosphate, 0.2 mM EDTA.

Figure 6.
Possible base pairing configurations of the ClU-G mispair.

Figure 7.

^1H NMR chemical shift study of CIU-G oligo as a function of pH at room temperature. pH dependent chemical shift of (a) H8 protons of G4 and G10 and (b) H6 proton of C9 and C11. The oligonucleotide was dissolved in a buffer containing 10% D_2O , 100 mM sodium chloride, 10 mM sodium phosphate, 0.2 mM EDTA.

Figure 8.

T_m of CIU-G and CIU-A oligo at different pH values. The values are obtained at a total strand concentration of 6.0 μM and a buffer containing 100 mM sodium chloride, 10 mM sodium phosphate, 0.1 mM ethylenediamine tetraacetic acid.

Table 1

T_m and thermodynamic parameters of the oligonucleotide, 5'-d[CGXGAATTCYCG]-3' (X= pyrimidine, Y= purine) at 10 mM sodium phosphate, 100 mM sodium chloride, 0.1 mM EDTA, pH 7.0. The values for CIU-A are from reference 12.

Oligo	T_m @ 28 μ M (°C)	ΔH° (kcal/mol)	ΔS° (cal/mol/k)	ΔG° (25 °C) (kcal/mol)
T-G	42.2 $\pm$ 0.2	-90.0 $\pm$ 3.7	-264 $\pm$ 12	-9.7 $\pm$ 0.2
CIU-G	41.8 $\pm$ 0.2	-86.7 $\pm$ 3.8	-254 $\pm$ 12	-9.5 $\pm$ 0.2
CIU-A	55.6 $\pm$ 0.3	-81 $\pm$ 14	-234 $\pm$ 4	-12.2 $\pm$ 1.0