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# Dynamics of Uracil and 5-Fluorouracil in DNA†

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ABSTRACT: The prodrug 5-fluorouracil (5-FU), after activation into 5-F-dUMP, is an extensively used anticancer agent that inhibits thymidylate synthase and leads to increases in dUTP and 5-F-dUTP levels in cells. One mechanism for 5-FU action involves DNA polymerase mediated incorporation of dUTP and 5-F-dUTP into genomic DNA leading to U/A, 5-FU/A, or 5-FU/G base pairs. These uracil-containing lesions are recognized and excised by several human uracil excision repair glycosylases (hUNG2, hSMUG2, and hTDG) leading to toxic abasic sites in DNA that may precipitate cell death. Each of these enzymes uses an extrahelical base recognition mechanism, and previous studies with UNG have shown that extrahelical recognition is facilitated by destabilized base pairs possessing kinetically enhanced base pair opening rates. Thus, the dynamic properties of base pairs containing 5-FU and U are an important unknown in understanding the role of these enzymes in damage recognition and prodrug activation. The pH dependence of the $^{19}$F NMR chemical shift of 5-FU imbedded in a model trinucleotide was used to obtain a p$K_a$ = 8.1 for its imino proton (10 °C). This is about 1.5 units lower than the imino protons of uracil or thymine and indicates that at neutral pH 5-FU exists significantly as an ionized tautomer that can mispair with guanine during DNA replication. NMR imino proton exchange measurements show that U/A and 5-FU/A base pairs open with rate constants ($k_{op}$) that are 6- and 13-fold faster than a T/A base pair in the same sequence context. In contrast, these same base pairs have apparent opening equilibrium constants ($\alpha K_{op}$) that differ by less than a factor of 2, indicating that the closing rates ($k_{cl}$) are enhanced by nearly equal amounts as $k_{op}$. These dynamic measurements are consistent with the previously proposed kinetic trapping model for extrahelical recognition by UNG. In this model, the enhanced intrinsic opening rates of destabilized base pairs allow the bound glycosylase to sample dynamic extrahelical excursions of thymidine and uracil bases as the first step in recognition.

The antimetabolite 5-fluorouracil (5-FU)[1] has been in clinical use since the late 1950s for the treatment of colorectal, breast, and head and neck cancers (*1*, *2*). After facilitated transport inside the cell (*3*), 5-FU is metabolized much like1 uracil and can be enzymatically converted to the active metabolite 5-FdUMP which potently inhibits thymidylate synthase (TS), blocking *de novo* production of dTMP and leading to accumulation of dUMP. One outcome of TS inhibition by 5-F-dUMP is the direct misincorporation of 5-F-dUMP and dUMP into cellular DNA (*2*, *4*−*8*). Although DNA polymerases readily utilize both dUTP and 5-F-dUTP as substrates, incorporation of these nucleotides during 5-FU treatment is more probable when the dTTP pool is depleted. Subsequent excision of genomic U and 5-FU is executed by the base excision repair or mismatch repair systems (*9*, *10*), but repair is ineffective because repair polymerases continue to reincorporate dUTP and 5-F-dUTP in a futile repair cycle (*1*, *2*, *11*). If substantial levels of U or 5-FU are incorporated and excised through a glycosylase-mediated excision mechanism, this will lead to the generation of abasic sites in DNA that are the substrate for a potent abasic site endonuclease (APE1) that introduces toxic DNA strand breaks. In addition to these DNA-centric cytotoxicity mechanisms, the insertion of 5-FU into RNA by RNA polymerase has been shown to exert a distinct toxic effect by disrupting diverse aspects of RNA metabolism. These disruptions include pre-rRNA processing, tRNA posttranslation modifications, and pre-mRNA splicing (*1*).

The involvement of DNA glycosylases in the removal of U and 5-FU from DNA raises the interesting question of whether these bases have special properties that allow for their enhanced detection in a large genome that is abundantly populated with structurally similar thymine bases. Previous NMR and crystallography studies with UNG have established that this enzyme extrahelically inspects thymine bases in T/A base pairs using a T- and U-specific exosite located on the enzyme surface that is distinct from the active site (*12*). This site serves as a transient docking point to trap these bases as they emerge spontaneously from the DNA base stack due to stochastic thermal fluctuations. We previously suggested that the intrinsic dynamic properties of T/A base pairs, as compared to more stable C/G base pairs, are utilized by UNG to selectively inspect T/A base pairs for the presence of U (*12*). A key feature of this mechanism is the kinetic

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[1]Abbreviations: SMUG1, single-strand selective monofunctional uracil DNA glycosylase; UNG2, nuclear uracil DNA glycosylase; TDG, thymine DNA glycosylase; dUTP, deoxyuridine triphosphate; 5-F-dUTP, 5-fluorodeoxyuridine triphosphate; dTTP, deoxythymidine triphosphate; 5-FU, 5-fluorouracil base; DFEA, difluoroethylamine base catalyst; U, uracil.

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propensity of thymine to emerge from the DNA duplex and obtain an extrahelical conformation that is recognized by the enzyme. Accordingly, the relative dynamic properties of U/A and 5-FU/A base pairs are anticipated to be important determinants of their enzymatic recognition. Here we explore the dynamic properties of U and 5-FU in DNA using NMR imino proton exchange methods and show that 5-FU/A and U/A base pairs are much more dynamic than T/A base pairs, making these lesions especially amenable for extrahelical recognition by hUNG2 and perhaps other uracil glycosylases.

## EXPERIMENTAL PROCEDURES

*DNA Substrates*. The palindromic 10-mer oligonucleotides used for imino exchange studies (5′-CTGGAXCCAG-3′, where X = T, U, 5-FU) and the 3-mer oligonucleotide (5′-AFC-3′, where F = 5-FU) used for the pH titration of 5-FU were synthesized on an Applied Biosystems 394 DNA synthesizer using standard phosphoramidites and supports obtained from Glen Research. All oligonucleotides were purified using a DNA Pac100 anion-exchange column (Dionex) and desalted using a $C_{18}$ reversed-phase column (Phenomenex) with acetonitrile elution. Oligonucleotide masses were confirmed by MALDI-TOF spectrometry, and purity was analyzed using 7 M urea and 19% PAGE. DNA duplexes (Table 1) were hybridized by heating to 90 °C and slow cooling to room temperature. Duplex formation was analyzed via 19% native PAGE and ethidium bromide visualization using a Typhoon (GE Healthcare).

*Determination of the Imino Proton p$K_a$ of 5-FU*. NMR spectra were acquired on a Varian Inova spectrometer tuned to $^{19}$F and operating at 470 MHz. The sample contained 1 mM 5′-AFC-3′ oligonucleotide (F = 5-FU), 10 mM $NaH_2PO_4$, 10 mM NaCl, and 10% $D_2O$. The chemical shift of the fluorine substituent of 5-FU was measured using a 90° pulse and recovery while adjusting pH via addition of NaOH. pH was measured, data were acquired at 10 °C, and the chemical shift was referenced to that of trifluoroacetic acid set to 0 ppm. Data were analyzed using Prism software, and the p$K_a$ was calculated by fitting the data to eq 1. In this equation, $\delta_{pH}$ is the chemical shift of the fluorine at the respective pH, and $\delta_{A-}$ and $\delta_{HA}$ are the fluorine chemical shifts of the unprotonated and protonated forms of 5-FU, respectively.

$$\delta_{pH} = \delta_{HA} + \left[\frac{\delta_{A-} - \delta_{HA}}{1 + 10^{\log(pK_a - pH)}}\right] \quad (1)$$

*Imino Proton Exchange Measurements*. NMR measurements were carried out at 10 °C using a 600 MHz Varian Inova spectrometer equipped with a 5 mm triple resonance probe. Samples were made up of 1 mM palindromic DNA duplex (5′CTGGAXCCAG3′, where X = T, U, 5-FU) in buffer containing 1 mM Tris-$d_{11}$-HCl, pH 8.0, 35 mM NaCl, and 10% $D_2O$. Imino proton exchange rates were measured using magnetization transfer from water as previously described (*13*). Data were processed and analyzed by line shape analysis using an in-house MatLab script and then fit to eq 2, where $I_Z(t_{mix})$ and $I_{Z,eq}$ are intensities of the imino proton peaks at a given value of $t_{mix}$ and at equilibrium, respectively, $k_{ex}$ is the chemical exchange rate, $R_{lw}$ is the longitudinal relaxation rate of water, $R_{li}$ is the sum of the imino proton longitudinal relaxation rate and $k_{ex}$, and $E$ is the efficiency of water inversion (value of −2 for 100% inversion efficiency).

Table 1: Rate and Equilibrium Constants for DNA Base Opening[a]

| kinetic parameter | T/A[b] | U/A | 5-FU/A |
|---|---|---|---|
| $k_{ex}$ ($s^{-1}$) | 0.63 ± 0.02 | 6.3 ± 0.1 | 126 ± 8 |
| $k_{int} \times 10^{-6}$ ($s^{-1}$) | 0.02 ± 0.03 | 0.4 ± 0.2 | 14 ± 3 |
| $k_{op}$ ($s^{-1}$) | 35 ± 6 | 200 ± 25 | 440 ± 20 |
| $k_{cl} \times 10^{-6}$ ($s^{-1}$) | 1.8 ± 0.3 | 7 ± 3 | 37 ± 7 |
| $\alpha K_{op} \times 10^6$ | 20 ± 4 | 27 ± 11 | 12 ± 2 |

[a]Abbreviations: 5-FU, 5-fluorouracil; U, uracil; A, adenine; T, thymine. [b]Kinetic parameters for the T·A base pair were previously reported (*13*) .

$$\frac{I_Z(t_{mix})}{I_{Z,eq}} = 1 + Ek_{ex}\left[\frac{\exp(-R_{li}t_{mix}) - \exp(-R_{lw}t_{mix})}{R_{lw} - R_{li}}\right] \quad (2)$$

To determine the rate of base opening ($k_{op}$), imino exchange rates were measured in the presence of the general base catalyst difluoroethylamine (DFEA, p$K_a$ = 7.6, 10 °C) (*13*). Exchange rates of 5-FU in the presence of catalyst were too fast for measurement by magnetization transfer and were determined by monitoring line broadening where the exchange contribution to the full line width at half-height ($\Delta$) is given by $k_{ex} = \pi\Delta$. Exchange rates were plotted against the concentration of the neutral basic form of DFEA, and the data were fit to eq 3. In this equation, $k_{cl}$ is the rate of base closing, $k_{int}$ is the rate of intramolecular catalysis, [B] is the concentration of DFEA, and $k_b$ is the second-order rate constant for exchange catalysis as calculated from eq 4.

$$k_{ex} = \frac{k_{op}(k_B[B] + k_{int})\alpha}{\alpha(k_B[B] + k_{int}) + k_{cl}} \quad (3)$$

$$k_B = \frac{k_D}{1 + 10^{pK_a^{Nu} - pK_a^{B}}} \quad (4)$$

In eq 4, $k_D$ is the bimolecular collision rate ($1.5 \times 10^9\ M^{-1}\ s^{-1}$ (*14*)) and $pK_a^{Nu}$ and $pK_a^{B}$ are the p$K_a$ values for the imino proton of interest and DFEA base catalyst, respectively. The p$K_a$ values for DFEA (p$K_a$ = 7.6) and uridine (p$K_a$ = 9.5) at 10 °C were previously reported (*13*, *14*). The value $\alpha$ is a constant that takes into account the accessibility of the imino proton in the open state to catalyst as compared to the free nucleoside and is likely not significantly different than unity (*14*).

## RESULTS

DNA glycosylases, without exception, use an extrahelical recognition mechanism to bind and excise damaged bases from DNA (*15*). Previous studies with UNG have highlighted the importance of DNA base pair stability and dynamics in the process of detecting and excising uracil from DNA (*12*, *13*, *16*−*19*). In general, bases that are located in less stable base pairs have a propensity to assume extrahelical conformations outside of the DNA duplex more often than more stable normal base pairs, providing a handle for recognition that can mark a site as damaged to an enzyme. Accordingly, we began the present studies of 5-FU recognition by examining its dynamic properties in duplex DNA using NMR imino proton exchange methods (*13*).

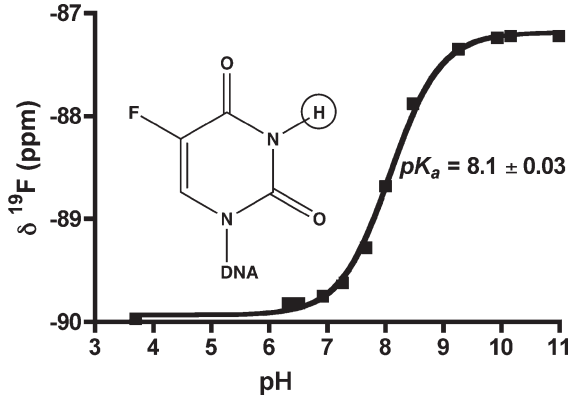


FIGURE 1: Determination of the p$K_a$ of the imino proton of 5-FU in ssDNA at 10 °C. 5-FU in DNA is displayed with the imino proton circled. The chemical shift ($\delta$) of the C-5 fluorine of 5-FU residing within a short oligonucleotide (5′-AFC-3′) was monitored via 1D $^{19}$F NMR while titrating pH. p$K_a$ was determined by fitting the data to eq 1 (Experimental Procedures).

*p$K_a$ of 5-FU in DNA*. Solvent magnetization transfer NMR methods may be used to monitor DNA base pair dynamics by measuring the exchange rate of imino protons with magnetically labeled solvent protons (*20*). The kinetics of the entire exchange process will depend on the lifetime of the open state of a base pair, which exposes the imino proton to solvent, and the efficiency of imino proton extraction by solvent or a solute proton acceptor (water, hydroxide ion, or a general base). Since the efficiency of imino proton extraction is dependent on the base strength of the solute proton acceptor relative to the acidity of the imino proton (eq 4), it is imperative to know the p$K_a$ values for the 5-FU base as well as the solute proton acceptors under the conditions of the experiment. To measure the imino proton p$K_a$, a short 3-mer DNA oligonucleotide was synthesized with 5-FU as the central base (5′-ApFpC-3′). $^{19}$F NMR was then used to monitor the protonation state by following the 5-$^{19}$F chemical shift as a function of pH (Figure 1). Using this approach, a p$K_a$ value of 8.10 ± 0.03 was determined from a nonlinear regression fit to eq 1, which is about 1.5 log units lower than T and U (*14*). Because p$K_a$ values for imino protons are known to decrease with increasing temperature (*21*), this value determined at 10 °C is consistent with the previously reported p$K_a$ of 7.68 at 25 °C (*22*). A further implication of this temperature-dependent p$K_a$ is that at physiological temperature and pH 5-FU will be ionized and in a tautomeric form that can pair with guanine in DNA.

*Imino Proton Exchange*. Imino proton exchange measurements were used to analyze the dynamics of a 5-FU/A base pair compared to U/A and T/A base pairs. We have previously determined the dynamic properties of a T/A base pair within a palindromic 10-mer oligonucleotide (*13*) (Figure 2A, X = T), and accordingly, we chose the same sequence and experimental conditions in this study to allow direct comparisons with the previous data. The imino proton resonances for the $T_6$ duplex have been previously assigned (*13*) (Figure 2A), allowing assignments for the $U_6$ and $F_6$ duplexes to be made by visual comparison (panels B and C of Figure 2, respectively). Both the $U_6$ and $F_6$ substitutions produced downfield chemical shift changes as compared to the imino proton shift of $T_6$ (0.1 and 0.7 ppm, respectively), indicating a relative decrease in the electron densities for the pyrimidine rings of U and 5-FU. In addition, significant line broadening was observed for the 5-FU resonance (Figure 2C).

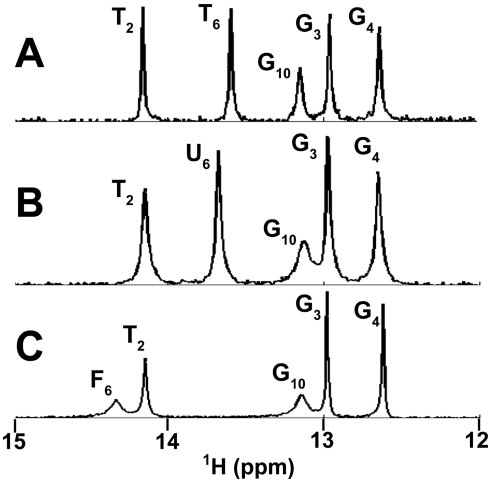


FIGURE 2: NMR spectra of the imino proton regions of T/A (A), U/A (B), and 5-FU/A (C) palindromic duplexes.

Imino proton exchange rates were then measured using magnetization transfer from water in the absence of DFEA exchange catalyst (Figure 3A). It was immediately apparent that the exchange rate of 5-FU was much greater than that of U or T, which may be easily discerned by comparing the initial rates for the decreases in imino proton intensities during the exchange time courses (Figure 3A) (i.e., before longitudinal relaxation of water returns magnetization to its equilibrium state).

To extract the rate of base opening ($k_{op}$), closing ($k_{cl}$), and the apparent opening equilibrium constant ($\alpha K_{op}$) from the observed exchange rate (eq 3), the concentration of the DFEA exchange catalyst was varied (Figure 3B). As DFEA concentration was increased, the value of $k_{ex}$ increased until a plateau was reached (Figure 3C), indicating a change in rate-limiting step from chemical exchange ($k_{ex} = \alpha K_{op} k_B[B]$) to base pair opening ($k_{ex} = k_{op}$). The kinetic parameters for exchange were determined by nonlinear regression fitting of the data to eq 3, and are reported in Table 1. Although 5-FU/A, U/A, and T/A all possess similar base pair opening equilibrium constants ($\alpha K_{op}$), the dynamics of these base pairs are markedly different. As compared to T/A (Figure 3D), U/A and 5-FU/A base pairs open ~6 and 13 times faster and have open lifetimes ($1/k_{cl}$) that are 4- and 20-fold shorter.

## DISCUSSION

*Dynamic Properties of 5-FU in DNA*. The origin of enzymatic specificity for damaged bases in DNA is a key question in DNA base excision repair. Although specificity may be partially encoded in the unique architecture of an enzyme that is perfectly matched to its cognate damaged base, another important aspect of specificity can be found in the different dynamic properties of damaged and normal base pairs (*15*). Although many DNA glycosylases have been suggested to rely on the thermodynamic instability of damaged base pairs as a recognition mechanism, the most extensively studied example is the role of spontaneous base pair opening dynamics in the damage search process of UNG (*12*, *13*). This work has established how this enzyme uses an exosite binding pocket that is specific for thymine and uracil to rapidly interrogate T/A and U/A base pairs for the presence of uracil. Since G/C base pairs are much less dynamic and the enzyme does not possess an exosite binding pocket that is complementary to G, C, or A, the enzyme

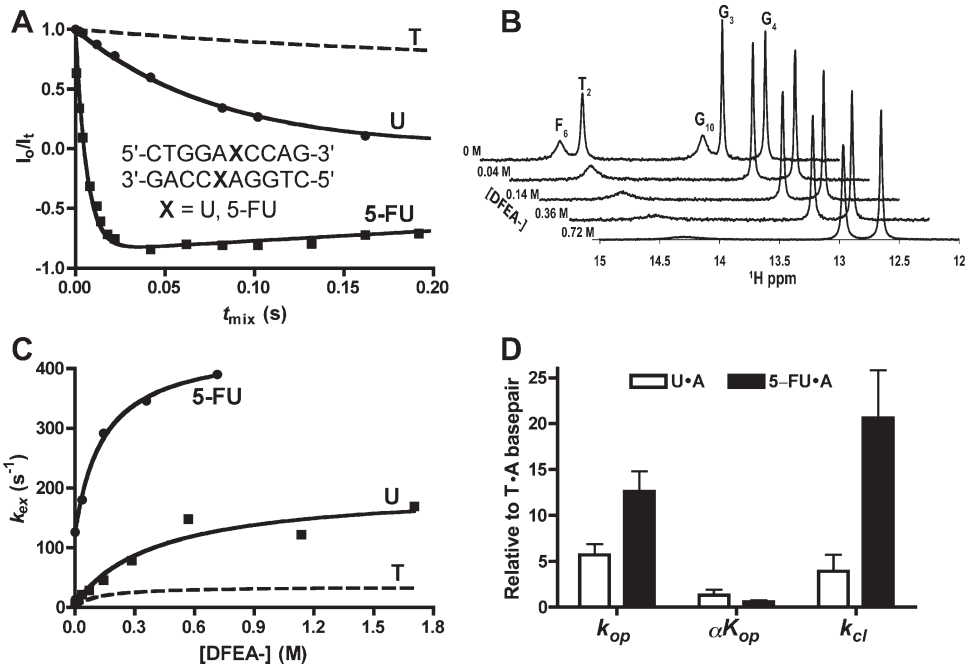


FIGURE 3: Imino exchange profiles and comparative kinetic analysis of U/A, 5-FU/A, and T/A base pairs. Data for the T/A base pair was simulated from previously determined parameters (*13*). (A) Imino proton exchange profiles of U/A, 5-FU/A, and T/A duplexes. The initial decrease in intensity reflects the exchange of an inverted water proton with an imino proton from an open base pair. Exchange rates were determined by fitting the data to eq 2 (Experimental Procedures). (B) Line broadening of imino exchange resonances upon addition of DFEA, general base catalyst. Broadening of imino proton resonances was correlated to an increase in the exchange rate as determined by $k_{ex} = \pi\Delta$, where $\Delta$ is the line width at half peak height. (C) Imino proton exchange catalysis upon addition of the general base catalyst DFEA. Addition of DFEA changes the rate-limiting step of imino proton exchange from base-catalyzed proton extraction to spontaneous opening of the base pair. (D) Comparison of the base pair dynamics of U/A and 5-FU/A relative to T/A.

does not spend time unproductively interrogating normal base pairs that are irrelevant to the search for uracil. This is a well-documented example of how the dynamic properties of base pairs can contribute to both specificity and efficiency of the damage search.

The increased dynamic properties of U/A and 5-FU/A relative to a T/A base pair provide a plausible dynamic handle for recognition of U and 5-FU by UNG and perhaps other glycosylases that act on these lesions (see DOI 10.1021/bi102046h). Upon landing in register with a base pair containing U or 5-FU, UNG could sample about 10 times more extrahelical excursions as compared to the normal base T based on the differences in the measured opening rates (Table 1). These excursions provide kinetically competent motions that move these bases rapidly onto the base flipping reaction coordinate (*15*). This mode of recognition is driven by dynamics ($k_{op}$), rather than thermodynamics ($\alpha K_{op}$), because the base pair opening equilibrium constants for T, 5-FU, and U are similar (Table 1), and duplexes containing T or 5-FU have the similar melting temperatures for (*23*). Given the isostructural nature of these three base pairs and their preservation of B DNA helical structure (*23*), the increased dynamics may be the most significant distinguishing feature that initiates their detection by glycosylase enzymes.

We note that the unfavorable equilibrium constants for spontaneous base pair opening yield an equilibrium population of T, U, and 5-FU in the extrahelical state of about 1 in $10^5$ (Table 1). It is not physically reasonable that UNG (or any enzyme) could come in contact with an already extrahelical base because its concentration is so low, and its extrahelical lifetime is so short ($1/k_{cl} \sim 50$ ns). Thus, even with diffusion-controlled rates of association, such as observed with UNG2114 4819 (*17*), there is not enough time for random diffusion to efficiently bring the enzyme and DNA together. (See Cao et al. (*13*) for a complete discussion of this aspect of recognition.) Accordingly, trapping of spontaneously emerging extrahelical bases requires that an enzyme be preassociated at the site, thereby allowing it to sample opening events that occur during its lifetime on the site (*15*). Such a mechanism is greatly facilitated by short-range sliding of the enzyme along the duplex because this pathway bypasses the kinetic limitations of 3D diffusion (*19*).

*Chemical Properties of 5-Substituted Uracils.* What are the chemical features of U/A and 5-FU/A base pairs that lead to their observed dynamic behavior? Both U and 5-FU have imino proton p$K_a$ values (9.5 and 8.1) that are reduced as compared to thymine (9.9), which may be reasonably attributed to the greater electron-donating property of the 5-methyl group as compared to a 5-H and 5-F substituent (p$K_a$ values are for 10 °C)(*24*). The reduced imino p$K_a$ values of U and 5-FU would be expected to strengthen the hydrogen bond between N3H and adenine N1 by reducing the p$K_a$ difference between this acid−base pair (*25*) but would also weaken the hydrogen bonding between the O4 carbonyl and the adenine 6-amino group by reducing the electron density on O4. It is therefore conceivable that the opposing effect of the electron-withdrawing group on the hydrogen bond donor and acceptor may largely cancel out energetically. Layered on these hydrogen-bonding effects are the effects of the 5-substituents on stacking interactions with adjacent bases in the DNA duplex. In this regard, electron-withdrawing groups would be expected to diminish the $\pi$ electron density of the pyrimidine ring, promoting base pair destabilization. The increased opening dynamics of both U and 5-FU would indicate that the destabilizing effects on stacking win out over the potentially stabilizing hydrogen-bonding effects.

An interesting and unexpected dynamic property of U and 5-FU base pairs is their decreased lifetime ($1/k_{cl}$) in the open state as compared to thymine (Table 1). Thus, these base pairs open more rapidly but also close more rapidly to an almost equal extent, leading to small differences in the opening equilibrium ($K_{op} = k_{op}/k_{cl}$). Since hydrogen bonds with the opposite adenine are not present in the open state, then differences in the hydrogen-bonding properties of these analogues cannot be reasonably attributed to the observed effects on closing. One plausible explanation is that the weaker stacking interactions of U and 5-FU lowers the activation energy for closing of these bases as compared to T. Accordingly, the ground state for imino exchange may occur in a partially stacked open state where the imino proton is exposed, but the base retains some electronic interactions with neighboring bases. The stronger stacking interactions of T would lead to a deeper free energy well and a larger activation barrier for its return into the base stack. The similar kinetic effects of the 5-substitutions on both base pair opening and closing also suggest that the weaker stacking interactions of U and 5-FU may be the primary factor that facilitates the reaction in both directions and that hydrogen-bonding differences may fortuitously cancel out (see Discussion above). Of course, other energetic effects such as differences in solvation, desolvation, or steric properties of these partially exposed bases could also contribute to the observed effects.

*Does Imino Exchange Result from Adenine, U, or 5-FU Opening?*. An intrinsic ambiguity exists in all imino proton exchange measurements. That is, does exchange result from movement of the purine or pyrimidine base in the pair? Computational work has suggested that the mechanism for exchange of the imino proton of thymine is preferentially initiated by breathing motions of the adenine base in the base pair (*26*). Since the most significant interaction that connects adenine to its partner base is the two Watson−Crick hydrogen bonds, then it would be expected that strengthening these hydrogen bonds by decreasing the p$K_a$ difference between the donor−acceptor groups would lead to decreased imino exchange. However, in the series studied here only increases in the opening and closing rates are observed, in the order T < U < 5-FU (Table 1), and such a trend cannot be easily attributed to interactions with the opposing adenine. The most sensible interpretation of this trend is that the 5-substituted uracil destabilizes the stacking interactions and increases the exchange contribution from the pyrimidine breathing pathway. This data set cannot assess the magnitude of the contribution from adenine breathing, but even a small change in the nature of the pyrimidine (T → U) brought about a 6-fold increase in the opening rate, suggesting that thymine breathing in the context of an A/T base pair also contributes significantly to imino exchange.

*Implications for DNA Repair Biology*. The low p$K_a$ of 5-FU indicates that a significant portion of 5-FU is ionized under physiological conditions, and therefore, this base exists in significant amounts as the ionized iminol tautomer (*22*, *27*). This tautomer is capable of forming an ionized base pair with an opposing guanine, and thus, a substantial population of 5-FU/G mispairs can arise in DNA from DNA polymerase catalyzed misincorporation. Studies performed with mismatch repair deficient HCT116 human colon cancer cells demonstrated that ~50% of the genomic 5-FU was incorporated opposite to guanine (*28*). The high propensity of 5-FU to pair with both adenine and guanine should therefore be considered when interpreting glycosylase-mediated excision of 5-FU (see DOI 10.1021/bi102046h). Of significance is fact that the toxic effects of 5-FU/G mismatches are precipitated by either the mismatch repair pathway (MMR) or the human thymine DNA glycosylase (hTDG).

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