Using Modified Nucleotides to Map the DNA Determinants of the Tus-TerB Complex, the Protein-DNA Interaction Associated with Termination of Replication in Escherichia coli*

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Laura J. Duggan, Thomas M. Hill‡, Su Wu§, Kristen Garrison, Xiaolin Zhang§, and Philip A. Gottlieb¶

From the Department of Chemistry and Biochemistry, University of Delaware, Newark, Delaware 19716 and the ¶Department of Bioscience and Biotechnology, Drexel University, Philadelphia, Pennsylvania 19104

A series of modified nucleotides was used to map the hydrogen-bonding and hydrophobic sites of the TerB DNA required for Tus interaction. Each of four consensuses guanine residues in the TerB-binding site was replaced by 7-deazaguanine, 2-aminopurine, or inosine nucleobase analogues, and each thymine by a uracil analogue. The observable equilibrium dissociation constant for the Tus protein-TerB DNA complex was measured at pH 7.5, 25 °C, and 150 mM potassium glutamate using a competition binding method. Substitutions made at position 10 with a 7-deazaguanine, 2-aminopurine, or inosine analogue had a large effect on the stability of the complex, approximately +3 kcal/mol in each case. Substitutions made at positions 13 and 17 had a varied response. For uracil substitutions, potential hydrophobic sites were identified at six positions in the TerB DNA. The energetic penalty for a removal of a single methyl group ranged between +1 and +2 kcal/mol. Rate dissociation measurements agree with these results. Overall, major and minor groove determinants are required for binding. An unusual result was that the conserved nucleotide at position 6 did not significantly affect in vitro binding of the complex.

In Escherichia coli, termination of replication is mediated by the specific interaction of a protein called Tus with a series of DNA sequences known as Ter. The Tus protein binds as a single subunit to its DNA-binding site (1, 2) and does not appear to have homology to any of the known DNA-binding motifs (3), although some similarities have been noted with some DNA- or RNA-binding proteins (4). A comparison of all Ter sites found on the chromosome and on plasmids revealed an 11-nucleotide sequence that is absolutely conserved for all sites (5). Despite the relatively small number of nucleotides that constitute the Ter-binding site, the observable binding constant for a 37-base pair fragment containing a Ter-binding site is 3.3 × 10^12 M^-1 in 150 mM potassium glutamate, pH 7.6. The measured dissociation rate for this site is 2.1 × 10^-5 s^-1, a half-life of 550 min (6).

The in vitro activity of the Tus-Ter complex impedes the progress of reconstituted replication forks on both the chromosome and the plasmids of E. coli (7, 8). This activity is orientation-dependent and is consistent with the asymmetric binding of this protein (2, 6). The action of this complex and its ability to impede replication forks are associated with antihelicase properties reported under some conditions (7, 10). The mechanism of action of this protein has not yet been elucidated, although two possibilities have been proposed (6). Either the protein-DNA complex produces a thermodynamically stable barrier to oncoming replication forks, or protein-protein interactions are required for stalling replication forks. Recently, evidence supporting the latter mechanism was reported (11, 12).

Modified nucleotides are an increasingly useful means of investigating interactions between proteins and DNA. Isosteric analogues inserted into the DNA report on the relative importance of specific groups required by the complex for replication and binding. In this work, we have used a series of modified nucleotides to analyze the Tus-TerB interaction. Two G analogues (2-aminopurine and 7-deazaguanine) were used to probe major groove sites, and a third analogue (inosine) was used to probe minor groove sites of G. A fourth analogue (uracil) allowed us to test the relative importance of methyl groups of T in the TerB binding sequence. From these results, we have mapped some of the chemical groups in the major and minor grooves of the TerB-binding site that are determinants for the interaction of Tus protein binding. Because we were able to measure the extent of the perturbation to the complex, our results show that the DNA major and minor grooves situated between nucleotides 8 and 19 are crucial for the interaction, although there are minor contributions from other nucleotides outside this region. Of particular interest is the need for six methyl groups that help stabilize the complex, presumably through van der Waals interactions. These measurements also demonstrate that analogue substitutions at position 6 do not appreciably affect the complex, and therefore, it is not essential for Tus binding in vitro.

MATERIALS AND METHODS

Tus Protein Preparation and Purification—The Tus protein was prepared and purified as described previously (8).

Nucleotide Analogues—Synthesis of the 2-aminopurine (2-AP)1 deoxyribonucleoside and 7-deazaguanine (7-deazaG) deoxyribonucleotide analogues and their conversion to the phosphoramidite were achieved by previously described protocols (13–15). Uracil and inosine nucleotide phosphoramidites were purchased from Glen Research Corp.

Synthesis and Purification of Oligonucleotides—All oligonucleotides were prepared using an Applied Biosystems DNA synthesizer. The protecting groups were removed with 1 ml of ammonium hydroxide at 50°C for 6 h. The material was dried, dissolved in 80% formamide, and purified by electrophoresis on a 7 M urea, 20% polyacrylamide gel. The

1 The abbreviations used are: 2-AP, 2-aminopurine; 7-deazaG, 7-deazaguanine.
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oligonucleotide was extracted from the gel with Tris/EDTA (TE buffer). The solution was desalted with a Sep-Pak C18 cartridge, concentrated, and redissolved in distilled water. The purity of the material was assessed by phosphorylation of 4 pmol of the DNA with T4 polynucleotide kinase and [γ-32P]ATP. The radiolabeled oligonucleotides were applied to a denaturing polyacrylamide gel and exposed to Kodak X-omat AR x-ray film.

DNA Duplex Preparation—The concentration of each oligonucleotide was determined by the UV absorbance at 260 nm of an aliquot of solution and the predicted extinction coefficient for the oligonucleotide. Complementary DNAs were annealed by combining 200 pmol of each strand in a 50-μl solution of 150 mM NaCl and TE buffer. The solution was heated to 90°C, slowly cooled to room temperature and then allowed to stand overnight at 4°C. DNA duplex formation was confirmed by labeling the DNA with Sequenase and applying the reaction to a 10% native polyacrylamide electrophoresis gel.

Protein Activity—Wild-type Tus DNA was labeled using Sequenase and [α-32P]dATP (see below). The labeled wild-type duplex, at a concentration of 1 × 10−12 M, was incubated with varying concentrations of Tus protein based on UV absorbance (1). The total volume of the samples in this assay was 800 μl. The protein and DNA were equilibrated for at least 3 h. 350 μl of the samples were filtered through nitrocellulose filters, washed, dried, and counted. The total counts retained on each filter were plotted versus the amount of protein in that sample. The activity of the protein was obtained from a best fit linear least-square plot through the data. All protein concentrations used in this work refer to the quantity of active protein. The filter efficiency was calculated by dividing the counts retained after filtration for saturated samples by the total counts of an equivalent amount of DNA used for this assay. The protein was found to retain its activity for ~3 weeks at −20°C after being removed from −70°C storage.

Equilibrium Dissociation Assays—The observed equilibrium binding constant for wild-type TerB and all TerB analogues was measured using the competition method of Lin and Riggs (16, 17). 8 pmol of Ter DNA duplex were labeled by incubation with Sequenase, [α-32P]dATP, dCTP, dGTP, and dTTP at 25°C for 15 min. The reaction was quenched by the addition of 160 μl of TE buffer, applied to a Sephadex G-50 column prepared in a 1-ml syringe, and centrifuged for 1 min at 1500 rpm. The concentration of the DNA was based on the eluent volume.

In the competition binding assay, a constant amount of labeled wild-type TerB (1 × 10−12 M) was incubated with the Tus protein (1.8 × 10−12 M) and competing amounts of analogue DNA. All protein and DNA dilutions were done in binding buffer containing 50 mM Tris-Cl, pH 7.6 (at 25°C), 150 mM potassium glutamate, 0.1 mM dithiothreitol, 0.1 mM EDTA, and 100 μg/ml bovine serum albumin. The total volume of the samples for this assay was 800 μl. The samples were incubated for at least 3 h. 500 μl of each sample were filtered through nitrocellulose filters pre-soaked in wash buffer (50 mM Tris-Cl, 150 mM potassium glutamate, and 0.1 mM EDTA) and washed with 650 μl of wash buffer. Background counts for each assay were determined by incubating 600 μl of wild-type DNA, 100 μl of competitor DNA, and 100 μl of binding buffer. The total quantity of Tus protein available for binding was determined by incubating saturating quantities of Tus protein with wild-type TerB DNA only. Each assay was repeated at least three times. Errors in the 1/2 dissociation constant were not corrected by the recently developed method of Wong and Lehman (18).

The results were fit to a binding curve using nonlinear regression analysis with the graphical program SIGMAPLOT (Jandel Scientific) using Equation 1:

\[
RO = \frac{1}{2} \left[ \frac{K_{Ct} + K_{Ot} + R_{t} + O_{t}}{K_{Ot} + K_{Ct} + R_{t} + O_{t}} \right] - 0.40R_{t} = \frac{c}{bkg}
\]

(Ro) is the background counts. The counts at time t, c, is the counts at time 0, k_d is the dissociation rate constant, and bkg is the background counts.

RESULTS

Wild-type and Modified TerB DNAs—The sequence of the wild-type TerB site used in this work is shown in Fig. 1. For all DNA duplexes, two complementary 33-mers were annealed, and labeling was achieved by an enzymatic fill-in reaction to form a 37-mer with blunt ends. Since the competitor DNA was not extended to a 37-mer enzymatically, we measured the wild-type DNA as a competitor before and after the fill-in reaction and found no difference in the binding affinity (data not shown). All competing DNAs used in this work have a 4-base overhang at both ends. For guanine base substitutions, each duplex was modified at position 13, 10, or 6 of the top strand or at position 17 of the bottom strand using 2-aminopurine, 7-deazaguanine, or inosine (Fig. 2A). For the 2-aminopurine derivative, the duplex was first paired with cytosine and then the uracil base. The latter base pair is more stable to thermal denaturation in duplex DNA, presumably due to a better hydrogen bonding scheme (Fig. 2B). Single substitutions of deoxyuridine were made for each thymine throughout the conserved sequence of TerB between positions 2 and 19.

Equilibrium Dissociation Constants for Singly Substituted 7-Deazaguanine, 2-Aminopurine, and Inosine TerB-binding Sites with Tus—We introduced into the TerB DNA-binding site analogues of G, using 7-deazaG, 2-AP, or inosine nucleotides. The effect on the observable binding constant was measured for each modified TerB site using a nitrocellulose competition filter binding assay. The first analogue replaces the N-7 atom with a carbon, and the second replaces the oxygen with hydrogen with deprotonation at N-1; both are located in the major groove (Fig. 2A). The inosine analogue removes the amino group from the minor groove (Fig. 2A).

Substitution with the analogue 7-deazaG affected the free energy of binding at positions 10, 13, and 17 and not at position 6 within experimental error (Fig. 3 and Table I). The free energy change at position 10 was the largest at ~3 kcal/mol, while modifications at positions 13 and 17 were ~1 kcal/mol. For substitutions containing the 2-AP analogue, the binding constants for TerB modified at position 10 or 13 were significantly different from the wild type, with ΔΔG° = 3.1 and 2.9 kcal/mol, respectively (Fig. 3 and Table I). The position 17 substitution had a measured free energy change of −0.7 kcal/mol, while the 2-AP nucleotide at position 6 resulted in only a small effect on the binding constant (Table I).

Equilibrium Dissociation Constants for 2-AP-U Base Pair-substituted TerB Sites—We measured changes in complex affinity when 2-aminopurine was paired with uracil. Our results paralleled those obtained with the cytosine base described
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Equilibrium Dissociation Constants for Uracil-substituted TerB Sites—Uracil nucleotides were inserted into the TerB-binding site at each thymine between positions 2 and 19 inclusive. Of those tested using the competition binding assay, six were significantly destabilized (Fig. 4 and Table I), with measured free energy changes ranging between 1 and 2 kcal/mol. Substitutions that perturbed the complex were located at positions 9, 12, and 14 in the top strand and at positions 8, 16, and 19 in the bottom strand (Table I). The largest energetic loss occurred at two sites, positions 12 and 16; the former was 1.8 kcal/mol, while the latter was 1.9 kcal/mol.

Dissociation Rate Constants for Wild-type and Modified DNAs—We measured the dissociation rate for each modified complex using the same salt concentration of potassium glutamate, but at pH 8.0 (Fig. 5 and Table I). In a few cases, the dissociation rate was too rapid for accurate analysis. These were the 7-deazaG substitution at position 10, the 2-AP-C base pair at positions 10 and 13, the 2-AP-U base pair at position 13, and inosine at position 10. For complexes for which we were able to determine dissociation rates, the relative changes in the activation free energy of dissociation were comparable to changes observed in the equilibrium dissociation binding constant.

**DISCUSSION**

The Tus protein is responsible for the termination of DNA replication in *E. coli*. The Tus protein interacts as a single subunit with Ter DNA and does not appear to have any symmetry, and the complex appears to function in a single orientation. Although we have used chemical protection methods to identify sites of interaction (6), this approach does not provide enough information about the relative importance of functional groups and is limited by the reactivity of the reagent to specific chemical groups of the DNA. If the mechanism of inhibition of replication by the Tus-Ter interaction is to be elucidated, then the relation between the complex's stability and the termination event is essential. We therefore used a series of nucleotide analogues that gives us information about the relative contributions made by specific functional groups and also allows us to test DNA sites that are not amenable to study by other methods. Furthermore, the effects of these modified nucleotides on the protein-DNA complex are useful for comparing the Tus-TerB interaction with other protein-DNA complexes that have been tested with similar analogues.

Chemical modification techniques have defined general features of the DNA-binding site (6). Dimethyl sulfate protection was observed at the four guanine residues between and including positions 6 and 17. The strongest protection sites were located at positions 10 and 13 in the DNA. Based on this information, we reasoned that interactions between the protein and the DNA are strongly indicated at positions 10 and 13 and that a protein segment might interact with N-7 of guanine residues at positions 6 and 17 as well (6). Our current measurements have confirmed the necessity of specific chemical groups at positions 10 and 13. The large energetic penalty of ~3 kcal/mol, brought about by the insertion of 2-AP at these two positions, clearly indicates that the carbonyl oxygen is required for complex formation. The substitution of 7-deazaG further underscores the role of the G residue at position 10. Two other sites (positions 13 and 17) contributed as well, but to a lesser extent, and are in agreement with the protection studies. When we tested for minor groove participation using inosine, once again, the position 10 substitution destabilized the complex ($\Delta G^\circ = 3.0$ kcal/mol) as well as the position 17 substitution ($\Delta G^\circ = 1.7$ kcal/mol).

The most surprising effect occurred at position 6. We anticipated that replacing the guanine residue at position 6 with...
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**Table I**

Observed equilibrium dissociation constant, half-life and rate of dissociation of the Tus-TerB wild-type complex or complexes with analogue substitution at the indicated sites

| TerB sequence and position | $t_{1/2}$ | $k_d$ | $\Delta G^\circ$ | $K_{obs}$ | $\Delta G^{\ddagger}$ |
|---------------------------|----------|-------|-----------------|-----------|------------------|
| Wild-type                 | 240 ± 6  | 4.8 ± 0.1 | 0.03 ± 0.01 | 0.3 ± 0.1 | 10.3 ± 0.4 |
| 7-DeazaG                  | 6        | 161 ± 3  | 7.2 ± 0.2      | 0.24 ± 0.05 | 63 ± 0.6 |
|                           | 10       | ND      | ND             | ND        | 755 ± 57 |
|                           | 13       | 16 ± 1  | 71 ± 1         | 1.6 ± 0.1  | 32 ± 3  |
|                           | 17       | 36 ± 4  | 32 ± 1         | 1.1 ± 0.1  | 19 ± 3  |
| 2-AP -C                   | 6        | 240 ± 8  | 4.8 ± 0.2      | 0.03 ± 0.01 | 3.3 ± 0.6 |
|                           | 10       | ND      | ND             | ND        | 742 ± 74 |
|                           | 13       | ND      | ND             | ND        | 467 ± 98 |
|                           | 17       | 53 ± 1  | 22 ± 1         | 0.9 ± 0.1  | 12 ± 1  |
| 2-AP -U                   | 6        | 124 ± 4  | 9.3 ± 0.3      | 0.4 ± 0.1  | 6.4 ± 0.5 |
|                           | 10       | 6 ± 1   | 170 ± 10       | 2.1 ± 0.1  | 199 ± 12 |
|                           | 13       | ND      | ND             | ND        | 5700 ± 460 |
|                           | 17       | 193 ± 8 | 6.0 ± 0.3      | 4.8 ± 0.6  | 0.2 ± 0.1 |
| Inosine                   | 6        | 114 ± 3  | 10.2 ± 0.2     | 0.45 ± 0.1 | 12.2 ± 2 |
|                           | 10       | ND      | ND             | ND        | 612 ± 34 |
|                           | 13       | 398 ± 96 | 2.9 ± 0.6     | -0.3 ± 0.1 | 2.6 ± 0.3 |
|                           | 17       | 6 ± 1   | 205 ± 25       | 2.2 ± 0.1  | 67 ± 4  |

* Dissociation rates were measured in 10 mM Tris, 150 mM potassium glutamate pH 8.0, at 25 °C. Constants and the standard deviation for sequences tested for at least three independent experiments are tabulated below.

* The change in the free energy of binding is based on the following equation: $\Delta G^\circ = -RT \ln K_{obs}$, where $R = 1.987$ cal/(mol·K), and $I = 298$ K.

* Equilibrium dissociation constants were measured in 10 mM Tris, 150 mM potassium glutamate, pH 7.6, at 25 °C.

* The change in the free energy of binding is based on the following equation: $\Delta G^{\ddagger} = -RT \ln K_{obs}$.

* ND, not determined.

**Fig. 4. Determination of the observable binding constant for the Tus protein using a nitrocellulose competition binding method.** Potential hydrophobic sites were identified by the insertion of uracil instead of thymine in the TerB-binding site. Labeled wild-type TerB DNA at a concentration of $1 \times 10^{-14}$ M was added to the indicated concentration of competitor and allowed to equilibrate with the Tus protein ($1.8 \times 10^{-13}$ M) in the following buffer: 50 mM Tris-HCl, pH 7.6 (at 25 °C), 150 mM potassium glutamate, 0.1 mM dithiothreitol, and 100 μg/ml bovine serum albumin. The results were plotted, and the line through the data is the best theoretical fit according to Equation 1. Blk. rpm in the absence of competitor DNA. ○, wild type; ◊, uracil (position 9); □, uracil (position 12); ●, uracil (position 14); ●, uracil (position 8); ●, uracil (position 16); ■, uracil (position 19); ●, uracil (positions 12/16).

7-deazaGuanine or 2-aminopurine nucleotides, a position that is conserved in all known Ter site sequences and is partially protected in chemical footprinting studies, would destabilize the complex. However, the complex was little affected by the presence of any of the modified nucleotides ($\Delta G^{\ddagger} = 0.3$ kcal/mol). The combined data of the analogues indicate that a unique base pair is not needed at this site. Apparently, chemical modification techniques, while providing information about the location of the protein on the DNA, are not reliable enough for identifying site-specific interactions in the protein-DNA complex. The question remains how a nucleotide position can be conserved and yet not significantly contribute to the protein-DNA complex's stability. One possibility is that the mechanism of termination requires weak interactions at this position that assist in orienting the protein properly to impede translocating proteins. Another possibility is that an additional factor is necessary to modulate the activity of the Tus protein that may interact with this conserved nucleotide and the Tus protein when positioned on the DNA (31).

To confirm the requirement of both major and minor groove interactions, we inserted a purine analogue at position 10. The purine analogue has both the O-6 carbonyl and the amino group at position 2 removed and is a structural combination of both the 2-AP and inosine analogues. We reasoned that if a significant effect on the binding resulted from structural perturbation to the DNA, then the insertion of the purine analogue should alter the free energy very little in comparison with either 2-AP or inosine analogues at this position. On the other hand, if the free energy change is a combination of the individual binding energies, then each group would contribute independently to the formation of the bimolecular complex. Our measurements showed that the insertion of purine at this site resulted in a free energy change of >6.0 kcal/mol, supporting the independent contributions of major and minor groove sites to the interaction.2 At this stage of analysis, we are unable to determine the origin of the perturbations to the complex. If they reflect steric clashes between the protein and DNA in the complex, then they appear to exceed estimates that are currently assumed for such disruptions (1.5–2.0 kcal/mol).

The use of uracil has served to further delineate methyl

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2 K. L. Garrison and P. A. Gottlieb, unpublished results.
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Tus protein (1.5) using isosteranalogues for the guanineresidues. Typically, the
from 1.0 to 1.9 kcal/mol (Table II). These sites are dispersed
through "hydrophobic" interactions. Of all the sites tested, six
were removed at the indicated times. Measurements were
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positions critical for full binding activity, ranging in value
to precisely define the thermodynamic origin of the

Table II

| TerB sequence | $t_{1/2}$ | $k_{s} \times 10^{-3}$ | $\Delta G^\ddagger$ | $K_{obs}$ | $\Delta G^\circ$ |
|---------------|----------|----------------------|---------------------|-----------|----------------|
| Wild-type     | 241 ± 6  | 4.8 ± 0.1            |                     |           |                |
| Position 8    | 41 ± 1   | 28 ± 1               | 1.0 ± 0.1           | 20 ± 1    | 1.0 ± 0.1      |
| Position 9    | 33 ± 1   | 35 ± 1               | 1.2 ± 0.1           | 20 ± 2    | 1.0 ± 0.1      |
| Position 12   | 7 ± 0.2  | 160 ± 10             | 2.1 ± 0.1           | 72 ± 10   | 1.8 ± 0.1      |
| Position 14   | 48 ± 0.2 | 24 ± 1               | 1.0 ± 0.1           | 26 ± 2    | 1.2 ± 0.1      |
| Position 16   | 11 ± 0.1 | 110 ± 10             | 1.9 ± 0.1           | 85 ± 11   | 1.9 ± 0.1      |
| Positions 12/16| 41 ± 1 | 28 ± 1               | 1.0 ± 0.1           | 24 ± 2    | 1.1 ± 0.1      |

a Dissociation rates were measured in 10 mM Tris, 150 mM potassium glutamate, pH 8.0, at 25°C. Constants and the standard deviation for sequences tested for at least three independent experiments are tabulated below.

b The change in the free energy of binding is based on the following equation: $\Delta G^\ddagger = -RT \ln \frac{k_{s}}{k_{d}}$, where $R = 1.987$ cal/(mol of potassium), and $T = 298$ K.

c The change in the free energy of binding is based on the following equation: $\Delta G^\circ = -RT \ln \frac{k_{s}}{k_{d}}$.

The Tus protein (1.5) $x$ was equilibrated with DNA at a concentration of $2 \times 10^{-12}$ M. Excess unlabeled DNA was added, and aliquots were removed at the indicated times. Measurements were made in 50 mM Tris-HCl, pH 8.0 (at 25°C), 150 mM potassium glutamate, 0.1 mM dithiothreitol, and 100 μg/ml bovine serum albumin. The results were plotted, and the line through the data is the best theoretical fit according to Equation 2. $\ddagger$, wild type; $\square$, 7-deazaG (position 13); $\blacklozenge$, 7-deazaG (position 17); ×, 2-AP-C (position 17); $\bullet$, 2-AP-U (position 10); $\bigcirc$, inosine (position 17).

By comparing the $\Delta G^0$ value for 2-AP paired with cytosine to ones paired with uracil, we are able to draw some conclusions about the effect of 2-AP on complex stability (Fig. 2B). The use of uracil instead of cytosine in the complementary strand potentially affects the stability of the complex in either of two ways. First, the introduction of uracil allows a better match between the base pair donor and acceptor hydrogen bonds (Fig. 2B). A recent study using 2-AP paired either with cytosine or uracil has demonstrated that, in general, the latter base pair leads to a thermodynamically more stable duplex DNA (19). The increased DNA stability may result in a more stable protein-DNA complex, as has already been demonstrated for the lac repressor-operator interaction (20). The position 10 substitution of 2-AP-U for 2-AP-C, which improved the binding of the Tus-TerB complex by approximately 0.7 kcal/mol, would appear to represent this effect. Although we are unable to precisely define the thermodynamic origin of the negative free energy, namely the reconstitution of wild-type requirements for binding or the introduction of new compensating effects, these results nonetheless establish that the complex is partially destabilized by DNA structural perturbations at this site.

On the other hand, the introduction of a carbonyl instead of an amino group may serve to further destabilize the protein-DNA interaction by removing a hydrogen bond donor of cytosine and replacing it with a hydrogen bond acceptor of uracil, directly perturbing the protein-DNA interface. An example of this is the substitution of uracil for cytosine at position 13, which resulted in an additional loss of free energy of 1.5 kcal/mol. This indicates that the amino group of cytosine is important for the formation of the complex, and its replacement in the complex offsets any possible gains made by stabilizing the DNA structure. The combined results of 2-AP paired with either cytosine or uracil imply a direct interaction along the protein-DNA interface in the major groove between positions 10 and 13, which is disrupted by the alignment of sterically incompatible groups.

The results from the substitution experiments are consistent with mutagenesis experiments performed with other Ter sites through this entire region, with three sites in the top strand and three sites in the bottom strand. Three of these sites (positions 12, 14, and 16) are located in the major groove region, where critical hydrogen-bonding sites are shown in this work. The need for six methyl groups to stabilize the complex through van der Waal contacts is unusual and may explain why the Tus-TerB complex is able to achieve tight binding within a relatively small segment of DNA.
modified by single natural base pair mutations at positions 10, 12, 13, and 14, which do not bind the Tus protein and consequently are incapable of functioning as replication arrest sites. However, Tus is able to bind and arrest replication at Ter sites containing single substitutions at positions 6, 8, 11, 16, and 18 (2). These data, on a whole, reinforce our earlier prediction that the central major groove region defined by bases 10–13 is directly involved in the interactions with the Tus protein.

In those cases where measurement of the dissociation rate was possible, the values for the dissociation rate paralleled those obtained from equilibrium measurements (Figs. 5 and 6 and Tables I and II). From these results and for this size fragment, analogues that affect binding do so by altering the dissociation rate, and characterization of this constant when possible is sufficient for characterizing the complex.

There are now a number of systems, both enzymatic and nonenzymatic, that have been analyzed using modified nucleotides (20–30). In those cases that allow for direct comparison of equilibrium dissociation constants, the free energy changes obtained from equilibrium measurements (Figs. 5 and 6) and Tables I and II). From these results and for this size fragment, analogues that affect binding do so by altering the dissociation rate, and characterization of this constant when possible is sufficient for characterizing the complex.

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Laura J. Duggan, Thomas M. Hill, Su Wu, Kristen Garrison, Xiaolin Zhang and Philip A. Gottlieb

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