Effects of site-specific substitution of 5-fluorouridine on the stabilities of duplex DNA and RNA

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ABSTRACT

The effects of 5-fluorouridine (FUr) and 5-fluorodeoxyuridine (FdUr) substitution on the stabilities of duplex RNA and DNA have been studied to determine how FUr substitution in nucleic acids may alter the efficiency of biochemical processes that require complementary base pairing for molecular recognition. The parent sequence, 5'-GCGAAUUCGC, contains two non-equivalent uridines. Eight oligonucleotides (four RNA and four DNA) were prepared with either zero, one or two Urd substituted by FUr. The stability of each self-complementary duplex was determined by measuring the absorbance at 260 nm as a function of temperature. T_m values were calculated from the first derivative of the absorbance versus temperature profiles and values for ΔH° and ΔS° were calculated from the concentration dependence of the T_m . Individual absorbance versus temperature curves were also analyzed by a parametric approach to calculate thermodynamic parameters for the duplex to single-stranded transition. Analysis of the thermodynamic parameters for each oligonucleotide revealed that FUr substitution had sequence-dependent effects in both A-form RNA and B-form DNA duplexes. Conservation of helix geometry in FUr-substituted duplexes was determined by CD spectroscopy. FUr substitution at a single site in RNA stabilized the duplex ($\Delta\Delta G_{37} = 0.8$ kcal/mol), largely due to more favorable stacking interactions. FdUr substitution at a single site in DNA destabilized the duplex ($\Delta\Delta G_{37} = 0.3$ kcal/mol) as a consequence of less favorable stacking interactions. All duplexes melt via single cooperative transitions.

INTRODUCTION

Many biochemical processes require complementary base pairing for molecular recognition (1). In many RNA-mediated processes, e.g. pre-mRNA splicing and translation of mRNA, complementary regions of RNA bind one another transiently. The efficiency of these processes depends, in part, on the stability of the duplexes formed. The stability of these duplexes, in turn,

depends on the number of base pairs formed, the G-C content of the resulting duplex and the number and type of non-standard bases (2). The stability of duplexes that form transiently between complementary portions of two different RNAs, e.g. U4 and U6 snRNA (3), can be modeled by using synthetic oligoribonucleotides. Information concerning the stability of these complexes can be obtained from an analysis of the temperature dependence of the UV spectra for the model duplexes. The present study investigates the effects of the non-natural nucleoside 5-fluorouridine (FUr) on the stability of eight self-complementary RNA and DNA duplexes. Each oligonucleotide is a self-complementary decamer containing two non-equivalent uridines (Fig. 1). FUr is an anti-cancer drug known to be incorporated into both the RNA and DNA of cells treated with this drug. The biological effectiveness of FUr is believed to be due, in part, to its incorporation into RNA and the disruption of rRNA assembly (4) and pre-mRNA splicing (5). The studies described herein were performed to determine whether changes in the stability of duplex RNA are of sufficient magnitude to account for the reduction in the efficiency of RNA-mediated processes in cells treated with FUr. Alternatively, changes in the structure and stability of non-duplex regions of RNA or RNA-protein interactions may account for the biological effects of FUr substitution on RNA-mediated processes (6).

Investigations into the effect of FdUr substitution for dT in duplex DNA have revealed that local structural disturbances occur near the site of the non-native base. Kremer *et al.* reported that FdUr substitution in the Pribnow recognition sequence resulted in local destabilization of A:FUra base pairs, but had a negligible effect on B-form DNA stability (7). Sowers *et al.* reported that FdUr substitutions increased the rate of base pair opening for A:FUra base pairs as a consequence of decreased stacking affinity of FUra for neighboring nucleoside bases (8). Detailed two-dimensional NMR investigations of the *EcoRI* DNA octamer containing FUr revealed that structural alterations were localized near the site of substitution, with little change in global structure (9). Several recent studies have shown that FUra substitution in snRNA (10) and mRNA (11) alters pre-mRNA splicing and mRNA translation. The molecular mechanisms by which these RNA-mediated processes are perturbed is not yet known.

The high sensitivity of ¹⁹F and its infrequent occurrence in biological molecules has made FUra an important probe for the study of RNA structure (12) and RNA-protein interactions by

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Figure 1. (A) The eight oligonucleotides used in this study. (B–D) UV absorbance (260 nm) versus temperature curves for UUDNA and UURNA (B), UUDNA and FUDNA (C) and UURNA and FURNA (D). The curves shown are representative of the 10 such curves collected for each duplex for eight different concentrations.

NMR spectroscopy (13,14). In many RNA systems Fura substitution is known to have a minimal effect on RNA structure (12). Nonetheless, the demonstrated biological effects of Fura substitution on RNA function (15) and the few reports of Fura-induced altered RNA secondary structure (16) warrant detailed investigations into the structural and thermodynamic consequences of Fura substitution in RNA. Fura has greater intrinsic affinity than does Ura to form nucleoside base pairs (17), but the stability of RNA duplexes containing Fura does not differ appreciably from the native sequences (2). To systematically investigate the effects of Fura substitution on the stability of duplex RNA and DNA four RNA and four DNA oligonucleotides were synthesized and analyzed in UV hyperchromicity experiments and by CD spectroscopy. The T_m , ΔH^0 and ΔS^0 values for dissociation of DNA and RNA substituted with FdUrd (FUr) have been determined. The results show that Fura substitution affects duplex nucleic acid stability in a sequence-specific manner. The magnitude of the observed changes are, however, unlikely to account for the effects of Fura substitution on RNA processing (18).

MATERIALS AND METHODS

Solid-phase synthesis of oligoribonucleotides

The synthesis and purification of 5'-O-(4,4'-dimethoxytri-tyl)-2'-O-(t-butyldimethylsilyl)-5-fluorouridine-3'-(2-cyanoethyl *N,N'*-diisopropyl-phosphoramidite) and purification of RNA con-

taining FUr was done as previously described (2). Synthesis was performed on an Applied Biosystems 380B DNA synthesizer on a 1 μ mol scale. The material was further purified on a denaturing 20% polyacrylamide gel. Bands were visualized by shadowing with UV light, excised with a razor and recovered using a manual crush and soak elution with 1.0 M ammonium acetate, 0.1 mM EDTA and 0.1% w/v SDS. The solution was passed through a 0.2 μ m filter and DNA was recovered following ethanol precipitation (19). The DNA and RNA sequences synthesized are shown in Figure 1.

Extinction coefficients

Extinction coefficients were calculated with the nearest neighbor approximation (20). Nearest neighbor values for FUr are not available, so the values for Urd were used. The extinction coefficient for Fura is similar to Ura (Fura = 7070/M/cm at 265 nm, Ura = 8200/M/cm at 260 nm) (21) and errors in oligonucleotide concentrations due to this assumption should be <3% for the double substituted oligonucleotides and <1.5% for the singly substituted oligonucleotides. The calculated extinction coefficients at 260 nm are 99140/M/cm.

Melting curves

All absorbance versus temperature curves were measured at 260 nm using a Cary 1 UV spectrophotometer. The buffer was 0.1 M NaCl, 2 mM sodium cacodylate, pH 7.3. Quartz cuvettes (10 and

Table 1. Summary of the thermodynamic data for the eight oligonucleotides studied

	Avg. Fitted Parameters ^a				1/T _m Vs.log(C _T)		
	ΔH(Kcal/mole)	ΔS(Cal/mole.K)	ΔG ₂₇ (Kcal/mole)	T _m (C)	ΔH(Kcal/mole)	ΔS(Cal/mole.K)	ΔG ₂₇ (Kcal/mole)
UUDNA	-60.0 ± 4.2	-166.8 ± 13.4	-8.3 ± 0.1	44.2 ± 0.1	-55.7	-158.2	-6.6
FUDNA	-54.9 ± 4.6 ^b	-151.1 ± 14.3 ^b	-8.0 ± 0.2	42.9 ± 0.3	-46.6	-130.2	-6.2
UFDNA	-61.1 ± 4.0	-169.9 ± 12.4	-8.4 ± 0.1	44.3 ± 0.1	-57.9	-165.1	-6.8
FFDNA	-53.7 ± 3.5	-147.9 ± 10.6	-7.9 ± 0.2	42.1 ± 0.2	-49.2	-138.2	-6.3
UURNA	-78.2 ± 1.2	-217.4 ± 3.9	-10.8 ± 0.1	53.0 ± 0.2	-67.6	-189.2	-9.0
FURNA	-83.9 ± 2.2	-234.5 ± 6.7	-11.2 ± 0.2	53.3 ± 0.2	-67.6	-189.2	-9.0
UFRNA	-87.4 ± 1.7	-244.5 ± 5.2	-11.6 ± 0.2	54.0 ± 0.1	-90.0	-257.5	-10.2
FFRNA	-82.5 ± 2.3	-230.7 ± 6.9	-11.0 ± 0.2	52.8 ± 0.1	-79.4	-226.3	-9.3

^aThe data were analyzed using Student's *t*-test, *P* < 0.0005.^b*P* < 0.025.

2.0 mm) were used to obtain melting curves. All initial and final absorbance values were between 0.5 and 2.0 OD. A temperature ramp from 10 to 85°C was performed at 1°C/min. Samples were cooled at 1°C/min and maintained at 10°C for 1 min. Ten measurements were made for each sample. *T_m* values were calculated from the first derivative of the absorbance versus temperature data.

Curve fitting

All eight oligonucleotides studied are self-complementary and the cooperative portion of the melting curves reflects the single-strand to double helix equilibrium

Numerical values for the thermodynamic parameters ΔH^0 and ΔS^0 associated with the single-strand to duplex transition for each of the self-complementary oligonucleotides were obtained by the method of Petersheim and Turner (22). Absorbance versus temperature data were fitted using the equation

$$\epsilon(T) = A(T)/(lC_T) = \alpha\epsilon_{ds} + (1 - \alpha)\epsilon_{ss} \quad 2$$

Where α is the fraction of the sample in duplex form at temperature *T*, *A(T)* is the absorbance observed at temperature *T* and *l* is the pathlength of the cuvette used in the measurement. The total strand concentration, *C_T*, is calculated from the *A*₂₆₀ at 80°C and the calculated value of the extinction coefficient ϵ (20). The extinction coefficients of the single- and double-stranded species are assumed to be linear functions of temperature.

$$\epsilon_{ds} = m_{ds}T + b_{ds} \quad 3$$

$$\epsilon_{ss} = m_{ss}T + b_{ss} \quad 4$$

Estimates for *m_{ds}*, *b_{ds}*, *m_{ss}* and *b_{ss}* are obtained from the linear regions of the absorbance versus temperature curves. The fraction of strands in the double-stranded state, α , is related to changes in enthalpy, ΔH^0 , and entropy, ΔS^0 , for the transition according to the equation

$$K = \alpha/[2(1 - \alpha)^2 C_T] = \exp[-(\Delta H^0/RT) + (\Delta S^0/R)] \quad 5$$

The software written by Petersheim and Turner (22) was used to determine the values of *m_{ds}*, *b_{ds}*, *m_{ss}*, *b_{ss}*, ΔH^0 and ΔS^0 . This uses the Marquardt non-linear least squares method to fit the experimental absorbance versus temperature data. Values of χ^2 for the calculated versus experimental curves were <10⁻⁵ for all curves.

Concentration dependence of *T_m*

Absorbance versus temperature curves were acquired with 10 different total oligonucleotide concentrations (*C_T*) ranging from 2.0 × 10⁻⁵ to 1.3 × 10⁻³ M for each of the eight oligonucleotide samples. Numerical values for ΔH^0 and ΔS^0 were obtained by fitting the *T_m* values to the equation (23)

$$1/T_m = (2.303R/\Delta H^0) \log(C_T) + (\Delta S^0/\Delta H^0) \quad 6$$

The *T_m* is defined as the temperature where the fraction of strands in double helical form is 0.5. *T_m* values are obtained from the first derivative of the absorbance versus temperature data. Values for ΔH^0 and ΔS^0 are obtained from plots of 1/*T_m* versus log(*C_T*). For two state behavior (equation 1) plots are linear, with the slope and intercept values containing the desired thermodynamic parameters.

Circular dichroism spectra

Spectra were recorded on a Jasco J-710 spectropolarimeter interfaced with an AT&T microcomputer. Spectra were recorded from 230 to 330 nm in 2 mM sodium cacodylate, pH 7.3, 100 mM NaCl at ambient temperature using a cylindrical cuvette with a path length of 1 mm (24). All CD data were baseline corrected for the cell and buffer and were processed through a noise reduction program.

RESULTS

UV hyperchromicity studies

Relative stability of duplex DNA and RNA. The two self-complementary duplex parent sequences, UUDNA and UURNA, are comprised of DNA and RNA respectively. The analysis of thermodynamic parameters for these sequences provides a measure of the relative stability of B-form DNA and A-form RNA duplexes of identical sequence (25). A typical plot of absorbance

Figure 2. Plot of $1/T_m$ versus log concentration for FUDNA and FURNA. Linear regression of these data yield values for ΔH^0 and ΔS^0 for the duplexes presented in Table 1.

versus temperature data for UUDNA and UURNA is shown in Figure 1. T_m values calculated by applying the first derivative method to the absorbance data show the DNA sequence to have a $T_m \sim 10^\circ\text{C}$ lower than the corresponding RNA sequence. This is true over the full range of concentrations studied. The results are in agreement with previous studies that have shown that RNA–RNA duplexes are more stable than DNA–DNA duplexes (26,27). Thermodynamic parameters for the double-stranded to single-stranded transition for UUDNA and UURNA were calculated by fitting the absorbance versus temperature data to equation 2 (28) and also from the concentration dependence of the T_m values obtained by the derivative method according to equation 6 (23). The numerical values for T_m , ΔH^0 , ΔS^0 and ΔG^0 obtained by both procedures are summarized in Table 1. The absolute values of ΔH^0 , ΔS^0 and ΔG^0 obtained from the concentration dependence of the T_m values are somewhat lower than those obtained from averaging the fitted parameters. Differences of similar magnitude for the thermodynamic parameters calculated by the two methods have been observed previously (29). These differences are also evident for the FURd-substituted DNA and RNA sequences (Table 1).

Effects of FURd in duplex DNA. The three FURd-substituted DNA sequences, FUDNA, UFDNA and FFDNA, as well as the parent DNA sequence (UUDNA), were analyzed by UV hyperchromicity methods to determine how FURd substitution effects the stability of B-form DNA duplexes. The T_m values for each self-complementary duplex were determined by curve fitting the experimental A_{260} values as a function of temperature as described in the Materials and Methods section. Typical experimental curves of A_{260} versus temperature for FUDNA and UUDNA at 2.4×10^{-4} M total strand concentration are shown in Figure 1. The T_m values, as well as estimates of ΔH^0 and ΔS^0 obtained by the curve fitting procedure (28), were obtained from the absorbance data at each of 10 concentrations covering the range 10^{-3} to 10^{-5} M. Several trends are evident in these data. Substitution of FURd at Urd₆ in FUDNA resulted in a very slight destabilization to the duplex, as is evident from the $\Delta\Delta G$ values

($\Delta G_{\text{substituted}} - \Delta G_{\text{control}}$), while substitution of FURd at Urd₇ in UFDNA had a very slight stabilizing effect on the duplex. Substitution of FURd at both Urd₆ and Urd₇ (FFDNA) also had a very slight destabilizing effect on the duplex. The values for T_m , ΔH^0 , ΔS^0 and ΔG^0 obtained by the curve fitting procedure are summarized in Table 1. The significance of the very slight differences in T_m , ΔG^0 , ΔH^0 and ΔS^0 for the FURd-substituted DNA sequences relative to the control (UUDNA) was established by performing a Student's *t*-test evaluation of the data. The probability of the T_m and other thermodynamic parameters for any of the FURd-substituted sequences being identical to the control sequence was <0.02 in all cases and <0.005 in all but two instances.

The four self-complementary DNA sequences analyzed by fitting the absorbance versus temperature data to obtain thermodynamic parameters were also investigated by the concentration dependence of T_m to obtain another measurement of the same thermodynamic parameters. The T_m values were obtained from the first derivative of the absorbance versus temperature data at each of the 10 concentrations. The plot of $1/T_m$ versus the log of concentration for FUDNA is shown in Figure 2. The plots for all DNA duplex samples are linear, with correlation coefficients >0.97 . The observed trends in stability for the DNA sequences due to site-specific substitution of FURd are the same for the two methods and in both cases the effects are slight. The numerical values for the thermodynamic parameters obtained from the concentration dependence of the T_m are also summarized in Table 1.

Effects of FURd in duplex RNA. The effects of FURd substitution on the stability of A-form duplex RNA were analyzed by methods similar to those applied to the four DNA sequences. The three FURd-substituted RNA sequences, FURNA, UFRNA and FFRNA, as well as the parent sequence (UURNA), were analyzed by fitting the absorbance versus temperature data to equation 2 (28) to obtain values for T_m , ΔH^0 , ΔS^0 and ΔG^0 . These values are summarized in Table 1. The absorbance versus temperature data for UURNA and for FURNA from typical experiments are shown in Figure 1. The numerical values for ΔH^0 , ΔS^0 and ΔG^0 were also

Figure 3. CD spectra for UUDNA and UFDNA (A) and UURNA and UFRNA (B). The CD spectra for the FdUrd (FUr) substituted duplexes differ subtly from the control duplexes, but the overall B-form helix of the DNA duplexes and A-form helix of the RNA duplexes are not affected by FdUrd (FUr) substitution.

obtained by analyzing the concentration dependence of the T_m values obtained by the derivative method according to equation 6 (23). The plot of $1/T_m$ versus the log of concentration for FURNA is shown in Figure 2. The plots for all RNA sequences were linear, with correlation coefficients of ≥ 0.97 . The numerical values for ΔH^0 , ΔS^0 and ΔG^0 obtained by this method are shown in Table 1. The statistical significance of the observed differences in T_m , ΔH^0 , ΔS^0 and ΔG^0 was verified by performing a Student's *t*-test analysis of the data. The effects of FUr substitution on the stability of duplex RNA are sequence dependent, similar to the trends that are observed for duplex DNA. The major difference between the effects in duplex RNA from those observed in duplex DNA is that single FUr substitutions at either Urd₆ (FURNA) or Urd₇ (UFRNA) had a slight stabilizing effect on the duplex relative to the parent sequence. Substitution of FUr at both Ura₆ and Ura₇ slightly destabilized the sequence. These trends are particularly evident in the ΔH^0 values for the four sequences, although an effect on ΔS^0 was also evident. The absolute values of ΔH^0 , ΔS^0 and ΔG^0 obtained from the concentration dependence of the T_m are lower than those obtained from averaging the fitted parameters. This is similar to observations made for the DNA sequences. The trends regarding the stabilizing effects of site-specific substitution of FUr in duplex RNA are the same irrespective of the method used to calculate them.

Circular dichroism

CD spectra were recorded for all eight self-complementary duplexes to determine whether FUr substitution affects the overall helical geometry of these duplexes. The CD spectra are shown in Figure 3. All the spectra for the DNA sequences show the specific features of a B-form helix. A CD maximum is observed at 284 nm and a minimum at 251 nm (30). The RNA sequences show the specific feature of an A-form helix, i.e. a CD maximum at 265 nm.

The shape of the CD spectra and positions of the peaks are not significantly altered by FUr substitution at either Urd₆ or Urd₇ or both these positions. This suggests that the FUr substitutions does not change the overall secondary structures of these RNA and DNA duplexes. A close study of the CD spectra show subtle differences caused by the FUr substitutions, which might be because of small local structural effects of the substitutions (31).

DISCUSSION

The effects of FUr substitution on the stabilities of the DNA and RNA duplexes analyzed in the present study are slight and the magnitude of the observed effects is unlikely to account for the reported biological effects of FUr in RNA. The effects measured, however, are not negligible.

While the T_m values and thermodynamic parameters for the FUr-substituted duplexes are indistinguishable from the control duplexes based on single UV hyperchromicity experiments, a more rigorous experimental approach, including Student's *t*-test analysis of multiple UV hyperchromicity experiments and measurements of T_m as a function of oligonucleotide concentration, reveals sequence-dependent effects on duplex stability due to FUr substitution. In the simple system chosen for analysis, 5'-GCGAAUUCGC, the T_m values and the values for the thermodynamic parameters ΔG^0 , ΔH^0 and ΔS^0 for the singly and doubly substituted DNA and RNA duplexes were all statistically distinguishable from the unsubstituted control duplexes. Single FUr substitutions in this decamer sequence represent an upper bound of substitution for modeling the physiological effects of FUr, since substitution of FUr for Urd in RNA at levels $>10\%$ is highly cytotoxic.

Enthalpy terms are responsible for the stabilizing effects of FUr substitution in the singly FUr-substituted DNA and RNA duplexes. Favorable enthalpy terms are larger in the duplex studied for FdUrd (FUr) substitution at sites flanked by pyrimidines and

particularly for A-form helices. Entropy terms for the duplexes studied are more favorable for FUr substitution in B-form helices. Favorable base stacking interactions are generally associated with favorable enthalpy terms, while restriction in rotation about torsional bonds is associated with disfavorable entropy terms (32,33). An analysis of ΔH^0 and ΔS^0 shows that the increased stability due to FUr substitution in RNA is due to more favorable base stacking interactions. Adoption of the preferred geometry for base stacking requires a restriction in torsional angles that is disfavored entropically and largely negates the enthalpic stabilization at physiological temperature. A single FUr substitution in the RNA duplex at Urd₇ is favored enthalpically by 9.2 kcal/mol, as calculated from curve fitting of the parameters, and 23 kcal/mol, from the concentration dependence of the T_m . Changes in enthalpy of this magnitude could be important in altering the rates of biochemical processes if offsetting entropy penalties associated with restrictions in torsional angles are not as large. The present study suggests that for RNA and DNA duplexes the substantial favorable changes in enthalpy associated with FUr substitution are largely countered by unfavorable changes in entropy that are similar in magnitude at physiological temperature.

The mechanism by which FUr substitution into RNA effects biochemical processes is not yet clear. Several possibilities exist and it is likely that more than one is important for the anti-cancer activity of FUr. FUr substitution may affect the stability of RNA-RNA and RNA-protein complexes. In complex, multi-step processes FUr-substituted RNA may differ in affinity for complementary sequences. Changes in the rates of association or dissociation due to FUr substitution may result in a decrease in the overall efficiency of RNA-mediated processes such as pre-mRNA splicing or translation of mRNA. Decreases in the efficiency of the splicing of pre-mRNA have been observed upon substitution of FUr into snRNA (5). Recent work has identified substitution of FUr in U2 and U6 snRNA as significantly affecting the overall efficiency of pre-mRNA splicing (10). The extent to which individual sites are affected by FUr substitution is not known. Decreases in the efficiency of complex biochemical processes may result from cumulative substitutions of FUr at several sites or a few sites may be hypersensitive to FUr substitution. Substitution at any one of the hypothetical sites hypersensitive to substitution by FUr may decrease the efficiency of RNA-mediated processes dramatically. The results of the present study on the thermodynamic effects of FUr substitution in the self-complementary decamer 5'-GCGAAUUCGC-3' suggest that substitution of FUr into duplex regions of RNA is relatively unimportant for the observed effects of FUr on RNA structure and function.

The physicochemical basis for the RNA-mediated effects of FUr remains unknown. The preferred base stacking geometry of FUr and its pK_a are both functions of the three-dimensional structure of nucleic acids substituted with FUr. The present study demonstrates that the identification of discrete structural elements that are affected by FUr substitution and an analysis of the thermodynamic and structural basis for the effectiveness of FUr substitution at these sites may play an important role in elucidating the mechanism of action of this widely used

anti-cancer drug (17). The subtle nature of the effects observed in the duplexes presented in this study is inconsistent with changes in the stability of RNA duplexes being responsible for the RNA-mediated effects of FUr.

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REFERENCES

- 1 Cantor, C.R. and Schimmel, P.R. (1980) *Biophysical Chemistry Part I: The Conformation of Biological Macromolecules*. W.H. Freeman, San Francisco, CA.
- 2 Gmeiner, W.H., Saharabudhe, P. and Pon, R.T. (1994) *J. Org. Chem.*, **59**, 5779-5783.
- 3 Legrain, P. and Chanfreau, G. (1994) *Bull. Inst. Pasteur*, **92**, 153-179.
- 4 Parker, W.B. and Cheng, Y.C. (1990) *Pharmacol. Ther.*, **48**, 381-395.
- 5 Dolnick, B.J. and Pink, J.J. (1985) *J. Biol. Chem.*, **260**, 3006-3014.
- 6 Weckbecker, G. (1991) *Pharmacol. Ther.*, **50**, 367-424.
- 7 Kremer, A.B., Mikita, T. and Beardsley, G.P. (1987) *Biochemistry*, **26**, 391-397.
- 8 Sowers, L.C., Eritja, R., Kaplan, B.E., Goodman, M.F. and Fazakerley, G.V. (1987) *J. Biol. Chem.*, **262**, 15436-15442.
- 9 Stolarski, R., Egan, W. and James, T.L. (1992) *Biochemistry*, **31**, 7027-7042.
- 10 Lenz, H.J., Manno, D.J., Danenberg, K.D. and Danenberg, P.V. (1994) *J. Biol. Chem.*, **269**, 31962-31968.
- 11 Takimoto, C.H., Voeller, D.B., Strong, J.M., Anderson, A., Chu, E. and Allegra, C.J. (1993) *J. Biol. Chem.*, **268**, 21438-21442.
- 12 Gollnick, P., Hardin, C.C. and Horowitz, J. (1986) *Nucleic Acids Res.*, **14**, 4659-4673.
- 13 Rastinejad, F. and Lu, P. (1993) *J. Mol. Biol.*, **232**, 105-122.
- 14 Kealey, J.T. and Santi, D.V. (1995) *Biochemistry*, **34**, 2441-2446.
- 15 Spiegelman, S., Sawyer, R., Nayak, R., Ritz, E., Stolfi, R. and Martin, D. (1980) *Proc. Natl. Acad. Sci. USA*, **77**, 4966-4970.
- 16 Armstrong, R.D., Takimoto, C.H. and Cadman, E.C. (1986) *J. Biol. Chem.*, **261**, 21-24.
- 17 Gmeiner, W.H., Anderson, J. and Saharabudhe, P. (1994) *Nucleosides Nucleotides*, **13**, 2329-2344.
- 18 Daher, G.C., Harris, B.E. and Diasio, R.B. (1990) *Pharmacol. Ther.*, **48**, 189-222.
- 19 Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd Edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- 20 Puglisi, J.D. and Tinoco, I., Jr (1989) *Methods Enzymol.*, **180**, 304-325.
- 21 Merck Inc. (1981) *Merck Index*.
- 22 Petersheim, M. and Turner, D.H. (1983) *Biochemistry*, **22**, 256-263.
- 23 Borer, P.N., Dengler, B., Uhlenbeck, O.C. and Tinoco, I. (1974) *J. Mol. Biol.*, **86**, 843-853.
- 24 Hall, K.B. and McLaughlin, L.W. (1991) *Biochemistry*, **30**, 10606-10613.
- 25 Saenger, W. (1984) *Principles of Nucleic Acid Structure*. Springer-Verlag, New York, NY.
- 26 Martin, F.H. and Tinoco, I., Jr (1980) *Nucleic Acids Res.*, **8**, 2295-2299.
- 27 Nelson, J.W. and Tinoco, I., Jr (1982) *Biochemistry*, **21**, 5289-5295.
- 28 Freier, S.M., Burger, B.J., Alkema, D., Neilson, T. and Turner, D.H. (1983) *Biochemistry*, **22**, 6198-6206.
- 29 LeBlanc, D.A. and Morden, K.M. (1991) *Biochemistry*, **30**, 4042-4047.
- 30 Johnson, W.C. and Tinoco, I. (1969) *Biopolymers*, **7**, 727-749.
- 31 Guenther, R.H., Hardin, C.C., Sierzputowska-Gracz, H., Dao, V. and Agris, P.F. (1992) *Biochemistry*, **31**, 11004-11011.
- 32 Breslauer, K.J. and Sturtevant, J.M. (1977) *Biophys. Chem.*, **7**, 205-209.
- 33 Freier, S.M., Hill, K.O., Dewey, T.G., Marky, L.A., Breslauer, K.J. and Turner, D.H. (1981) *Biochemistry*, **20**, 1419-1426.