Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 3746–3750, June 1986
Biochemistry

Predicting DNA duplex stability from the base sequence

(nearest-neighbor interactions/DNA structure/thermodynamics)

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Communicated by I. Tinoco, Jr., February 6, 1986

ABSTRACT **We report the complete thermodynamic library of all 10 Watson–Crick DNA nearest-neighbor interactions. We obtained the relevant thermodynamic data from calorimetric studies on 19 DNA oligomers and 9 DNA polymers. We show how these thermodynamic data can be used to calculate the stability and predict the temperature-dependent behavior of any DNA duplex structure from knowledge of its base sequence. We illustrate our method of calculation by using the nearest-neighbor data to predict transition enthalpies and free energies for a series of DNA oligomers. These predicted values are in excellent agreement with the corresponding values determined experimentally. This agreement demonstrates that a DNA duplex structure thermodynamically can be considered to be the sum of its nearest-neighbor interactions. Armed with this knowledge and the nearest-neighbor thermodynamic data reported here, scientists now will be able to predict the stability ($\Delta G°$) and the melting behavior ($\Delta H°$) of any DNA duplex structure from inspection of its primary sequence. This capability should prove valuable in numerous applications, such as (*i*) predicting the stability of a probe–gene complex; (*ii*) selecting optimal conditions for a hybridization experiment; (*iii*) deciding on the minimum length of a probe; (*iv*) predicting the influence of a specific transversion or transition on the stability of an affected DNA region; and (*v*) predicting the relative stabilities of local domains within a DNA duplex.**

It is well established that under a given set of solution conditions the relative stability of a DNA duplex structure depends on its base sequence (1–4). More specifically, the stability of a DNA duplex appears to depend primarily on the identity of the nearest-neighbor bases. Ten different nearest-neighbor interactions are possible in any Watson–Crick DNA duplex structure. These pairwise interactions are AA/TT; AT/TA; TA/AT; CA/GT; GT/CA; CT/GA; GA/CT; CG/GC; GC/CG; GG/CC. The overall stability and the melting behavior of any DNA duplex structure can be predicted from its primary sequence if one knows the relative stability ($\Delta G°$) and the temperature-dependent behavior ($\Delta H°$, $\Delta C_p°$) of each DNA nearest-neighbor interaction (5, 6). Tinoco and coworkers already have demonstrated the power of this predictive ability with RNA molecules for which they and others have determined the appropriate thermodynamic data (7–11). Unfortunately, comparatively few corresponding studies on DNA oligomers have been performed so that the relevant thermodynamic data required to predict DNA structural stability are rather sparse. The seriousness of this deficiency is dramatized by the fact that investigators attempting to evaluate sequence-dependent structural preferences in DNA molecules have resorted to the use of the more available RNA thermodynamic data. This use of RNA data does not reflect a belief that DNA and RNA are thermodynamically equivalent but rather is born of necessity due to a lack of the relevant DNA thermodynamic data. In fact, available comparisons suggest that serious errors may be introduced by applying RNA data to the analysis of sequence-dependent structural preferences in DNA molecules (10, 12–14). Consequently, a meaningful evaluation of sequence-dependent *DNA* structural preferences requires a *DNA* data base.

Several years ago, we initiated a program with the expressed objective of obtaining the required DNA thermodynamic data. To this end, we have employed differential scanning calorimetry (DSC) and UV spectroscopy to characterize thermally induced helix-to-coil transitions in specially designed and synthesized oligomeric and polymeric DNA molecules (5, 6, 15–23). By combining the results from these studies, we now are able to resolve and to assign thermodynamic profiles for all 10 DNA nearest-neighbor interactions. Furthermore, we can demonstrate that DNA duplex structures thermodynamically can be considered to be the sum of their nearest-neighbor pairwise interactions. Consequently, using our nearest-neighbor DNA thermodynamic library we now can calculate the stability and predict the melting behavior of any DNA double helix from its primary sequence. This predictive ability should prove valuable in a number of important biochemical applications, such as calculating the minimum length of a probe oligomer required to form a stable duplex with a target gene at a given hybridization temperature, estimating the melting temperature of a duplex structure formed between an oligomeric probe and its complementary gene segment, identifying potential sites of local melting within a polymer duplex by predicting the sequence-dependent melting temperatures of local DNA domains, predicting the influence of a specific transition or transversion on the stability and melting temperature of a DNA sequence, and calculating and comparing the stability of a DNA duplex in the B conformation with the stability of the same sequence in alternative conformational states (e.g., Z, B′, etc.) once these non-B conformations are thermodynamically characterized.

In this article, we report the complete thermodynamic characterization of all 10 nearest-neighbor interactions possible in a Watson–Crick DNA duplex structure. More significantly, we demonstrate how these data can be used to predict the stability and the melting behavior of any DNA duplex from knowledge of its primary sequence.

MATERIALS AND METHODS

DNA Polymers and Oligomers. The three trimer-repeat polymers were synthesized by Robert Ratliff and were the kind gift of Tom Jovin. The remaining six polymers were obtained from P-L Biochemicals. We synthesized 12 of the 19 oligomers using the standard phosphotriester method (24). Three of the sequences [d(CGCGCG); d(CGTACG); and d(ATGCAT)] were the kind gift of our colleague Roger Jones.

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Abbreviation: DSC, differential scanning calorimetry.

The sequences d(GCGAATTCGC) and d(ATATATATAT) were purchased from P-L Biochemicals. All of the oligomer studies were conducted in pH 7 buffer solutions consisting of 0.01 M sodium phosphate, 1 mM Na_2EDTA, and 1 M NaCl. The polymer studies were performed in the same buffer system but at lower NaCl concentrations in order to obtain melting temperatures below 100°C. These polymer results then were extrapolated to 1 M NaCl using our salt-dependent data.

Calorimetry. The thermally induced order–disorder transitions of each oligomer and polymer were monitored and characterized using DSC (Microcal 1). The experimental protocols and data analysis associated with this method have been described in detail (5, 6, 15–23).

UV Spectrophotometry. Absorbance versus temperature profiles for each oligomer and polymer duplex were measured using a thermoelectrically controlled Perkin–Elmer 575 spectrophotometer interfaced with a Tektronix 4051 computer. The resulting melting curves were analyzed as described (5, 6, 15–23).

Circular Dichroism (CD) Spectroscopy. CD spectra were recorded using a Cary 60 instrument equipped with a Cary 6001 CD accessory and a programmable, thermoelectrically controlled cell holder (AVIV Associates, Lakewood, NJ).

RESULTS

Selecting the DNA Oligomer Basis Set. To decide which oligomer sequences to investigate as part of our effort to characterize thermodynamically all 10 nearest-neighbor interactions, the following criteria were considered: (*i*) The oligomers should be long enough so as to exhibit experimentally convenient melting temperatures. (*ii*) The oligomers should have G·C base pairs at the ends to minimize fraying of terminal A·T base pairs (17). (*iii*) The oligomers should exhibit CD spectra characteristic of the B conformation. (*iv*) The oligomers should exhibit "all-or-none" (two-state) melting behavior. In other words, the calorimetric and the van't Hoff transition enthalpies must be equal (25).

These criteria were adhered to quite strictly. Only 2 of the 19 oligomers studied have terminal A·T base pairs, thereby causing them to violate criteria *ii* and *iv*. For these two sequences, the thermodynamic data were derived from van't Hoff treatments of the optical data and are included in our basis set to enable us to obtain the best possible solution for all 10 nearest-neighbor interactions. Sequences containing three consecutive guanosine or cytidine residues should be avoided. Such sequences systematically yield low transition enthalpies and free energies. Consequently, we have included only one such sequence in our basis set. The origin of this effect requires further investigation.

Systems Studied. We have thermodynamically characterized the helix-to-coil transitions of 19 oligonucleotides and 9 polynucleotides using DSC and temperature-dependent UV absorption spectroscopy. The specific sequences studied are listed in the first column of Table 1. Since all of the oligomers are self-complementary, only one strand is shown. The remaining columns in Table 1 list in a matrix format the identity and the frequency of the nearest-neighbors found in each sequence. For the nine polymeric duplexes, the frequencies listed represent weighting factors. These factors were assigned so as to reflect the greater accuracy of the polymer data and to balance the overall representation of each nearest-neighbor in the matrix. For most of the polymers, these weighting factors also correspond approximately to the size of the cooperative melting unit.

For each sequence listed in Table 1, we have calorimetrically and spectroscopically measured the free energy ($\Delta G°$), the enthalpy ($\Delta H°$), and the entropy ($\Delta S°$) change associated with its thermally induced helix-to-coil transition. The pro-

Table 1. Nearest-neighbor frequencies

		Nearest neighbors present in duplex									
		AA/TT	AT/TA	TA/AT	CA/GT	GT/CA	CT/GA	GA/CT	CG/GC	GC/CG	GG/CC
Entry no.	Sequence										
1	d(GCGCGC)	0	0	0	0	0	0	0	2	3	0
2	d(CGCGCG)	0	0	0	0	0	0	0	3	2	0
3	d(CGTACG)	0	0	1	0	2	0	0	2	0	0
4	d(ATGCAT)	0	2	0	2	0	0	0	0	1	0
5	d(GCCCGGGC)	0	0	0	0	0	0	0	1	2	4
6	d(GCGATCGC)	0	1	0	0	0	0	2	2	2	0
7	d(CGGTACCG)	0	0	1	0	2	0	0	2	0	2
8	d(GGCATGCC)	0	1	0	2	0	0	0	0	2	2
9	d(CGAGCTCG)	0	0	0	0	0	2	2	2	1	0
10	d(CGTCGACG)	0	0	0	0	2	0	2	3	0	0
11	d(GCAGCTGC)	0	0	0	2	0	2	0	0	3	0
12	d(GTGGCCAC)	0	0	0	2	2	0	0	0	1	2
13	d(GGAATTCC)	2	1	0	0	0	0	2	0	0	2
14	d(GGTATACC)	0	1	2	0	2	0	0	0	0	2
15	d(CATCGATG)	0	2	0	2	0	0	2	1	0	0
16	d(GAAGCTTC)	2	0	0	0	0	2	2	0	1	0
17	d(GCGAATTCGC)	2	1	0	0	0	0	2	2	2	0
18	d(GAAGATCTTC)	2	1	0	0	0	2	4	0	0	0
19	d(ATATATATAT)	0	5	4	0	0	0	0	0	0	0
20	poly(dG)·poly(dC)	0	0	0	0	0	0	0	0	0	15
21	poly[d(GC)]·poly[d(GC)]	0	0	0	0	0	0	0	10	10	0
22	poly[d(AC)]·poly[d(GT)]	0	0	0	10	10	0	0	0	0	0
23	poly[d(AG)]·poly[d(CT)]	0	0	0	0	0	10	10	0	0	0
24	poly(dA)·poly(dT)	20	0	0	0	0	0	0	0	0	0
25	poly[d(AT)]·poly[d(AT)]	0	10	10	0	0	0	0	0	0	0
26	poly[d(AAT)]·poly[d(ATT)]	5	5	5	0	0	0	0	0	0	0
27	poly[d(ATC)]·poly[d(GAT)]	0	5	0	5	0	0	5	0	0	0
28	poly[d(AGC)]·poly[d(GCT)]	0	0	0	5	0	5	0	0	5	0

tocols used to obtain the thermodynamic data have been described in detail (5, 6, 15–23, 26, 27). By combining the data from all 28 sequences, we have been able to resolve thermodynamic profiles for all 10 possible nearest-neighbor Watson–Crick interactions. Table 2 lists these nearest-neighbor values.

Thermodynamic Data. Our calorimetric measurements reveal no significant heat capacity changes for the helix-to-coil transitions of the sequences listed in Table 1. In other words, the tabulated enthalpy values listed in Table 2 are temperature-independent. The free energy data listed in the final column of Table 2 were calculated at 25°C. The $\Delta G°$ values at any other temperature can be calculated by using the tabulated enthalpy and entropy data and the standard thermodynamic relationship:

$$\Delta G° = \Delta H° - T\Delta S°. \quad [1]$$

For example, the relative stability of the AA/TT pair at 37°C is 1.7 kcal [(9.1 kcal) − (310 K)(0.024 kcal K^{-1})] compared with 1.9 kcal at 25°C (1 kcal = 4.184 kJ). Thus, use of Eq. 1 in conjunction with the thermodynamic data in Table 2 allows one to calculate the stability ($\Delta G°$) of each nearest-neighbor interaction at any temperature of interest. In the sections that follow, we describe how such free energy data can be used to predict the stability of any Watson–Crick duplex structure from its primary sequence.

Base Sequence Not Base Composition Determines Stability. Inspection of the thermodynamic data listed in Table 2 reveals that base sequence rather than base composition dictates the energetics ($\Delta H°$) and the relative stabilities ($\Delta G°$) of nearest-neighbor interactions in DNA molecules. For example, the first three entries in Table 2 (AA/TT, AT/TA, and TA/AT) each contain two A·T base pairs and therefore have identical base compositions. Nevertheless, as the data in Table 2 show, these three pairwise interactions exhibit significantly different thermodynamic profiles. Consistent with this observation, we find that the poly(dA)·poly(dT) duplex is more stable than the poly[d(AT)]·poly[d(AT)] duplex despite their identical base compositions. Further support for the dependence of stability on base sequence can be gleaned from a comparison of oligomers 13 and 14 in Table 1. These two self-complementary octamers both form duplexes possessing identical base compositions—namely, 4 guanines, 4 cytosines, 4 adenines, and 4 thymines. Nevertheless, both duplexes exhibit significantly different stabilities. Specifically, $\Delta G° = 9.4$ kcal/mol for disruption of the duplex formed by sequence 13 and $\Delta G° = 7.4$ kcal/mol for disruption of the duplex formed by sequence 14. Several additional examples can be extracted from the sequences listed in Table 1. The significant point is that in evaluating the energetics ($\Delta H°$) and the relative stabilities ($\Delta G°$) of DNA molecules, one must consider not only the base composition but also the base sequence.

Table 2. Nearest-neighbor thermodynamics

Interaction	$\Delta H°$	$\Delta S°$	$\Delta G°$
AA/TT	9.1	24.0	1.9
AT/TA	8.6	23.9	1.5
TA/AT	6.0	16.9	0.9
CA/GT	5.8	12.9	1.9
GT/CA	6.5	17.3	1.3
CT/GA	7.8	20.8	1.6
GA/CT	5.6	13.5	1.6
CG/GC	11.9	27.8	3.6
GC/CG	11.1	26.7	3.1
GG/CC	11.0	26.6	3.1

All values refer to the disruption of the interaction in an existing duplex at 1 M NaCl, 25°C, and pH 7. The units for $\Delta G°$ and $\Delta H°$ are kcal/mol of interaction, whereas the units for $\Delta S°$ are cal/K per mol of interaction (1 cal = 4.184 J).

Predicting Transition Enthalpies for DNA Oligomers. The thermodynamic data in Table 2 allow us to calculate the transition enthalpy of any duplex from knowledge of its base sequence. Scheme I shown below illustrates this calculation for 1 of the 19 oligomers in our basis set, assuming that the helix initiation enthalpy (Δh_i) equals zero (7–9).

Scheme I
Predicting transition enthalpies
of DNA oligomers

$$\Delta H_{total} \stackrel{?}{=} \Delta h_i + \Sigma_x \Delta h_x$$

```
11.0  9.1  9.1  11.0
  ↓    ↓    ↓    ↓
G–G–A–A–T–T–C–C
* * * * * * * *
C–C–T–T–A–A–G–G
   ↑    ↑    ↑
  5.6  8.6  5.6
```

$$\Delta H_{predicted} = 0 + (2 \times 11.0) + (2 \times 9.1) + (2 \times 5.6) + (1 \times 8.6)$$

$$\Delta H_{predicted} = 60.0 \text{ kcal}$$

$$\Delta H_{observed} = 58.3 \text{ kcal}$$

The excellent agreement shown in Scheme I between the predicted and the calorimetrically determined transition enthalpy supports the validity of this calculation method and provides confidence in the nearest-neighbor thermodynamic data listed in Table 2. The corresponding comparisons between the predicted and observed transition enthalpies for other oligomeric duplexes are shown in Table 3. The first six entries in Table 3 represent sequences contained in our basis set of 28. Comparison of the predicted versus the observed values for these six duplexes reveals that we can calculate transition enthalpies with considerable confidence. Howev-

Table 3. Comparison of calculated and observed ΔH_T

	Oligomeric duplex	ΔH_{pred}	ΔH_{obs}
1	GCGCGC CGCGCG	57.1	59.6
2	CGTCGACG GCAGCTGC	60.0	64.1
3	GAAGCTTC CTTCGAAG	56.2	57.4
4	GGAATTCC CCTTAAGG	60.0	58.3
5	GGTATACC CCATATGG	55.6	54.5
6	GCGAATTCGC CGCTTAAGCG	84.0	80.0
7	CAAAAAG GTTTTTC	50.0	49.0
8	CAAACAAAG GTTTGTTTC	62.3	64.5
9	CAAAAAAAG GTTTTTTTC	68.2	68.0
10	CAAATAAAG GTTTATTTC	64.6	58.6
11	CAAAGAAAG GTTTCTTTC	63.4	62.8
12	CGCGTACGCGTACGCG GCGCATGCGCATGCGC	143	158

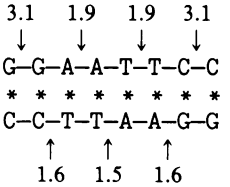
All values refer to the disruption of the duplex in 1 M NaCl at pH 7. The ΔH_{obs} was determined by DSC. The ΔH_{pred} was calculated by using the data in Table 2. The units of ΔH are kcal/mol of interaction.

er, to truly test our predictive powers, we must examine oligomeric duplex sequences not contained in the basis set used to fit the thermodynamic data. From a survey of the literature, we were able to find six oligomeric DNA duplexes for which transition enthalpies have been reported (10, 12, 28). These sequences are listed as duplexes 7–12 in Table 3. Duplexes 7–11 have been studied by Tinoco and coworkers (10, 12), whereas duplex 12 has been studied indirectly by Hilbers and coworkers (28). The excellent agreement between our predicted and their observed transition enthalpies for these non-basis set duplexes provides further confidence in our nearest-neighbor enthalpy data.

Predicting Free Energies for DNA Oligomers. In a manner similar to that described for predicting transition enthalpies, transition free energies can be calculated for any DNA duplex from its primary sequence. However, as shown below, a symmetry term (Δg_{sym}) must be included in the free energy calculation. This term accounts for the entropic difference between a duplex formed from a self-complementary sequence and a duplex formed from two complementary sequences. Scheme **II** illustrates the free energy calculation for one of the octamers in our basis set.

Scheme **II**
Predicting transition free energies
of DNA oligomers

$$\Delta G_{total} \stackrel{?}{=} -(\Delta g_i + \Delta g_{sym}) + \Sigma_x \Delta g_x$$



$$\Delta G_{predicted} = -(5.0 + 0.4) + (2 \times 3.1) + (2 \times 1.6) + (2 \times 1.9) + (1 \times 1.5)$$

$$\Delta G_{predicted} = 9.3 \text{ kcal}$$

$$\Delta G_{observed} = 9.4 \text{ kcal}$$

This calculation assumes that the free energy of a duplex results from the sum of its nearest-neighbor interactions. We have assigned a helix initiation free energy (Δg_i) of 5 kcal for duplexes containing G·C base pairs and 6 kcal for duplexes composed exclusively of A·T base pairs (7–9, 29). For a duplex formed from a self-complementary sequence, Δg_{sym} equals 0.4 kcal, whereas for a duplex formed from two complementary sequences, Δg_{sym} equals 0. The excellent agreement between the predicted and observed free energies shown in Scheme **II** supports the validity of the nearest-neighbor analysis and the assigned free energies of initiation and symmetry. The corresponding comparison between predicted and observed transition free energies for other oligomeric duplexes is shown in Table 4. The first six entries represent sequences contained in the basis set, whereas the final six entries correspond to sequences not found in the basis set. These comparisons reveal that in most cases we can predict duplex free energies with a high level of accuracy. This accuracy is particularly important since the $\Delta G°$ data often are used to predict melting temperatures, which are very sensitive to small variations in free energy.

CONCLUDING REMARKS

Expansion of our thermodynamic library will include characterizations of hairpins (23, 30), cruciforms (30), bulge and interior loops (18, 22), dangling ends (31), selectively modified bases (32), and non-B conformational states (33, 34). This expanded thermodynamic library ultimately will provide us with an empirical basis for predicting the complete secondary structure of a DNA molecule based purely upon its primary sequence. Such a predictive ability is important since sequence-dependent structural preferences can result in the selective formation of specific secondary structural features along local regions of the polymer chain. These local, sequence-specific structural domains may serve as unique binding sites and/or control switches for biological events (35). For example, bulge loops resulting from imperfect sequence complementarity have been proposed as intermediates in frameshift mutagenesis (36, 37), whereas sequences favoring hairpin loops have been found near functional loci in DNA, thereby suggesting a structural basis for control mechanisms (37). Consequently, in evaluating and proposing possible biological roles for specific sequences in naturally occurring DNAs, it would be extremely useful if we could predict the formation of particular secondary structural features along the polymer chain simply by inspecting the primary base sequence. The thermodynamic data that we obtain will make such predictions possible.

Table 4. Comparison of calculated and observed ΔG_T

	Oligomeric duplex	ΔG_{pred}	ΔG_{obs}
1	GCGCGC CGCGCG	11.1	11.1
2	CGTCGACG GCAGCTGC	11.2	11.9
3	GAAGCTTC CTTCGAAG	7.9	8.7
4	GGAATTCC CCTTAAGG	9.3	9.4
5	GGTATACC CCATATGG	6.7	7.4
6	GCGAATTCGC CGCTTAAGCG	16.5	15.5
7	CAAAAAG GTTTTTC	6.1	6.1
8	CAAACAAAG GTTTGTTTC	9.3	10.1
9	CAAAAAAAG GTTTTTTTC	9.9	9.6
10	CAAATAAAG GTTTATTTC	8.5	8.5
11	CAAAGAAAC GTTTCTTTC	9.3	9.5
12	CGCGTACGCGTACGCG GCGCATGCGCATGCGC	32.9	34.1

All values refer to the disruption of the duplex in 1 M NaCl, 25°C, at pH 7. The units of $\Delta G°$ are kcal/mol of interaction.

We thank Drs. Doug Turner and Phil Borer for providing the sequences d(GCGCGC) and d(GGTATACC), respectively. We also thank Prof. Ignacio Tinoco, Jr., for helpful discussions and Dr. Soo Freier for the computer program used to fit the data. This work was supported by the National Institutes of Health (Grants GM 23509 and GM 34469).

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