Nucleic Acids Research, 2006, Vol. 34, No. 13 ***3609–3614***
doi:10.1093/nar/gkl232

Nearest neighbor parameters for Watson–Crick complementary heteroduplexes formed between 2′-*O*-methyl RNA and RNA oligonucleotides

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Received January 20, 2006; Revised January 30, 2006; Accepted March 28, 2006

ABSTRACT

Results from optical melting studies of Watson–Crick complementary heteroduplexes formed between 2′-*O*-methyl RNA and RNA oligonucleotides are used to determine nearest neighbor thermodynamic parameters for predicting the stabilities of such duplexes. The results are consistent with the physical model assumed by the individual nearest neighbor-hydrogen bonding model, which contains terms for helix initiation, base pair stacking and base pair composition. The sequence dependence is similar to that for Watson–Crick complementary RNA/RNA duplexes, which suggests that the sequence dependence may also be similar to that for other backbones that favor A-form RNA conformations.

INTRODUCTION

Oligonucleotides are used for many applications, ranging from diagnostics (1–4) to therapeutics (5–9) to nanotechnology (10,11). The thermodynamics of nucleic acid duplex formation facilitates rational design of sequences for the various applications (12–14). The thermodynamics of duplex formation is dependent on the backbone of the nucleic acid. For example, the sequence dependence of the thermodynamics of DNA/DNA (15,16), RNA/RNA (17,18) and DNA/RNA (19) duplexes differ. All, however, can be approximated well by nearest neighbor models when only Watson–Crick base pairs are formed. Thus, it is relatively easy to predict the thermodynamics of Watson–Crick paired duplexes from sequence (16,18–22). Here, optical melting studies are analyzed to provide nearest neighbor thermodynamic parameters for formation of 2′-*O*-methyl RNA/RNA duplexes that are Watson–Crick complementary. The 2′-*O*-methyl RNA and other 2′-*O*-alkyl backbones are particularly useful for hybridization to RNA because they favor A-form helical structure and are more resistant than RNA or DNA to nuclease digestion (23–26).

MATERIALS AND METHODS

Experimental

Synthesis and purification of oligonucleotides was done as previously described (27). The buffer for melting experiments was 100 mM NaCl, 20 mM sodium cacodylate and 0.5 mM Na_2EDTA, pH 7.0. Oligonucleotide single strand concentrations were determined from absorbances >80°C with extinction coefficients approximated by a nearest neighbor model (28,29). The sequence dependence of extinction coefficients for 2′-*O*-methyl and RNA strands was assumed to be identical. Melting curves were measured at 260 nm with a heating rate of 1°C/min from 0 to 90°C on a Beckman DU640 spectrophotometer with a water cooled Peltier thermoprogrammer. Melting curves were analyzed and thermodynamic parameters were calculated on the basis of a two-state model with the program MeltWin 3.5 (30). With one exception, agreement within 15% was found for thermodynamic parameters calculated from averaging parameters derived from the shapes of melting curves and from the following equation (20):

$$T_M^{-1} = (R/\Delta H^\circ)\ln(C_T/4) + \Delta S^\circ/\Delta H^\circ \quad \mathbf{1}$$

This agreement is consistent with the two-state model.

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Parameter fitting

The measured thermodynamic parameters were fit to the individual nearest neighbor-hydrogen bonding (INN-HB) model (18) by multiple linear regression with the program Analyse-It v.1.71 (Analyse-It Software, Ltd, Leeds, England; www.analyse-it.com), which expands Microsoft Excel. Only duplexes that melted in a two-state manner were included in the fit. Measured parameters from T_M^{-1} versus ln(C_T/4) plots were used as the data for the calculations. Error limits reported for the experimental data reflect the scatter in T_M^{-1}'s when fit to Equation 1. Systematic errors are typically larger, however, and difficult to estimate (18). For example, the melting is not truly two-state because the stacking in the single strand conformations is dependent on temperature and sequence. Therefore, all duplexes included in the fit were given equal weight.

RESULTS

Table 1 lists measured thermodynamic parameters for several 2′-*O*-methyl RNA/RNA duplexes. Only the duplex m(5′-CGAAGUGAA)/r(3′-GCUUCACUU) does not melt in an apparent two-state manner as revealed by a >15% difference between the ΔH°s derived from averaging fits to the shapes of melting curves and from the T_M^{-1} versus ln (C_T/4) plot. With the exception of m(5′-CGAAGUGAA)/r(3′-GCUUCACUU), results in Table 1 were combined with previously reported results (27) listed in Table 2 and fit to the INN-HB nearest neighbor model (18) to give the nearest neighbor thermodynamic parameters listed in Table 3.

On a percentage basis, the errors in nearest neighbor parameters for ΔG°_{37} are much smaller than for ΔH° and ΔS°. This is expected from the high correlation of errors in ΔH° and ΔS°, typically with $R^2 > 0.99$ (18,31,32). While the individual errors in nearest neighbor parameters for ΔH° and ΔS° are large, the percentage errors in predicting ΔH° and ΔS° for duplex formation are smaller. This is because the values of ΔH° and ΔS° are given by the sums of the nearest neighbors, but the errors propagate as the square root of the sum of the squares of the errors. Table 2 lists the predicted values of ΔG°_{37}, ΔH° and ΔS° and their percentage differences from measured values for the oligonucleotides studied. The range of percentage differences is 0–15, 0.04–23 and 0.1–26% for ΔG°_{37}, ΔH° and ΔS°, respectively, while the average differences are, respectively, 2.4, 6.7 and 7.7%. The worst percentage prediction of ΔG°_{37} differs from the measured value by 0.73 kcal/mol, which translates to a difference of 3-fold in measured and predicted association constants. The thermodynamics for the duplex m(5′-CGAAGUGAA)/r(3′-GCUUCACUU) (Table 1) are also predicted reasonably well even though it does not melt in a two-state manner and

Table 1. Thermodynamic parameters of heteroduplex formation between 2′-*O*-methyl RNA and oligoribonucleotides in 0.1 M NaCl, pH 7[a]

2′-*O*-methyl RNA (5′→3′)	RNA (5′→3′)	Average of curve fits				T_M^{-1} versus log(C_T/4) plots			
		$-\Delta H^\circ$ (kcal/mol)	$-\Delta S^\circ$ (eu)	$-\Delta G^\circ_{37}$ (kcal/mol)	T_M^b (°C)	$-\Delta H^\circ$ (kcal/mol)	$-\Delta S^\circ$ (eu)	$-\Delta G^\circ_{37}$ (kcal/mol)	T_M^b (°C)
CAUGGG	CCCAUG	61.6 ± 17.7	173.9 ± 56.1	7.72 ± 0.34	43.1	60.9 ± 1.5	172.3 ± 4.9	7.52 ± 0.01	42.1
ACAACCA	UGGUUGU	49.4 ± 2.7	139.1 ± 8.7	6.20 ± 0.13	34.9	42.7 ± 0.8	117.3 ± 2.6	6.31 ± 0.02	35.4
ACACCCA	UGGGUGU	51.5 ± 5.3	138.5 ± 16.7	8.52 ± 0.15	49.5	47.7 ± 1.5	126.9 ± 4.6	8.38 ± 0.04	49.5
ACAGCCA	UGGCUGU	54.4 ± 2.3	147.4 ± 6.9	8.70 ± 0.14	49.9	50.7 ± 0.8	135.7 ± 2.5	8.56 ± 0.02	49.9
ACCACCA	UGGUGGU	51.1 ± 3.1	137.0 ± 9.4	8.62 ± 0.18	50.2	47.4 ± 2.6	126.5 ± 8.1	8.50 ± 0.09	50.3
ACCGCCA	UGGCGGU	60.0 ± 3.2	158.3 ± 9.6	10.86 ± 0.23	61.1	57.7 ± 2.7	151.4 ± 8.2	10.69 ± 0.16	61.1
ACGACCA	UGGUCGU	53.5 ± 3.6	145.5 ± 11.1	8.40 ± 0.14	48.2	49.6 ± 1.3	133.3 ± 4.1	8.28 ± 0.03	48.3
ACGCCCA	UGGGCGU	54.4 ± 2.3	142.2 ± 7.1	10.32 ± 0.11	60.2	58.9 ± 1.7	155.9 ± 5.2	10.56 ± 0.10	59.8
ACGGCCA	UGGCCGU	57.5 ± 5.2	150.9 ± 15.9	10.71 ± 0.30	61.3	57.5 ± 1.7	151.0 ± 5.0	10.68 ± 0.10	61.1
ACGUACA	UGUACGU	52.5 ± 3.3	146.5 ± 10.5	7.09 ± 0.11	40.3	49.2 ± 1.6	135.8 ± 5.2	7.09 ± 0.02	40.5
ACGUGCA	UGCACGU	58.3 ± 1.5	158.4 ± 4.6	9.20 ± 0.10	51.9	59.1 ± 1.4	160.8 ± 4.4	9.22 ± 0.05	51.8
ACGUUCA	UGAACGU	52.9 ± 0.43	147.9 ± 14.1	7.05 ± 0.14	40.1	47.2 ± 0.8	129.3 ± 2.7	7.09 ± 0.01	40.7
ACUACAU	AUGUAGU	46.5 ± 2.6	132.3 ± 8.7	5.41 ± 0.10	29.7	45.9 ± 1.7	130.4 ± 5.8	5.45 ± 0.07	29.9
ACUACUU	AAGUAGU	52.1 ± 1.9	149.3 ± 5.9	5.78 ± 0.10	32.6	46.9 ± 1.2	132.3 ± 4.1	5.91 ± 0.04	32.9
AUUACCA	UGGUAAU	47.8 ± 1.6	137.0 ± 5.2	5.34 ± 0.11	29.5	44.5 ± 1.3	125.8 ± 4.5	5.47 ± 0.05	29.8
CGGCAUG	CAUGCCG	69.0 ± 3.6	191.7 ± 11.1	9.59 ± 0.15	51.4	65.5 ± 1.1	180.7 ± 3.6	9.48 ± 0.04	51.6
CUUACCA	UGGUAAG	54.9 ± 3.8	154.7 ± 12.1	6.89 ± 0.08	39.0	50.2 ± 0.8	139.6 ± 2.6	6.68 ± 0.01	39.1
GCUAAGG	CCUUAGC	66.5 ± 6.4	188.2 ± 20.7	8.15 ± 0.11	44.7	71.1 ± 2.0	202.0 ± 6.4	8.17 ± 0.04	44.3
GUUACCA	UGGUAAC	55.3 ± 3.5	156.0 ± 11.1	6.88 ± 0.10	39.0	49.9 ± 1.2	138.6 ± 3.9	6.85 ± 0.01	39.0
UUUACCA	UGGUAAA	43.2 ± 3.7	122.2 ± 12.4	5.27 ± 0.21	28.2	43.0 ± 2.1	121.7 ± 7.0	5.28 ± 0.11	28.3
CGAGCAAG	CUUGCUCG	76.3 ± 8.9	213.2 ± 27.6	10.15 ± 0.35	52.4	70.8 ± 4.4	196.0 ± 13.7	10.01 ± 0.17	53.0
CGUUGAAG	CUUCAACG	67.1 ± 11.5	190.3 ± 36.9	8.09 ± 0.09	44.4	76.4 ± 13.1	219.6 ± 41.4	8.27 ± 0.39	44.2
GAGUGAAG	CUUCACUC	78.3 ± 5.1	22.6 ± 16.1	9.33 ± 0.21	48.5	79.6 ± 5.9	226.4 ± 18.8	9.37 ± 0.18	48.5
AGAAGUAAG	CUUACUUCU	84.5 ± 6.8	245.0 ± 22.4	8.49 ± 0.11	44.4	85.6 ± 7.6	248.5 ± 24.1	8.49 ± 0.17	44.3
CCAAGAUUG	CAAUCUUGG	94.2 ± 4.9	271.6 ± 15.3	9.95 ± 0.23	48.7	92.5 ± 5.8	266.4 ± 18.2	9.89 ± 0.17	48.7
CGAAAGAUG	CAUCUUUCG	78.3 ± 3.4	223.9 ± 10.6	8.87 ± 0.12	46.6	75.8 ± 1.6	216.2 ± 5.0	8.80 ± 0.02	46.6
GAAGAUUCG	CGAAUCUUC	79.1 ± 5.1	225.3 ± 16.0	9.21 ± 0.22	47.9	76.1 ± 6.6	216.1 ± 20.8	9.11 ± 0.21	47.9
GAUGUAAGU	ACUUACAUC	81.7 ± 8.4	235.1 ± 26.7	8.76 ± 0.25	45.7	79.9 ± 7.4	229.6 ± 23.7	8.71 ± 0.19	45.7
GGAAUGUAG	CUACAUUCC	87.0 ± 4.8	247.7 ± 14.9	10.19 ± 0.24	50.6	79.9 ± 13.0	225.5 ± 40.6	9.98 ± 0.56	51.0
Non-two-state duplex									
CGAAGUGAA	UUCACUUCG	96.6 ± 6.1	277.6 ± 19.1	10.56 ± 0.23	50.5	77.2 ± 2.4	216.8 ± 7.6	9.99 ± 0.07	51.5

[a]Solutions are 100 mM NaCl, 20 mM sodium cacodylate and 0.5 mM Na_2EDTA, pH 7.
[b]Calculated for 10^{-4} M total strand concentration.

Table 2. Measured and predicted (in parenthesis) ΔG°_{37}, ΔH°, ΔS° and the percentage difference between measured and predicted values for Watson–Crick complementary 2′-*O*-methyl RNA/RNA duplexes in 0.1 M NaCl, pH 7

2′-*O*-methyl RNA (5′→3′)	ΔG°_{37} (kcal/mol)	% of difference	ΔH° (kcal/mol)	Percentage of difference	ΔS° (eu)	Percentage of difference
$C^MG^MG^MC^MA^M$	−6.19 (−6.46)	4.36	−46.0 (−45.19)	1.76	−128.4 (−125.0)	2.65
$U^MC^MG^MG^MC^M$	−6.38 (−6.47)	1.41	−51.5 (−49.63)	3.63	−145.4 (−139.3)	4.20
$C^MA^MU^MG^MG^MG^M$	−7.52 (−6.99)	7.05	−60.9 (−55.80)	8.37	−172.3 (−157.5)	8.59
$C^MG^MG^MC^MA^MU^M$	−6.98 (−7.30)	4.58	−45.3 (−51.52)	13.73	−123.5 (−142.7)	15.55
$G^MC^MA^MU^MG^MG^M$	−7.19 (−7.19)	0.00	−51.9 (−57.33)	10.46	−144.1 (−161.7)	12.21
$G^MU^MU^MC^MG^MG^M$	−6.95 (−6.86)	1.29	−54.6 (−53.63)	1.78	−153.8 (−150.9)	1.89
$G^MG^MC^MA^MU^MG^M$	−7.19 (−7.19)	0.00	−65.2 (−57.33)	12.07	−187.1 (−161.7)	13.58
$U^MC^MG^MG^MC^MA^M$	−8.06 (−8.06)	0.00	−65.5 (−51.70)	21.07	−185.1 (−140.9)	23.88
$U^MU^MC^MG^MG^MC^M$	−7.76 (−7.41)	4.51	−53.5 (−55.06)	2.92	−147.3 (−153.8)	4.41
$A^MC^MA^MA^MC^MC^MA^M$	−6.31 (−6.39)	1.27	−42.7 (−45.94)	7.59	−117.3 (−127.6)	8.78
$A^MC^MA^MC^MC^MC^MA^M$	−8.38 (−8.62)	2.86	−47.7 (−47.34)	0.75	−126.9 (−125.0)	1.50
$A^MC^MA^MG^MC^MC^MA^M$	−8.56 (−9.07)	5.96	−50.7 (−57.27)	12.96	−135.7 (−155.5)	14.59
$A^MC^MA^MU^MC^MC^MA^M$	−6.86 (−6.98)	1.75	−43.5 (−48.12)	10.62	−118.2 (−132.8)	12.35
$A^MC^MC^MA^MC^MC^MA^M$	−8.50 (−8.62)	1.41	−47.4 (−47.34)	0.13	−126.5 (−125.0)	1.19
$A^MC^MC^MG^MC^MC^MA^M$	−10.69 (−10.50)	1.78	−57.7 (−56.47)	2.13	−151.4 (−148.4)	1.98
$A^MC^MC^MU^MC^MC^MA^M$	−9.20 (−9.20)	0.00	−50.9 (−55.05)	8.15	−134.6 (−148.0)	9.96
$A^MC^MG^MA^MC^MC^MA^M$	−8.28 (−8.36)	0.97	−49.6 (−48.49)	2.24	−133.3 (−129.5)	2.85
$A^MC^MG^MC^MC^MC^MA^M$	−10.56 (−10.50)	0.57	−58.9 (−56.47)	4.13	−155.9 (−148.4)	4.81
$A^MC^MG^MG^MC^MC^MA^M$	−10.68 (−10.54)	1.31	−57.5 (−57.25)	0.43	−151.0 (−150.8)	0.13
$A^MC^MG^MU^MA^MC^MA^M$	−7.09 (−6.89)	2.82	−49.2 (−46.93)	4.61	−135.8 (−129.2)	4.86
$A^MC^MG^MU^MC^MC^MA^M$	−8.96 (−8.77)	2.12	−55.8 (−52.67)	5.61	−151.1 (−141.7)	6.22
$A^MC^MG^MU^MG^MC^MA^M$	−9.22 (−9.05)	1.84	−59.1 (−57.47)	2.76	−160.8 (−156.2)	2.86
$A^MC^MG^MU^MU^MC^MA^M$	−7.09 (−6.93)	2.26	−47.2 (−49.22)	4.28	−129.3 (−136.5)	5.57
$A^MC^MU^MA^MA^MC^MA^M$	−5.23 (−5.09)	2.68	−48.9 (−47.91)	2.02	−140.9 (−138.1)	1.99
$A^MC^MU^MA^MC^MA^MU^M$	−5.45 (−5.38)	1.28	−45.9 (−46.76)	1.87	−130.4 (−133.5)	2.38
$A^MC^MU^MA^MC^MC^MA^M$	−7.13 (−7.32)	2.66	−44.4 (−49.31)	11.06	−120.2 (−135.5)	12.73
$A^MC^MU^MA^MC^MC^MC^M$	−8.29 (−8.51)	2.65	−61.2 (−56.12)	8.30	−170.4 (−153.6)	9.86
$A^MC^MU^MA^MC^MC^MG^M$	−8.04 (−8.08)	0.50	−53.0 (−56.71)	7.00	−145.3 (−156.9)	7.98
$A^MC^MU^MA^MC^MC^MU^M$	−7.37 (−7.60)	3.12	−62.2 (−53.69)	13.68	−176.8 (−148.7)	15.89
$A^MC^MU^MA^MC^MG^MU^M$	−7.05 (−7.17)	1.70	−50.9 (−51.31)	0.81	−141.3 (−142.4)	0.78
$A^MC^MU^MA^MC^MU^MU^M$	−5.91 (−5.76)	2.54	−46.9 (−50.24)	7.12	−132.3 (−143.5)	8.47
$A^MC^MU^MA^MG^MC^MA^M$	−8.22 (−7.77)	5.47	−75.2 (−59.24)	21.22	−216.0 (−166.0)	23.15
$A^MC^MU^MA^MU^MC^MA^M$	−5.82 (−5.68)	2.41	−52.1 (−50.09)	3.86	−149.2 (−143.3)	3.95
$A^MC^MU^MC^MA^MC^MA^M$	−7.22 (−7.13)	1.25	−63.6 (−48.82)	23.24	−181.7 (−134.5)	25.98
$A^MC^MU^MC^MC^MC^MA^M$	−9.51 (−9.20)	3.26	−55.9 (−55.05)	1.52	−149.5 (−148.0)	1.00
$A^MC^MU^MC^MG^MC^MA^M$	−9.04[a] (−9.01)	0.33	−56.0 (−57.95)	3.48	−152.5 (−157.9)	3.54
$A^MC^MU^MC^MU^MC^MA^M$	−7.66 (−7.71)	0.65	−53.0 (−56.53)	6.66	−146.1 (−157.5)	7.80
$A^MC^MU^MG^MA^MC^MA^M$	−7.36 (−7.34)	0.27	−48.2 (−51.87)	7.61	−131.8 (−143.6)	8.95
$A^MC^MU^MG^MC^MC^MA^M$	−9.34 (−9.48)	1.50	−58.7 (−59.85)	1.96	−159.2 (−162.5)	2.07
$A^MC^MU^MG^MG^MC^MA^M$	−9.76 (−9.52)	2.46	−59.6 (−60.63)	1.73	−160.7 (−164.9)	2.61
$A^MC^MU^MG^MU^MC^MA^M$	−7.83 (−7.75)	1.02	−52.5 (−56.05)	6.76	−144.1 (−155.8)	8.12
$A^MC^MU^MU^MA^MC^MA^M$	−5.65 (−5.48)	3.01	−46.7 (−45.86)	1.80	−132.2 (−130.3)	1.44
$A^MC^MU^MU^MC^MC^MA^M$	−7.36 (−7.36)	0.00	−47.6 (−51.60)	8.40	−129.6 (−142.8)	10.19
$A^MC^MU^MU^MG^MC^MA^M$	−7.59 (−7.64)	0.66	−52.7 (−56.40)	7.02	−145.3 (−157.3)	8.26
$A^MC^MU^MU^MU^MC^MA^M$	−5.76 (−5.52)	4.17	−39.6 (−48.15)	21.59	−109.2 (−137.6)	26.01
$A^MG^MU^MA^MC^MC^MA^M$	−7.33 (−7.53)	2.73	−44.5 (−53.96)	21.26	−119.9 (−149.8)	24.94
$A^MU^MU^MA^MC^MC^MA^M$	−5.47 (−5.33)	2.56	−44.5 (−45.16)	1.48	−125.8 (−128.6)	2.23
$C^MC^MU^MA^MC^MC^MA^M$	−9.07 (−8.80)	2.98	−60.5 (−55.01)	9.07	−165.9 (−149.1)	10.13
$C^MG^MG^MC^MA^MU^MG^M$	−9.48 (−9.54)	0.63	−65.5 (−66.80)	1.98	−180.7 (−184.7)	2.21
$C^MU^MU^MA^MC^MC^MA^M$	−6.86 (−6.96)	1.46	−50.2 (−51.56)	2.71	−139.6 (−143.9)	3.08
$G^MC^MU^MA^MA^MG^MG^M$	−8.17 (−8.25)	0.98	−71.1 (−71.13)	0.04	−202.0 (−202.7)	0.35
$G^MC^MU^MA^MC^MC^MA^M$	−9.78 (−9.04)	7.57	−54.3 (−57.32)	5.56	−146.7 (−155.7)	6.13
$G^MC^MU^MA^MC^MU^MG^M$	−8.51 (−8.78)	3.17	−66.2 (−68.10)	2.87	−186.0 (−191.2)	2.80
$G^MG^MC^MA^MU^MG^MG^M$	−9.69 (−10.01)	3.30	−64.1 (−66.99)	4.51	−175.6 (−183.8)	4.67
$G^MG^MU^MA^MU^MG^MG^M$	−8.34 (−8.47)	1.56	−57.2 (−63.68)	11.33	−157.4 (−178.1)	13.15
$G^MU^MU^MA^MC^MC^MA^M$	−6.85 (−6.60)	3.65	−49.9 (−48.45)	2.91	−138.6 (−135.0)	2.60
$U^MC^MU^MA^MC^MC^MA^M$	−7.18 (−7.62)	6.13	−45.2 (−52.64)	16.46	−122.5 (−145.3)	18.61
$U^MU^MU^MA^MC^MC^MA^M$	−5.28 (−5.43)	2.84	−43.0 (−44.26)	2.93	−121.7 (−125.4)	3.04
$U^MU^MU^MC^MA^MC^MU^M$	−4.79 (−5.52)	15.24	−56.4 (−48.15)	14.63	−166.3 (−137.6)	17.26
$C^MG^MA^MG^MC^MA^MA^MG^M$	−10.01 (−10.17)	1.60	−70.8 (−79.80)	12.71	−196.0 (−224.4)	14.49
$C^MG^MU^MU^MG^MA^MA^MG^M$	−8.27 (−8.50)	2.78	−76.4 (−73.65)	3.60	−219.6 (−210.0)	4.37
$G^MA^MG^MU^MG^MA^MA^MG^M$	−9.37 (−9.08)	3.09	−79.6 (−78.46)	1.43	−226.4 (−223.5)	1.28
$A^MG^MA^MA^MG^MU^MA^MA^MG^M$	−8.49 (−8.34)	1.77	−85.6 (−85.30)	0.35	−248.5 (−248.0)	0.20
$C^MC^MA^MA^MG^MA^MU^MU^MG^M$	−9.89 (−9.49)	4.04	−92.5 (−77.98)	15.70	−266.4 (−220.8)	17.12
$C^MG^MA^MA^MA^MG^MA^MU^MG^M$	−8.80 (−8.84)	0.45	−75.8 (−81.18)	7.10	−216.2 (−233.1)	7.82
$G^MA^MA^MG^MA^MU^MU^MC^MG^M$	−9.11 (−9.19)	0.88	−76.1 (−76.64)	0.71	−216.1 (−217.4)	0.60
$G^MA^MU^MG^MU^MA^MA^MG^MU^M$	−8.71 (−9.12)	4.71	−79.9 (−75.03)	6.10	−229.6 (−212.4)	7.49
$G^MG^MA^MA^MU^MG^MU^MA^MG^M$	−9.98 (−10.07)	0.90	−79.9 (−81.21)	1.64	−225.5 (−229.3)	1.69

[a]The error in this value is 0.07 kcal/mol, but was originally reported as 0.7 kcal/mol (27).

Table 3. Thermodynamic parameters for INN-HB nearest neighbor model applied to 2′-*O*-methyl RNA/RNA heteroduplexes in 0.1 M NaCl, pH 7

Parameters	ΔG°_{37} [a] (kcal/mol)	ΔH° (kcal/mol)	ΔS° [b] (eu)	Number of occurrences
m(5′-AA)/r(3′-UU)	−0.55 ± 0.15 (−0.93)	−7.48 ± 3.20	−22.3 ± 9.8	14
m(5′-AU)/r(3′-UA)	−0.84 ± 0.13 (−1.10)	−6.33 ± 2.85	−17.7 ± 8.8	17
m(5′-UU)/r(3′-AA)	−0.94 ± 0.11 (−0.93)	−5.43 ± 2.41	−14.5 ± 7.4	19
m(5′-UA)/r(3′-AU)	−1.20 ± 0.15 (−1.33)	−6.47 ± 3.31	−17.0 ± 10.2	25
m(5′-AC)/r(3′-UG)	−1.60 ± 0.16 (−2.24)	−6.32 ± 3.47	−15.2 ± 10.7	62
m(5′-AG)/r(3′-UC)	−1.81 ± 0.16 (−2.08)	−13.94 ± 3.47	−39.1 ± 10.7	17
m(5′-CA)/r(3′-GU)	−1.89 ± 0.15 (−2.11)	−5.21 ± 3.33	−10.7 ± 10.2	56
m(5′-UC)/r(3′-AG)	−1.90 ± 0.16 (−2.35)	−9.65 ± 3.51	−25.0 ± 10.8	20
m(5′-UG)/r(3′-AC)	−1.94 ± 0.16 (−2.11)	−12.14 ± 3.56	−32.9 ± 10.9	19
m(5′-GA)/r(3′-CU)	−2.06 ± 0.18 (−2.35)	−5.77 ± 3.90	−11.9 ± 12.0	14
m(5′-CU)/r(3′-GA)	−2.17 ± 0.16 (−2.08)	−9.59 ± 3.40	−23.9 ± 10.4	34
m(5′-GU)/r(3′-CA)	−2.17 ± 0.16 (−2.24)	−6.62 ± 3.37	−14.3 ± 10.4	16
m(5′-CG)/r(3′-GC)	−2.35 ± 0.16 (−2.36)	−9.47 ± 3.48	−23.0 ± 10.7	22
m(5′-CC)/r(3′-GG)	−2.78 ± 0.10 (−3.26)	−8.88 ± 2.15	−19.7 ± 6.6	35
m(5′-GG)/r(3′-CC)	−2.82 ± 0.17 (−3.26)	−9.66 ± 3.69	−22.1 ± 11.4	19
m(5′-GC)/r(3′-CG)	−3.02 ± 0.18 (−3.42)	−11.19 ± 3.90	−26.3 ± 12.0	23
Initiation	3.32 ± 0.55 (4.09)	−12.80 ± 11.98	−52.0 ± 36.8	68
Per terminal AU	0.30 ± 0.08 (0.45)	3.14 ± 1.82	9.1 ± 5.6	92

[a]Values in parentheses are for RNA/RNA duplexes in 1 M NaCl [Xia *et al*. (18)].
[b]Calculated from $\Delta S^{\circ} = (\Delta H^{\circ} - \Delta G^{\circ}_{37})/310.15$ and given in eu = cal K^{-1} mol^{-1}.

was not used in fitting the nearest neighbor parameters. The predicted values for ΔG°_{37}, ΔH° and ΔS° are −9.87 kcal/mol, −78.3 kcal/mol and −220.6 eu while the measured values are −10.56 kcal/mol, −96.6 kcal/mol and −277.6 eu, respectively.

DISCUSSION

Thermodynamic parameters for nucleic acid duplexes are useful for designing sequences for many applications, including diagnostics, therapeutics and nanotechnology (12–14,33). The 2′-*O*-methyl backbone and other 2′-*O*-alkyl backbones are particularly useful for binding to RNA because they favor A-form helixes and are chemically stable relative to DNA and RNA backbones. Thus for example, 2′-*O*-alkyl backbones have been used to flank a 'gap' DNA sequence in order to decrease nuclease digestion of the oligonucleotide while providing a long enough pairing between DNA and RNA to induce RNase H to cleave an RNA target (34). Reduction of gene expression by an RNA interference mechanism has been demonstrated with siRNA duplexes having completely 2′-*O*-methyl modified sense strands (35). The 2′-*O*- methyl modification is also used in aptamers (7), including the commercially successful therapeutic, Macugen (8).

There are several ways to analyze the data in Table 2. In principle, it is possible to fit the data to 20 parameters each for ΔG°_{37}, ΔH° and ΔS° (21,22). We chose, however, to fit the data to the 18 parameters of the INN-HB model, which ascribes separate parameters to the 16 different nearest neighbor stacks, to the average difference between a CG and UA pair, and to duplex initiation (18). This simplifies the 20 parameter model by assuming that the parameters for initiation are independent of the nature of the terminal base pairs and that terminal base pairs are equivalent to internal base pairs. With the 18 parameter fit, the highest *P*-values for ΔG°_{37} were 0.0007 and 0.0005 for the terminal AU and m(5′-AA)/r(3′-UU) parameters, respectively. All other *P*-values were <0.0001. For ΔH°, however, *P*-values were 0.29, 0.15 and 0.12 for the initiation, m(5′-GA)/r(3′-CU), and m(5′-CA)/r(3′-GU) parameters, respectively. After correction for salt dependence, the parameters in Table 3 can be coupled with the existing parameters for RNA loops and incorporated into dynamic programming algorithms for predicting and designing secondary structures (14,17,36–38) and can be directly compared with the 12 INN-HB parameters for RNA (18).

A comparison of the ΔG°_{37} parameters for 2′-*O*-methyl RNA/RNA duplexes at 0.1 M NaCl in Table 3 with ΔG°_{37} parameters determined for RNA/RNA duplexes at 1 M NaCl (18) shows that on average the RNA/RNA parameters for ΔG°_{37} of base pair stacks are more favorable by a value of 0.26 kcal/mol (Table 3). This is likely because of higher salt concentration. The sequence dependence of stability is similar for 2′-*O*-methyl RNA/RNA and RNA/RNA duplexes. For example, base pair stacks with two AU pairs are less stable than base pair stacks with one AU and one GC pair; the 5′-CG/3′-GC nearest neighbor has stability similar to nearest neighbors with one AU and one GC pair, but other stacks with two GC pairs are more stable. The parameters for terminal AU pairs are 0.30 and 0.45 kcal/mol for 2′-*O*-methyl RNA/RNA and RNA/RNA duplexes, respectively. In the INN-HB model, this parameter is used to account for the fact that two sequences can have the same nearest neighbor base pair stacks but differ by one in the number of AU and GC pairs (18). Thus it accounts for half of the difference in hydrogen bonding free energy between AU and GC pairs. The magnitude and sign of the 0.30 kcal/mol is consistent with this physical interpretation of the model. The similarity of the sequence dependence of parameters suggests that the programs for predicting RNA secondary structure (17,36–38) can approximate 2′-*O*-methyl modified nucleotides as unmodified RNA, at least in base paired regions. This is because the uncertainties in the sequence dependence of loop stabilities is probably larger

than the difference in sequence dependence of RNA/RNA and 2′-*O*-methyl RNA/RNA base pairs. Moreover, natural RNA typically has relatively few 2′-*O*-methyl substitutions.

The similarity of the free energy parameters for 2′-*O*-methyl RNA/RNA and RNA/RNA duplexes also suggests that either set may be useful for predicting the sequence dependence of stabilities of base paired duplexes with other backbones that favor A-form RNA conformations. This would include other 2′-*O*-alkyl, 2′-fluoro and N3′–P5′ phosphoramidate backbones (24,39,40) and chimeras that include a mixture of such backbones. For example, hammerhead ribozymes and aptamers have been designed with a mixture of RNA and 2′-fluoro backbones in order to increase resistance to nuclease digestion (8,41,42). The predictions would be rough approximations, but sufficient for many applications. As expected, 2′-*O*-methyl RNA/RNA duplexes appear more stable than DNA/RNA duplexes. The nearest neighbour stacking parameters for 2′-*O*-methyl RNA/RNA in 0.1 M NaCl average 0.4 kcal/mol more stable at 37°C than those for DNA/RNA duplexes in 1 M NaCl (19).

The average error limits for the 2′-*O*-methyl RNA/RNA nearest neighbor parameters for ΔG°_{37} and ΔH° are 0.17 and 3.7 kcal/mol, respectively (Table 3). These are roughly 2- and 5-fold larger than the average error limits for RNA/RNA (18) and DNA/DNA (16) parameters, respectively, where only 12 parameters can be fit and the number of duplexes is larger. Direct comparison with 17 and 18 parameter fits to data for DNA/RNA duplexes is not possible because error limits for individual parameters were not reported (19,22).

The error limits for the nearest neighbor parameters for ΔH° and ΔS° for 2′-*O*-methyl RNA/RNA are large. There are many potential reasons why the nearest neighbor model does not fit the experimental ΔH° and ΔS° values particularly well. The two-state model for fitting the melting curve data is an approximation (43–46). The high temperature state for a duplex transition is the single strand, but stacking in these single strands is temperature dependent, thereby producing a temperature dependent ΔH° that is not included in the model. While the agreement between ΔH°s determined by fitting melting curves and by plotting T_M^{-1} versus $\ln(C_T/4)$ is consistent with two-state behavior over the temperature range of the transition, it is not sufficient to insure two-state behavior even over this range. In addition, the INN-HB nearest neighbor model is a simple approximation. The thermodynamics may depend on more than the stacking of nearest neighbors and number of hydrogen bonds. In general, parameters obtained from optical melting data are best near the melting temperatures of the oligonucleotides. The range of melting temperatures at 0.1 mM total strand concentration for the duplexes used to determine the nearest neighbor parameters range from 28 to 55°C with an average of 44°C. Thus the predicted ΔG°s should be reasonable from ~25 to 65°C.

ACKNOWLEDGEMENTS

This work was supported by Polish State Committee for Scientific Research (KBN) grant 2 PO4A03729 to R.K., NIH grant 1RO3 TW1068 to R.K. and D.H.T., and NIH grant GM22939 to D.H.T. A.C. is a recipient of a fellowship from the President of the Polish Academy of Sciences. Funding to pay the Open Access publication charges for this article was provided by NIH.

Conflict of interest statement. None declared.

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