

# **Conformation transition of Nucleic Acids**

# **Helix to coil transition: melting study of nucleic acids**

- **Why are we interested in thermodynamic data from melting studies?**
  1. Thermodynamic data provided the fundamental basis for defining the nature of the inter- and intramolecular forces that stabilize single- and double-stranded DNA and RNA structures (data to fit the models for helix to coil transition);
  2. To interpret data from other studies such as kinetic studies and conformation transitions;
  3. Drug-DNA interactions;
  4. Protein-DNA interactions;
  5. Molecular biology (cloning or PCR);
  6. Prediction of DNA conformation from the sequences;
  7. More.

### THERMALLY INDUCED HELIX – COIL TRANSITION

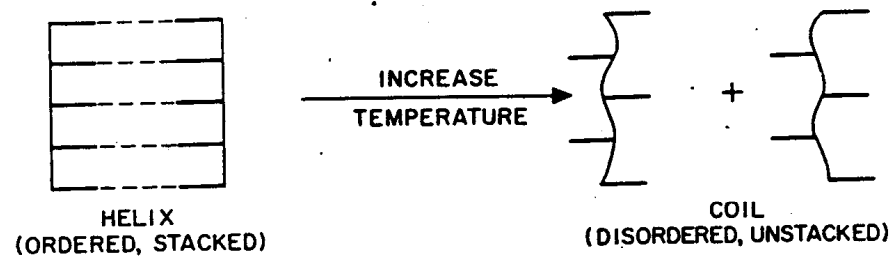
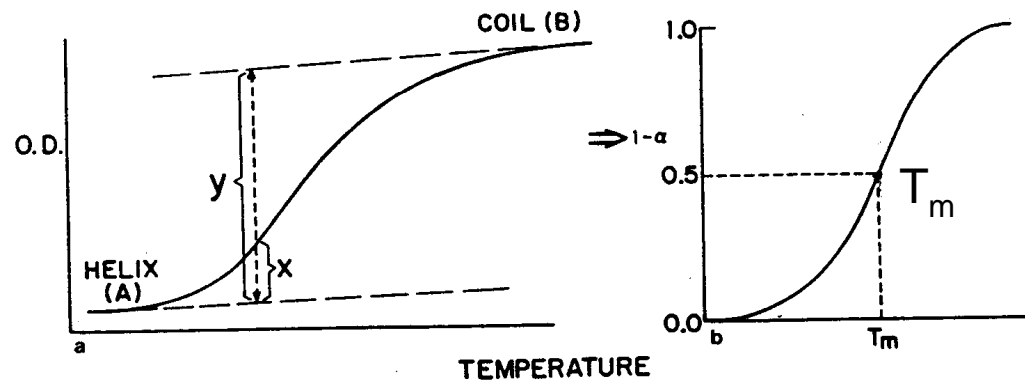


Fig. 1. Schematic representation of a thermally induced transition from a fully base-paired and base-stacked double helix to two unpaired and unstacked single strands



Note:  $1-\alpha = \frac{x}{y}$  = fraction in coil (B) state

Fig. 2. a Schematic of an optical density (O.D.) vs. temperature profile (a UV melting curve) showing the hyperchromic effect associated with a transition from a base stacked (helix) to an unstacked (coil) state. b A derived melting curve showing the fraction in the coil state ( $1-\alpha$ ) as a function of temperature. The two figures together illustrate how the experimental absorbance vs. temperature profile can be converted into a ( $1-\alpha$ ) versus temperature melting curve

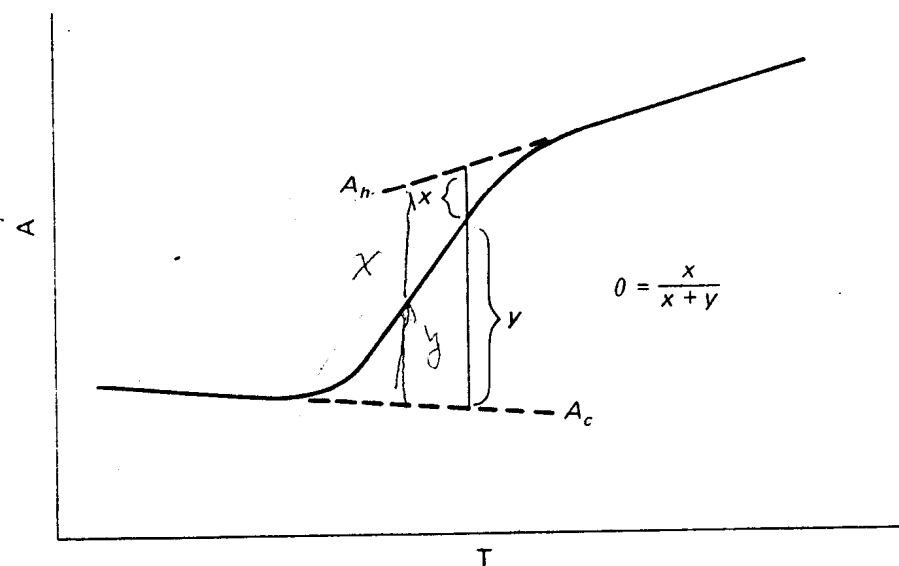
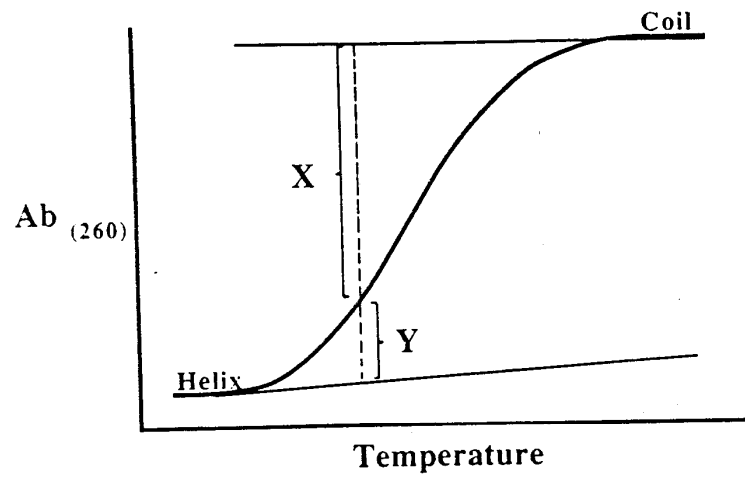


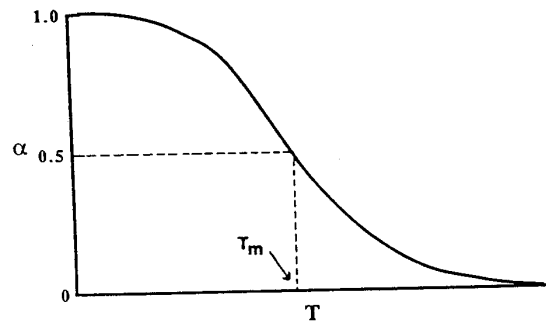
FIGURE 6-4 Graphical construction for calculating fraction helix ( $\theta$ ) when the absorbance of helix and coil forms depends linearly on temperature.

**Table 1.** Dependence of van't Hoff transition enthalpies on lower baseline selection

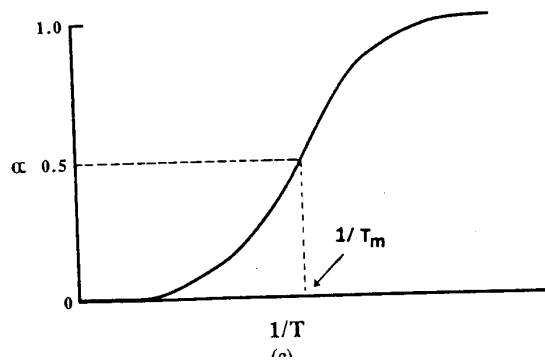
Oligomer sequence	Method of analysis	$\Delta H_{vH}$ (kJ)	Reference
$rA_7U_7$	Eq. (5) using a flat lower baseline	-240	[37]
$rA_7U_7$	Eq. (5) using a sloping lower baseline	-294	[37]
$d(GC)_3$	Eq. (5) using a flat lower baseline	-173	[38]
$d(GC)_3$	Eq. (5) using a sloping lower baseline	-238	[38]
$dCA_5G + dCT_5G$	Eq. (5) using a flat lower baseline	-176	[62]
$dCA_5G + dCT_5G$	Eq. (5) using a sloping lower baseline	-213	[62]



(a)



(b)



(c)

$$\alpha = X/(X+Y)$$

$$\Delta H_{VH} = RT^2 \left[ \frac{d \ln K}{dT} \right] \quad \text{or} \quad \Delta H_{VH} = -R \left[ \frac{d \ln K}{d(1/T)} \right]$$

**For two state transition:**

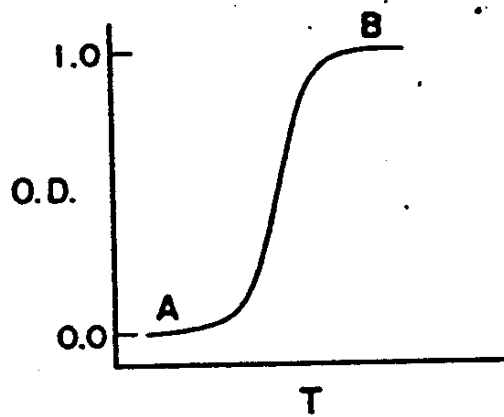
$$\Delta H_{VH} = (2 + 2n)RT^2 \left[ \frac{d\alpha}{dT} \right]_{T=T_m}$$

or

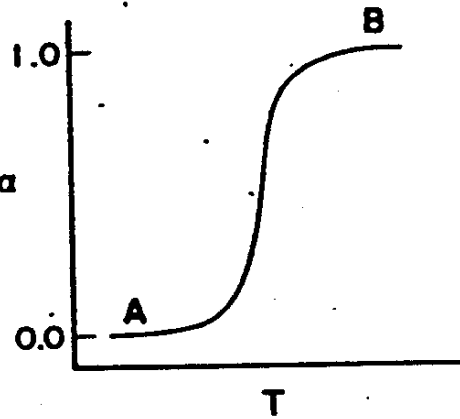
$$\Delta H_{VH} = -(2 + 2n)R \left[ \frac{d\alpha}{d(1/T)} \right]_{T=T_m}$$

R = 1.987 caldeg<sup>-1</sup>mol<sup>-1</sup>; unit for  $\Delta H_{VH}$  is kcal/mol  
Mole of what? “cooperative unit”

### A COOPERATIVE TRANSITION

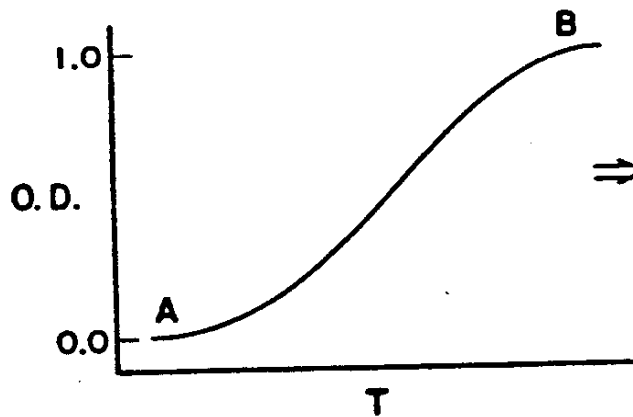


$\Rightarrow 1-a$

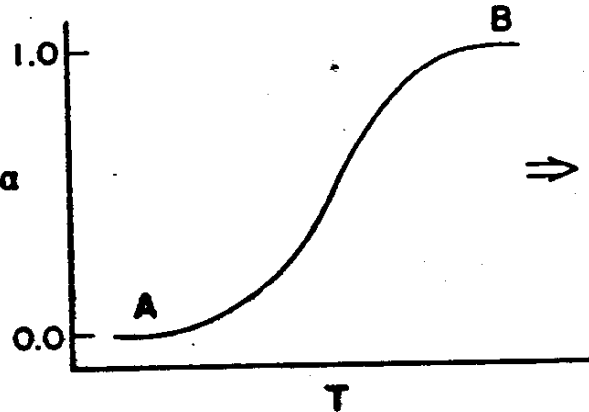


$\Rightarrow \left(\frac{\partial a}{\partial T}\right)_{T_m}$  is high  
 $\therefore \Delta H_{V.H.}$  is high

### A NON-COOPERATIVE TRANSITION



$\Rightarrow 1-a$



$\Rightarrow \left(\frac{\partial a}{\partial T}\right)_{T_m}$  is low  
 $\therefore \Delta H_{V.H.}$  is low

# Calculating $\Delta H_{vH}$ from the concentration dependence of the melting temperature

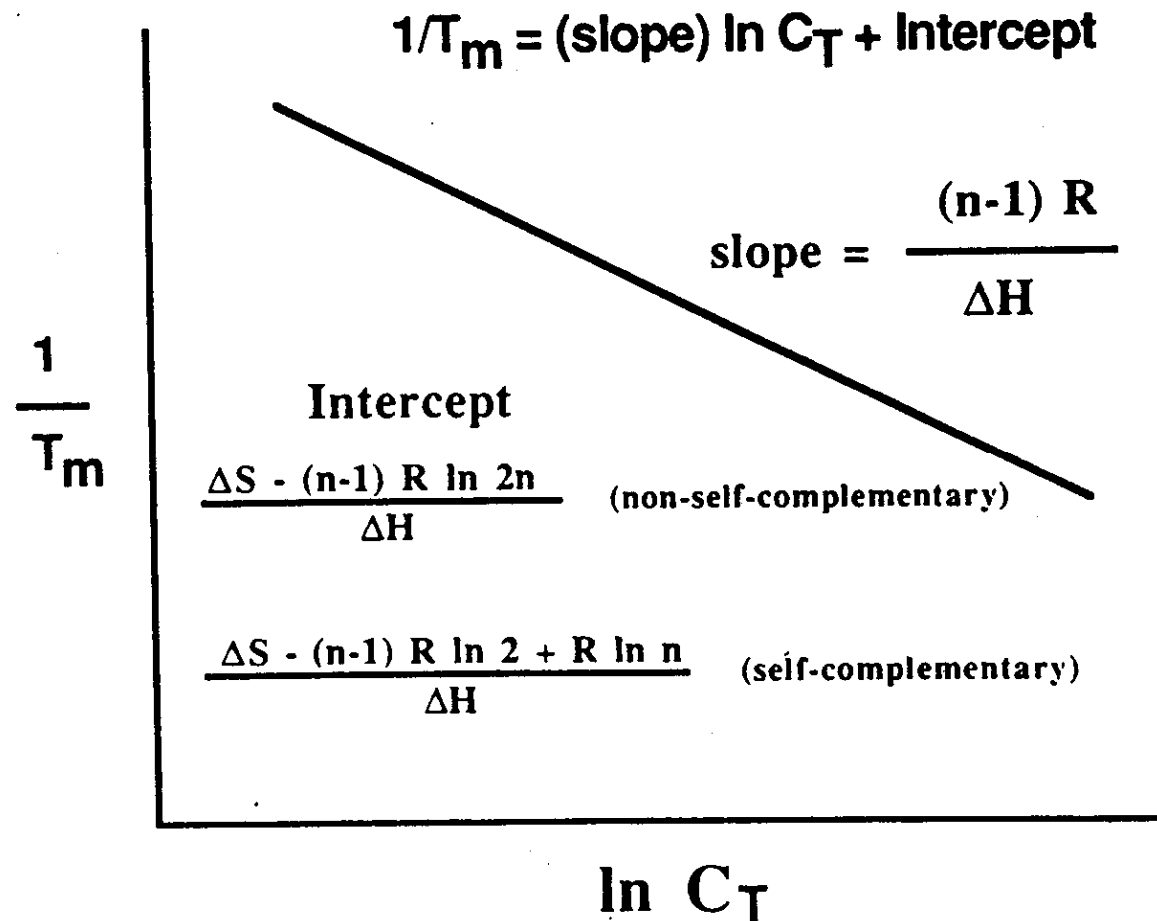
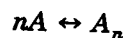


TABLE II  
TWO-STATE ANALYSIS OF NUCLEIC ACID TRANSITIONS

Reaction type	Equilibrium constants	$\Delta H^\circ$ from slope of $f$ versus $T$	Concentration dependence of $T_m$
Monomolecular $S = H$	$K = \frac{[H]}{[S]} = \frac{f}{(1-f)}$	$\Delta H^\circ = 4RT_m^2 \left( \frac{df}{dT} \right)_{T=T_m}$	
Bimolecular (self-complementary) $2S = D$	$K = \frac{[D]}{[S]^2} = \frac{f}{2(1-f)^2 c_i}$	$\Delta H^\circ = 6RT_m^2 \left( \frac{df}{dT} \right)_{T=T_m}$	$\frac{1}{T_m} = \frac{R}{\Delta H^\circ} \ln c_i + \frac{\Delta S^\circ}{\Delta H^\circ}$
Bimolecular (non-self-complementary) $S_A + S_B = D$	$K = \frac{[D]}{[S_A][S_B]} = \frac{2f}{(1-f)^2 c_i}$	$\Delta H^\circ = 6RT_m^2 \left( \frac{df}{dT} \right)_{T=T_m}$	$\frac{1}{T_m} = \frac{R}{\Delta H^\circ} \ln c_i + \frac{(\Delta S^\circ - R \ln 4)}{\Delta H^\circ}$
Trimolecular (identical strands) $3S = T$	$K = \frac{[T]}{[S]^3} = \frac{f}{3c_i^2(1-f)^3}$	$\Delta H^\circ = 8RT_m^2 \left( \frac{df}{dT} \right)_{T=T_m}$	$\frac{1}{T_m} = \frac{2R}{\Delta H^\circ} \ln c_i + \frac{[\Delta S^\circ - R \ln 4/3]}{\Delta H^\circ}$
Trimolecular (nonidentical strands) $S_A + S_B + S_C = T$	$K = \frac{[T]}{[S_A][S_B][S_C]} = \frac{9f}{c_i^3(1-f)^3}$	$\Delta H^\circ = 8RT_m^2 \left( \frac{df}{dT} \right)_{T=T_m}$	$\frac{1}{T_m} = \frac{2R}{\Delta H^\circ} \ln c_i + \frac{(\Delta S^\circ - 2R \ln 6)}{\Delta H^\circ}$
Tetramolecular (identical strands) $4S = Q$	$K = \frac{[Q]}{[S]^4} = \frac{f}{4c_i^3(1-f)^4}$	$\Delta H^\circ = 10RT_m^2 \left( \frac{df}{dT} \right)_{T=T_m}$	$\frac{1}{T_m} = \frac{3R}{\Delta H^\circ} \ln c_i + \frac{(\Delta S^\circ - R \ln 2)}{\Delta H^\circ}$
Tetramolecular (nonidentical strands) $S_A + S_B + S_C + S_D = Q$	$K = \frac{[Q]}{[S_A][S_B][S_C][S_D]} = \frac{64f}{c_i^4(1-f)^4}$	$\Delta H^\circ = 10RT_m^2 \left( \frac{df}{dT} \right)_{T=T_m}$	$\frac{1}{T_m} = \frac{3R}{\Delta H^\circ} \ln c_i + \frac{(\Delta S^\circ - 3R \ln 8)}{\Delta H^\circ}$

# Calculating $\Delta H_{\text{VH}}$ from differential equilibrium melting curve



the relevant general forms of the van't Hoff equation are

$$\Delta H_{\text{VH}} = \frac{B}{(1/T_1) - (1/T_2)} \quad \left( \text{for the full width at the half-height} \right)$$

$$\Delta H_{\text{VH}} = \frac{B'}{(1/T_{\text{max}}) - (1/T_2)} \quad \left( \text{for the upper half width at the half-height} \right)$$

TABLE I

Values of the Constants  $B$  and  $B'$  in Eqs. (10) and (11) for Association Reactions Exhibiting Molecularities ( $n$ ) Between 1 and 5.  $B$  and  $B'$  in cal/K-mol.

$n$	$-B$	$-B'$
1	7.00	3.50
2	10.14	4.38
3	12.88	5.06
4	15.40	5.63
5	17.79	6.14

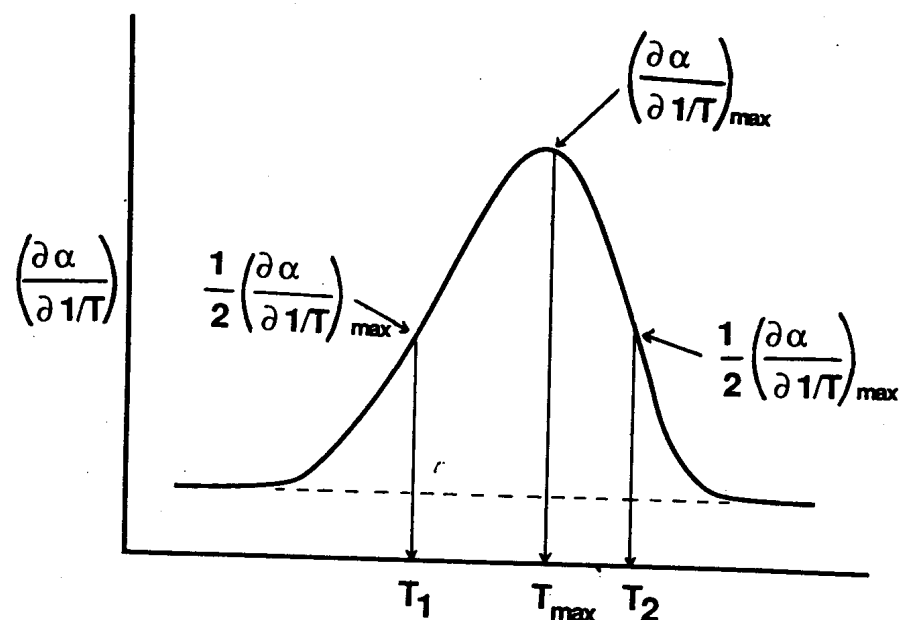


Fig. 3. A typical differentiated melting curve. Several reference temperature points are specifically defined in the figure and described in the text.

**Table 4.** Comparison of various van't Hoff transition enthalpies for selected DNA oligomers

Oligomer sequence	$\Delta H_{vH}^{\text{con. dep. a}}$	$\Delta H_{vH}^{\text{shape b}}$	$\Delta H_{vH}^{\text{cal c}}$
ATGCAT	163	164	146
GCGCGC	240	238	217
CGCGCG	180	209	192
CGTACG	191	183	197
GGAATTCC	245	225	248
GCGAATTCGC	301	318	362
ATATATATAT	344	144	171
TATGCGCGCATA	238	234	310
CGCGAATTCGCG	273	272	318

<sup>a</sup> Calculated using Eq. (9).

<sup>b</sup> Calculated using Eq. (5).

<sup>c</sup> Calculated using Eq. (8).

$\Delta H_{vH}$  in kJ (mol cooperative unit)<sup>-1</sup>.

## Calculating thermodynamic parameters ( $\Delta G^0$ , $\Delta H^0$ , and $\Delta S^0$ ) from noncalorimetric melting curves

If we assume  $\Delta H_{VH}$  is independent from the temperature,  $\Delta H^0$  can be calculated from the following van't Hoff equation:

$$\ln[K(T_m)/K(T)] = \Delta H^0/R(1/T - 1/T_m)$$

$$\Delta G^0 = -RT \ln K$$

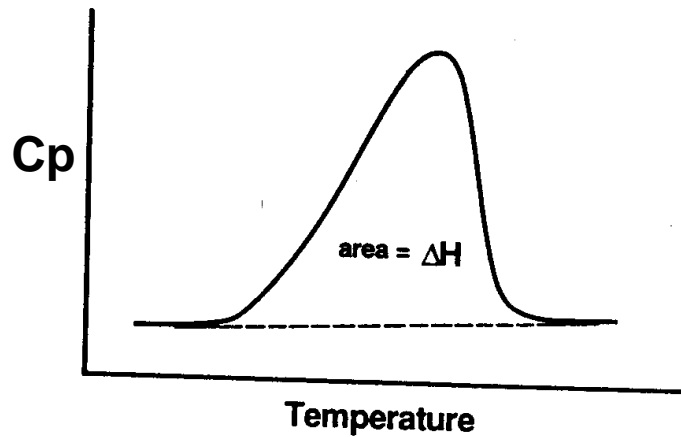
$$\Delta G^0 = \Delta H^0 - T\Delta S^0$$

If  $\Delta H_{VH}$  was determined,  $\Delta S^0 = \Delta H^0/T_m$ ,

$$\Delta G^0 = \Delta H^0 - T\Delta S^0$$

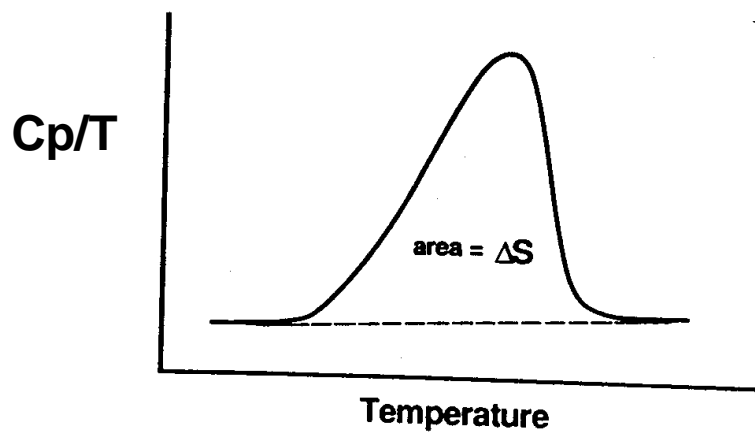
Note: We assume the transition is a two-state transition (helix to coil).

# Obtaining thermodynamic data from Differential Scanning Calorimetry (DSC)



(a)

$$\Delta H^0 = \int C_p dT$$



(b)

$$\Delta S^0 = \int (C_p / T) dT$$

$$\Delta G^0 = \Delta H^0 - \Delta S^0$$

**Table 3.** Comparison of calorimetric and optical  $\Delta H^0$  and  $\Delta S^0$  values<sup>a</sup>

Oligomer sequences	Transition	Optical data <sup>b</sup>		Calorimetric data	
		$\Delta H_{vH}^0$	$\Delta S_{vH}^0$	$\Delta H_{cal}^0$	$\Delta S_{cal}^0$
(GC) <sub>3</sub>	Two-state	240	659	249	649
GGAATTCC	Two-state	245	699	244	799
CGTACG	Two-state	191	544	203	640
rA <sub>7</sub> U <sub>7</sub>	Multi-state	317	931	415	1163
GCGAATTCGC	Multi-state	301	816	335	1151
CGCGAATTCGCG	Multi-state	273	711	427	1246
CGTGAATTCGCG	Multi-state	330	933	444	1277
CGCAGAATTCGCG	Multi-state	284	791	435	1364
TATGCGCGCATA	Multi-state	238	611	312	872

<sup>a</sup>  $\Delta H_{vH}^0$  in kJ (mol cooperative unit)<sup>-1</sup>,  $\Delta H_{cal}^0$  in kJ (mol duplex)<sup>-1</sup>,  $\Delta S_{vH}^0$  in J (mol cooperative unit · K)<sup>-1</sup>,  $\Delta S_{cal}^0$  in J (mol duplex · K)<sup>-1</sup>.

<sup>b</sup> From Eq. (9).

# Advantages for DSC

- Model-free determination of  $\Delta H^0$  and  $\Delta S^0$ ;
- Direct measure of the heat capacity change  $\Delta C_p^0$ ;
- We can compare the  $\Delta H_{\text{cal}}$  and  $\Delta H_{\text{VH}}$ , and determine the nature of the transition. If  $\Delta H_{\text{VH}} < \Delta H_{\text{cal}}$ , then the transition involves intermediate states; however, if  $\Delta H_{\text{VH}} = \Delta H_{\text{cal}}$ , it is a real two-state transition. The ratio of  $\Delta H_{\text{VH}}/\Delta H_{\text{cal}}$  also provide information for the melting cooperativity.

# Thermodynamic of Single-stranded Stacking

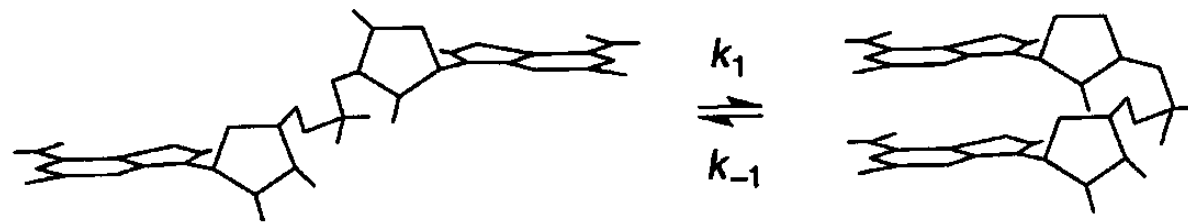


Figure 8-1  
Transition from unstacked to stacked conformation in single-stranded dinucleoside monophosphate.

Since the transition of single strand stacking occur over a very wide range, the enthalpy for their transition are always varied significantly. For example, the enthalpy for poly(A) range from -3 to -13 kcal mol<sup>-1</sup>.

# Non-cooperative transition

- No Interaction between segments



$$s = [b]/[a]$$

$$s = \exp(-\Delta H/RT + \Delta S/R)$$

$\theta$  = fraction unstacked

$$= s/(1+s)$$

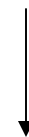
$$d \ln s / dT = \Delta H_u / RT^2, \Delta H_u \text{ is for a segment}$$

~hhhhhh~



step by step

~chhhhh~



~cchhhh~

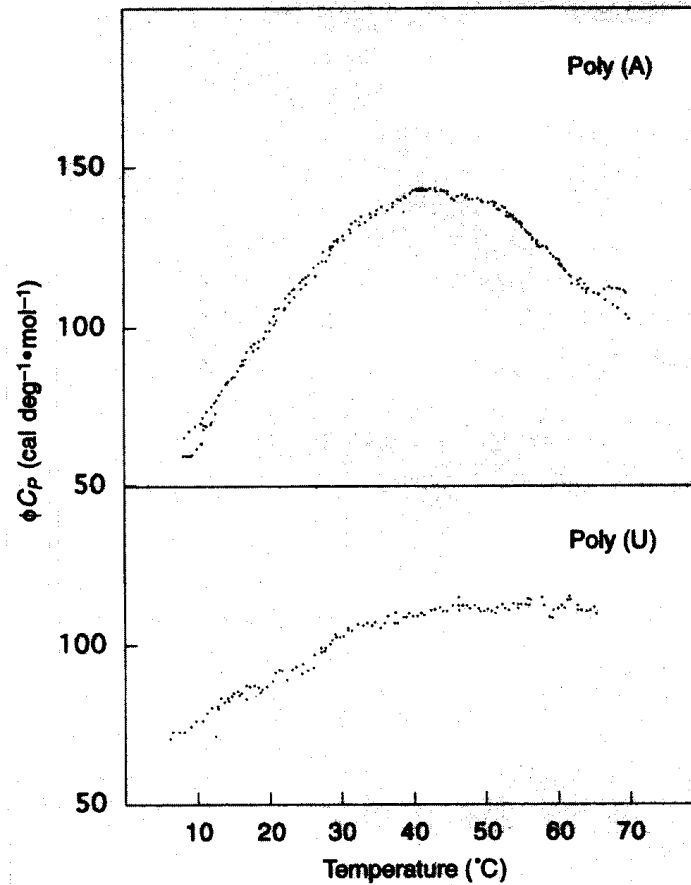
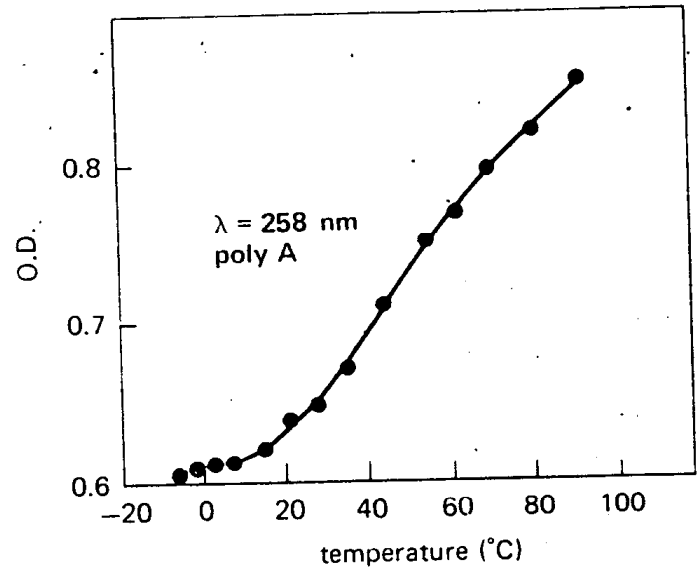


Figure 8-2  
 Excess heat capacity,  $\phi C_p$ , versus temperature for poly(A) and poly(U) as measured by differential scanning calorimetry. [Calorimetric Determination of the Heat capacity Changes Associated with the Conformational Transitions of Polyriboadenylic Acid and Polyribouridylic Acid, Suurkuusk, J., Alvarez, J., Freire, E., and Biltonan, R., *Biopolymers*, **16**, 2641–2652. Copyright ©1997. Reprinted by permission of John Wiley & Sons, Inc.]



$$\Delta H_{V.H} = \Delta H_{cal}$$

Figure 6-2 Absorbance at 258 nm of poly A as a function of temperature. Solvent is 0.1 M-LiCl-0.01 M-cacodylate buffer. [From M. Leng and G. Felsenfeld, *J. Mol. Biol.*, 15, 455 (1966). Reprinted with permission.]

Table 8.1  
Thermodynamic Parameters for Single-Strand Stacking

Molecule	Reference	Method <sup>a</sup>	[NaCl]	$\beta$	$\Delta H^\circ$ (kcal mol <sup>-1</sup> )	$\Delta S^\circ$ (eu) <sup>c</sup>	$t_M$ °C
dApA	Olsthoorn et al. (1981).	CD			-7.3	-23	49
ApA	Olsthoorn et al. (1981).	CD			-7.2	-25	22
ApA	Powell et al. (1972).	A	0.01-1		-8.5	-28	26
ApU	Olsthoorn et al. (1981).	CD			-7.3	-25	22
CpC	Powell et al. (1972).	A	0.01-1		-8.5	-30	13
UpU	Simpkins and Richards (1967).	A,ORD	0.1		No stacking		< 0
Poly(A)	Filimonov and Privalov (1978).	C	0.01-0.1		-3.0	-10	40
Poly(A)	Suurkuusk et al. (1977).	C	0.02	0.6	-8.5	-27	46
Poly(A)	Freier et al. (1981).	A,C	0.05-0.1	0.5 <sup>b</sup>	-6.8 <sup>b</sup>	-22	38
Poly(C)	Freier et al. (1981).	A,C	0.2	1	-9.0	-28	53
Poly(U)	Richards et al. (1963); Inners and Felsenfeld (1970).	V,S	0.0002-2		No stacking		< 0

<sup>a</sup>Methods: A = absorbance; C = calorimetry; CD = circular dichroism; ORD = optical rotatory dispersion; S = sedimentation; V = viscosity.

<sup>b</sup>Average of two values.

<sup>c</sup>eu = cal k<sup>-1</sup> mol<sup>-1</sup>

# Thermodynamics of duplex formation

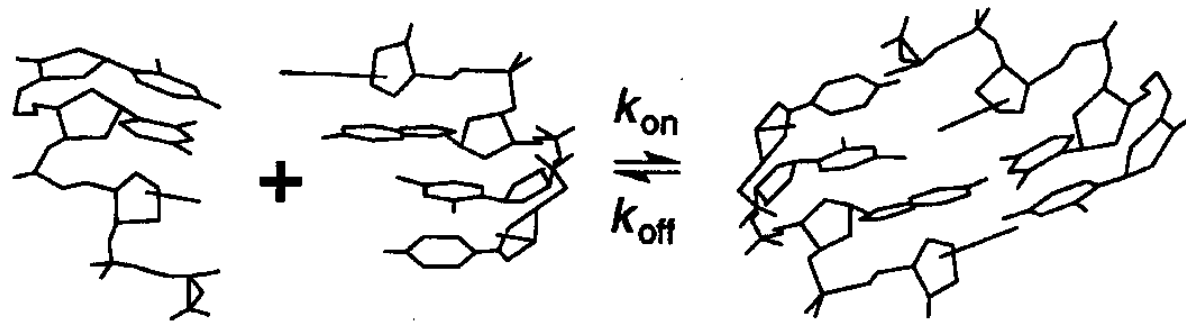


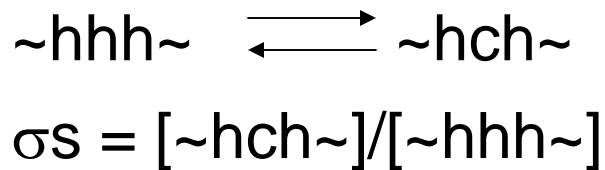
Figure 8-4

Transition from two single strands to a double helix.

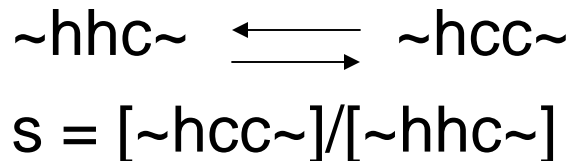
# Cooperative transition of a long chain

For a two state transition (all or none):

- **Nucleation:**



- **Growth:**



Equilibrium constants depend on the state of the nearest neighbors.

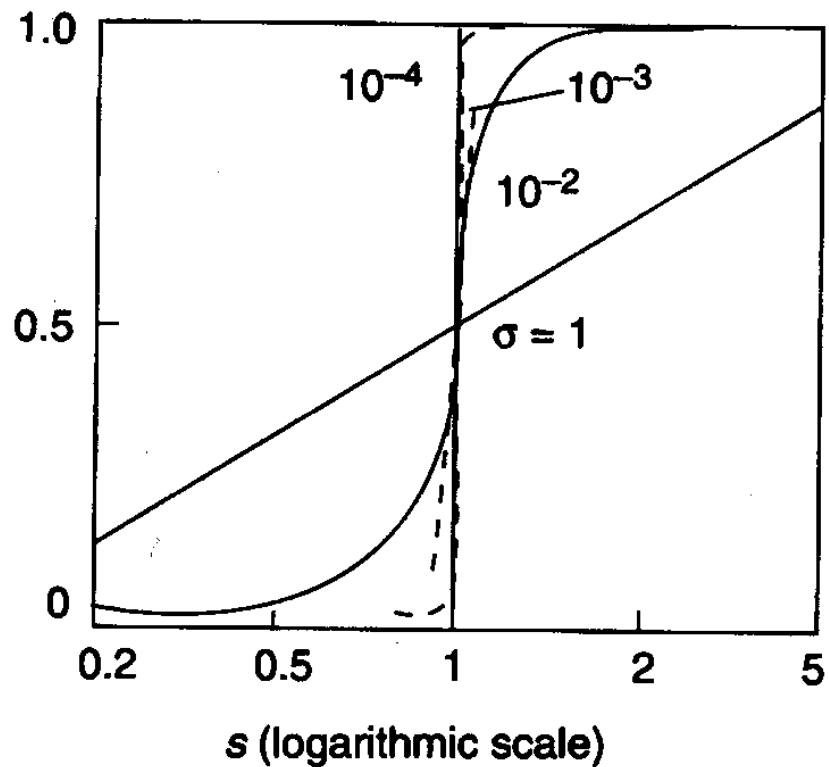
# Thermodynamics: Matrix model

- Helical fraction  $\theta$  is given by the following equation:

$$\theta = \left(\frac{s}{\lambda}\right) \frac{d\lambda}{ds}$$

$$\lambda = \frac{1}{2} [1 + s + \sqrt{(1-s)^2 + 4\sigma s}]$$

$$\frac{d\lambda}{ds} = \frac{1}{2} \left\{ 1 + \frac{s-1+2\sigma}{\sqrt{(1-s)^2 + 4\sigma s}} \right\}$$



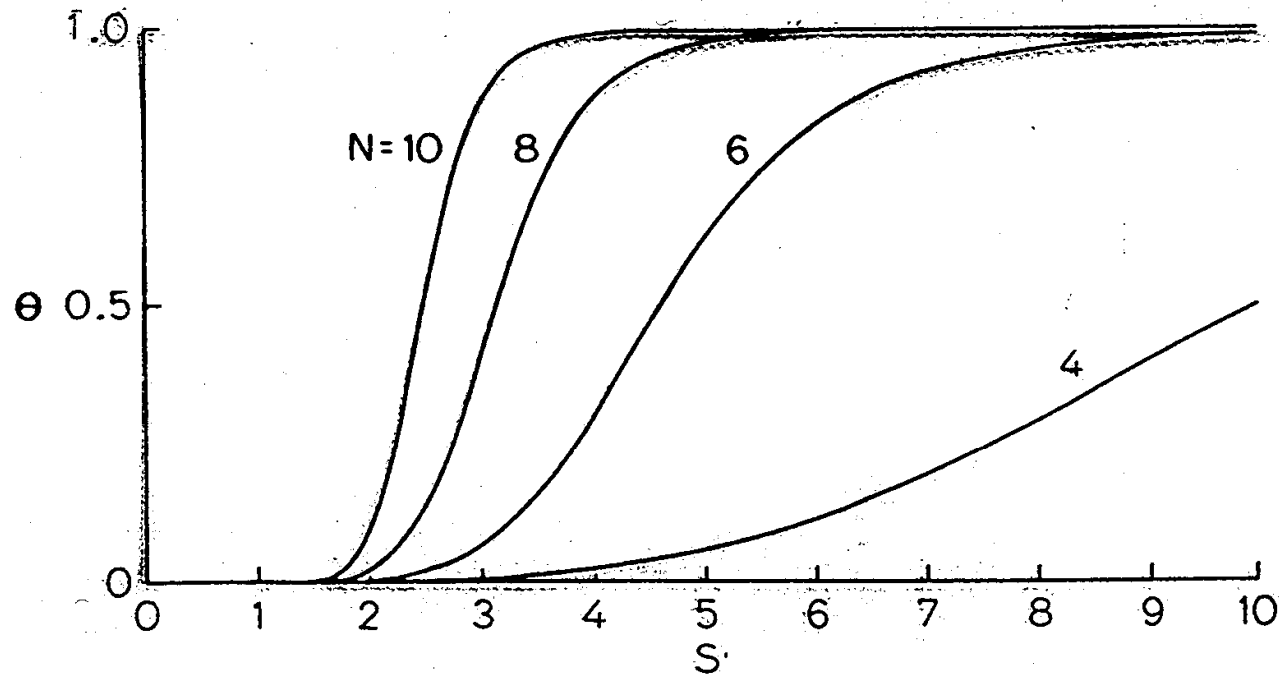
$\sigma$  governs the steepness of the transition.

For  $\sigma \ll 1$ ,  $\Delta s = 4\sigma^{1/2}$

$s = \exp(-\Delta G/RT)$

$d(\ln s) = \Delta H/RT^2$

$\Delta T = 4\sigma^{1/2}RT^2/\Delta H$



**Fig. 4.12.** Chain-length dependence of the equilibrium of the transition, the equilibrium constants of which are  $\sigma s$  for nucleation and  $s$  for propagation. Calculations were performed by the all or none approximation [Eq. (4.62)] with a nucleation parameter  $\sigma = 10^{-4}$

The average length of an uninterrupted sequence in the “C” conformation is

$$N_c = \frac{1}{\sqrt{\sigma s}} \frac{\theta}{1 - \theta}$$

At  $\theta = 0.5$  ( $s=1$ )

$$N_c = N_h = 1/(\sigma^{1/2}) = N_0$$

This is the “cooperative unit” length. If  $\sigma = 10^{-4}$ ,  $N_0 = 200$ ,  
i.e. 200 units undergo the transition in concert.

- From experiments

$$\left(\frac{d\theta}{dT}\right)_{\theta=0.5} = \frac{[\Sigma\Delta H_u]}{4RT_m^2}$$

$$\Delta H_{VH} = \Sigma\Delta H_u$$

$$\Delta H_u = \Delta H_{cal}$$

$$\frac{\Delta H_{VH}}{\Delta H_u} = N_0 = \frac{1}{\sqrt{\sigma}}$$

Calorimetry gives both enthalpy estimates, and determines  $N_0$  and  $\sigma$  in a direct way.

TABLE 6-2 HEATS OF HELIX FORMATION

Reaction	$\Delta H$ (kcal/mole) <sup>a</sup>	Ref. <sup>b</sup>
$A_n + U_n \rightarrow A_n \cdot U_n$	-5.2 to -8.5	55-59
$(dA-dT)_{2n} \rightarrow (dA-dT)_n \cdot (dA-dT)_n$	-7.9	60
$A_n \cdot U_n + U_n \rightarrow A_n \cdot 2U_n$	-2.7 to -4.3	56, 58, 59
$2\{A_n \cdot U_n\} \rightarrow A_n \cdot 2U_n + A_n$	1.4 to 3.0	56, 58, 59
$A_n + 2U_n \rightarrow A_n \cdot 2U_n$	-9.1 to -12.7	58, 59
DNA (coil) $\rightarrow$ DNA (helix)	-8	57
(corrected for heat of protonation)		
DNA (coil) $\rightarrow$ DNA (helix)	-9.2	61
poly A(coil) $\rightarrow$ poly A(helix)	-3.4 to -7	56, 62, 63
$I_n + C_n \rightarrow I_n \cdot C_n$	-5.6	64
adenosine + $2U_n \rightarrow$ adenosine $\cdot 2U_n$	-12.8	64
2-aminoadenosine + $2U_n \rightarrow$ 2-aminoadenosine $\cdot 2U_n$	-15.8	64
adenine + $2U_n \rightarrow$ adenine $\cdot 2U_n$	-12.8	64
2,6-diaminopurine + $2U_n \rightarrow$ 2,6-diaminopurine $\cdot 2U_n$	-15.9	64
deoxyadenosine + $2U_n \rightarrow$ deoxyadenosine $\cdot 2U_n$	-12.8	64
2-methylaminoadenosine + $2U_n \rightarrow$ 2-methylaminoadenosine $\cdot 2U_n$	-14.5	64

<sup>a</sup> The mole in a base pair or base triplet.

<sup>b55</sup> M. A. Rawitscher et al., *J. Amer. Chem. Soc.*, **85**, 1915 (1963).

<sup>56</sup> P. D. Ross and R. L. Scruggs, *Biopolymers*, **3**, 491 (1965).

<sup>57</sup> L. C. Bunville et al., *Biopolymers*, **3**, 213 (1965).

<sup>58</sup> E. Neumann and T. Ackermann, *J. Phys. Chem.*, **71**, 2377 (1967).

<sup>59</sup> H. Krabauer and J. M. Sturdevant, *Biopolymers*, **6**, 491 (1968).

<sup>60</sup> I. Scheffler and J. M. Sturdevant, *J. Mol. Biol.*, **42**, 577 (1969).

<sup>61</sup> P. L. Privalov et al., *Biopolymers*, **8**, 559 (1969).

<sup>62</sup> H. Klump et al., *Biopolymers*, **7**, 423 (1969).

<sup>63</sup> D. W. Hennage, Thesis Yale University.

<sup>64</sup> R. L. Scruggs and P. D. Ross, *J. Mol. Biol.*, **47**, 29 (1970).

# Prediction of oligomer stability: possible to predict melting by simple addition (Short oligomers).

- **Nearest neighbor models: the independent nearest neighbor-hydrogen bonding or INN-HB model:** the stability of a given base pair depends on the identity of adjacent base pair, and the stability of a helix depends on these nearest neighbor interactions and the base composition of the helix as reflected by the terminal base pairs (the predicted thermodynamic parameters are generally within about 10%).

**Bimolecular, self-complementary**

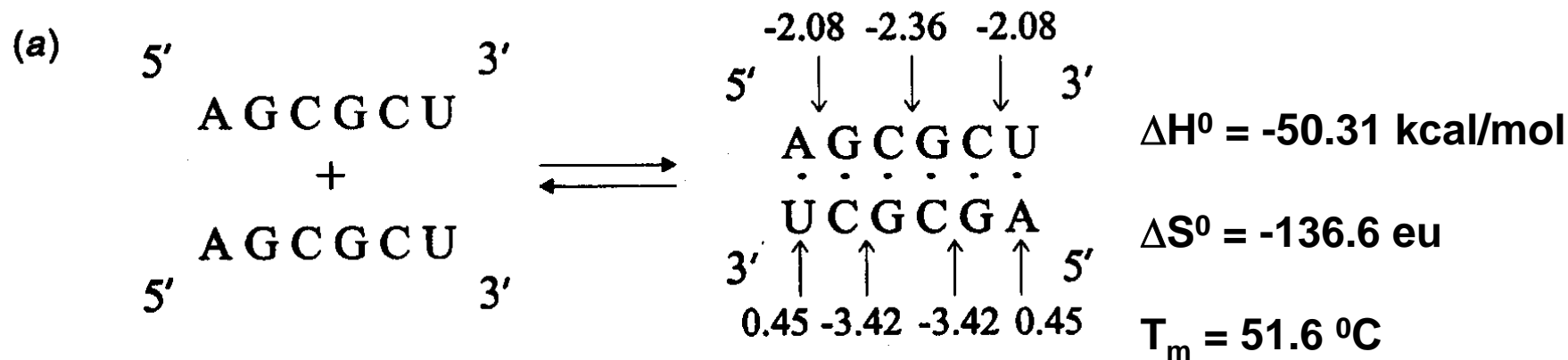
$$T_m = \frac{\Delta H^0}{\Delta S^0 + R \ln(C_T)} - 273.15$$

**Bimolecular nonself-complementary**  
**( $[A]_0 = [B]_0 = C_T/2$ )**

$$T_m = \frac{\Delta H^0}{\Delta S^0 + R \ln(C_T / 4)} - 273.15$$

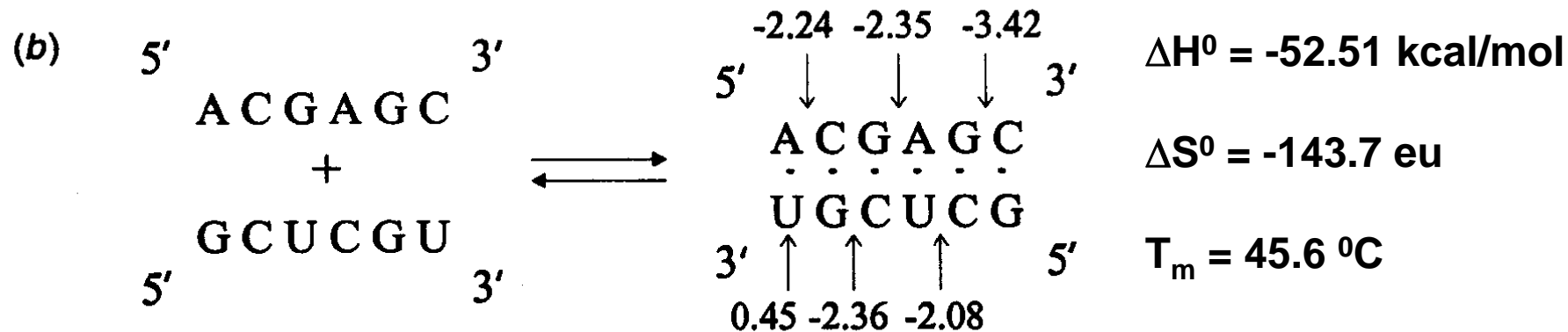
Table 1. Nearest-neighbor frequencies

Entry no.	Sequence	Nearest neighbors present in duplex									
		AA	AT	TA	CA	GT	CT	GA	CG	GC	GG
		TT	TA	AT	GT	CA	GA	CT	GC	CG	CC
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



$$\begin{aligned}
 \Delta G_{\text{TOT}}^0 &= \Delta G_{\text{INIT}}^0 + \Delta G_{\text{SYM}}^0 + \sum \Delta G_{\text{NN}}^0 + 2\Delta G_{\text{TERM-AU}}^0 \\
 &= 4.09 + 0.43 + (-1336) + 2 \times 0.45 \\
 &= -7.94 \text{ kcal/mol}
 \end{aligned}$$

$$C_T = 10^{-4} \text{ M strand}$$



$$\begin{aligned}
 \Delta G_{\text{TOT}}^0 &= \Delta G_{\text{INIT}}^0 + \Delta G_{\text{SYM}}^0 + \sum \Delta G_{\text{NN}}^0 + \Delta G_{\text{TERM-AU}}^0 \\
 &= 4.09 + 0 + (-12.45) + 0.45 \\
 &= -7.91 \text{ kcal/mol}
 \end{aligned}$$

$$C_T = 10^{-4} \text{ M strand}$$

Table 8.4  
Thermodynamic Parameters for Helix Initiation and Propagation in 1M NaCl

Propagation Sequence	RNA <sup>a</sup>			DNA <sup>b</sup>			RNA/DNA <sup>c</sup>		
	$\Delta H^\circ$ (kcal mol <sup>-1</sup> )	$\Delta S^\circ$ (eu)	$\Delta G_{37}^\circ$ (kcal mol <sup>-1</sup> )	$\Delta H^\circ$ (kcal mol <sup>-1</sup> )	$\Delta S^\circ$ (eu)	$\Delta G_{37}^\circ$ (kcal mol <sup>-1</sup> )	$\Delta H^\circ$ (kcal mol <sup>-1</sup> )	$\Delta S^\circ$ (eu)	$\Delta G_{37}^\circ$ (kcal mol <sup>-1</sup> )
RNA → GC CG ↑									
	-14.88	-36.9	-3.42	-9.8	-24.4	-2.24	-8.0	-17.1	-2.7
→ GG CC ↑									
	-13.39	-32.7	-3.26	-8.0	-19.9	-1.84	-12.8	-31.9	-2.9
→ CG GC ↑							-9.3	-23.2	-2.1
	-10.64	-26.7	-2.36	-10.6	-27.2	-2.17	-16.3	-47.1	-1.7
→ GA CU ↑									
	-12.44	-32.5	-2.35	-8.2	-22.2	-1.30	-5.5	-13.5	-1.3
→ GU CA ↑							-8.6	-22.9	-1.5
	-11.40	-29.5	-2.24	-8.4	-22.4	-1.44	-7.8	-21.6	-1.1
→ CA GU ↑							-5.9	-12.3	-2.1
	-10.44	-26.9	-2.11	-8.5	-22.7	-1.45	-9.0	-26.1	-0.9
							-10.4	-28.4	-1.6



Table 8.3  
Thermodynamic Parameters for Duplex Formation by Oligonucleotides

Sequence	Reference	Measured <sup>a</sup>				Predicted <sup>b</sup>			
		$\Delta H^\circ$ (kcal mol <sup>-1</sup> )	$\Delta S^\circ$ (eu)	$\Delta G_{37}^\circ$ (kcal mol <sup>-1</sup> )	$t_m$ (°C)	$\Delta H^\circ$ (kcal mol <sup>-1</sup> )	$\Delta S^\circ$ (eu)	$\Delta G_{37}^\circ$ (kcal mol <sup>-1</sup> )	$t_m$ (°C)
<b>RNA, 3'-5', Antiparallel Strands, 1 M NaCl</b>									
CCGG	Petersheim and Turner (1983).	-34.2	-95.6	-4.6	27.1	-33.8	-95.0	-4.4	25.3
CGCG	Xia et al. (1998).	-33.3	-95.6	-3.7	19	-32.6	-93.2	-3.6	18.8
GCGC	Freier et al. (1985b).	-30.5	-83.4	-4.6	26.5	-36.8	-103.4	-4.7	29.1
GGCC	Freier et al. (1983).	-35.8	-98.1	-5.4	34.4	-38.1	-105.2	-5.4	34.9
CGCGCG	Freier et al. (1986b).	-54.5	-146.4	-9.1	57.9	-58.1	-156.8	-9.4	58.5
GCAUGC	Groebe, Cameron, Freier, Turner, and Uhlenbeck, unpublished results.	-62.3	-177.2	-7.4	45.7	-56.4	-157.2	-7.6	48.3
GCCGGC	Freier et al. (1985b).	-62.7	-166.0	-11.2	67.2	-63.6	-168.8	-11.2	66.6
GCGCGC	Freier et al. (1985b).	-66.0	-178.5	-10.6	62.1	-62.3	-167.0	-10.5	63.1
GGCGCC	Freier et al. (1986b).	-67.8	-182.0	-11.3	65.2	-63.6	-168.8	-11.2	66.6
CA <sub>7</sub> G + CU <sub>7</sub> G	Nelsen et al. (1981); Freier et al. (1986b).	-59.8	-175.1	-5.5	31.6	-58.2	-169.5	-5.7	32.4
<b>RNA, 2'-5', Antiparallel Strands, 1 M NaCl</b>									
GCGCGC	Kierzek et al. (1992).	-22.2	-56.2	-4.8	24.4				
CGGCGCCG	Kierzek et al. (1992).	-44.3	-121.2	-6.8	45.3				
<b>DNA, 3'-5', Antiparallel Strands, 1 M NaCl</b>									
CGCGCG	Senior et al., (1988b).	-46.4	-122.8	-8.3	55.7	-51.2	-137.4	-8.6	55.7
GCATGC	Williams et al. 1989.	-42.4	-118	-5.9	38.3	-43.6	-121.6	-5.9	38.5
GCGCGC	Albergo et al. (1981).	-59.6	-162.7	-9.1	56.1	-50.4	-134.6	-8.7	56.5
CA <sub>7</sub> G+CT <sub>7</sub> G	Aboulela et al. (1985)	-68.0	-196	-7.2	40.1	-63.5	-182.5	-6.8	43.1
<b>DNA, 3'-5', Antiparallel Strands, 0.1 M NaCl</b>									
5'A <sub>10</sub> TA <sub>2</sub> T <sub>4</sub> A <sub>3</sub> TAT <sub>3</sub> 3'									
3'T <sub>10</sub> AT <sub>2</sub> A <sub>4</sub> T <sub>3</sub> ATA <sub>3</sub> 5'	Rippe and Jovin (1992); Rentzeperis (1992).	-151	-457						
5'A <sub>5</sub> GA <sub>2</sub> GTAGT <sub>4</sub> A <sub>2</sub> GTAT <sub>3</sub> 3'									
3'T <sub>3</sub> CG <sub>3</sub> CATCA <sub>4</sub> T <sub>2</sub> CATA <sub>3</sub> 5'	Rippe and Jovin (1992); Rentzeperis (1992).	-167	-500						
<b>DNA, 3'-5', Parallel Strands, 0.1 M NaCl</b>									
5'A <sub>10</sub> TA <sub>2</sub> T <sub>4</sub> A <sub>3</sub> TAT <sub>3</sub> 3'									
5'T <sub>10</sub> AT <sub>2</sub> A <sub>4</sub> T <sub>3</sub> ATA <sub>3</sub> 3'	Rippe and Jovin (1992); Rentzeperis (1992).	-116	-374			-116	-354	-6.2	
5'A <sub>5</sub> GA <sub>2</sub> GTAGT <sub>4</sub> A <sub>2</sub> GTAT <sub>3</sub> 3'									
5'T <sub>3</sub> CT <sub>3</sub> CATCA <sub>4</sub> T <sub>2</sub> CATA <sub>3</sub> 3'	Rippe and Jovin (1992); Rentzeperis (1992).	-116	-381						

<sup>a</sup>Thermodynamic parameters determined from  $T_m^{-1}$  versus  $\log C_T$  plots. The  $t_m$  is for  $1 \times 10^{-4} M$  strand concentration.

<sup>b</sup>Thermodynamic parameters predicted from Table 8.4 for 1 M NaCl and from Rippe and Jovin (1992). Values for  $\Delta G_{37}^\circ$  calculated from the predicted  $\Delta H^\circ$  and  $\Delta S^\circ$  using  $\Delta G_{37}^\circ = \Delta H^\circ - 310.15\Delta S^\circ$  may differ from the listed  $\Delta G_{37}^\circ$  due to round off errors. The  $t_m$  is for  $1 \times 10^{-4} M$  strand concentration.

Table 2. Nearest-neighbor thermodynamics

Interaction	$\Delta H^\circ$	$\Delta S^\circ$	$\Delta G^\circ$
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  $\Delta G^\circ$  and  $\Delta H^\circ$  are kcal/mol of interaction, whereas the units for  $\Delta S^\circ$  are cal/K per mol of interaction (1 cal = 4.184 J).

**Scheme 1**  
**Predicting transition enthalpies**  
**of DNA oligomers**

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

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

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

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

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

Table 3. Comparison of calculated and observed  $\Delta H_T$

Oligomeric duplex	$\Delta H_{pred}$	$\Delta H_{obs}$
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 CAAAAAAG GTTTTTTTC	68.2	68.0
10 CAAATAAAG GTTTATTTC	64.6	58.6
11 CAAAGAAAG GTTTCTTTC	63.4	62.8
12 CGCGTACGGGTACGGC GCGCATGCGCATGCGC	143	158

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

Table 4. Comparison of calculated and observed  $\Delta G_T$

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

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

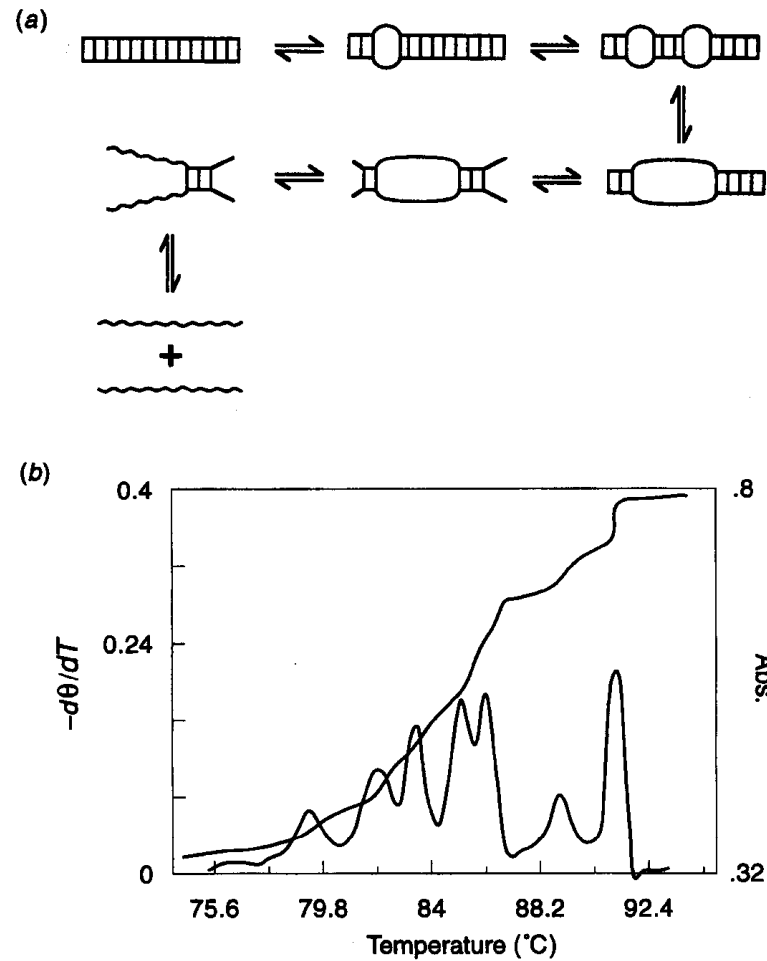


Figure 8-12

(a) Typical pathway for melting of DNA. (b) Absorbance versus temperature and differentiated melting curves for the 1630 bp Hinf I restriction endonuclease fragment of plasmid pBR322. [Reprinted from Wartell, R. M. and Benight, A. S., Thermal Denaturation of DNA Molecules: A Comparison of Theory with Experiment, *Phys. Rep.*, **126**, 67-107. Copyright ©1985 with permission of Elsevier Science-NL, Amsterdam, The Netherlands.]

TABLE II  
*Nearest neighbor stacking energies in 0.075 M Na<sup>+</sup>*

5'-NM-3'	$T_{MN}^a$	$\delta\Delta G_{MN}^b$	$\Delta H_{MN}^{b,c}$	Optimized potential stacking energies <sup>b,d</sup>
	°C			
TA(TA)	56.31	+186	-8,187	-6,570
AA(TT)	63.88	-35	-8,375	-5,370
CA(TG)	68.70	+399	-8,495	-10,510
AT(AT)	70.82	-175	-8,548	-3,820
AG(CT)	76.50	+205	-8,689	-9,810
CG(CG)	88.74	+421	-8,993	-14,590
GA(TC)	89.45	-117	-9,011	-6,780
GG(CC)	99.45	+155	-9,259	-8,260
AC(GT)	104.72	-497	-9,390	-6,570
GC(GC)	134.92	-726	-10,141	-9,990

<sup>a</sup> The standard deviation for  $T_{MN}$ ,  $\sigma = \pm 0.23$  °C.

<sup>b</sup> (cal(mol MN)<sup>-1</sup>).

<sup>c</sup> From the product  $\Delta S \cdot T_{MN}$ , where  $\Delta S = 24.85(\pm 1.74)$  cal(mol·deg)<sup>-1</sup>. Therefore, the relative uncertainty in  $\Delta H_{MN}$  is  $\pm 10$  cal whereas the absolute uncertainty is  $\pm 606$  cal (mol·bp)<sup>-1</sup>.

<sup>d</sup> From Ornstein *et al.*, 1978.

$T_m = \sum f_{MN} T_{MN}$ , where  $f_{MN}$  is the fraction neighbor frequencies, and  $T_{MN}$  is the stacking energies for pair M on N.

**Table 4.** Dependence of various thermodynamic parameters on the percentage of G-C base pairs present in DNA [71, 72]

DNA from	%-GC	$\langle m \rangle^a$	$\Delta H_{cal}$ kJ (mol base pair) <sup>-1</sup>	$T_m$ °C	$b_c^b$ Å	$\Delta G$ kJ (mol base pair) <sup>-1</sup>	$\Delta S$ JK <sup>-1</sup> (mol base pair) <sup>-1</sup>
1 Salmon sperm	41.2	24	33.5	67.0	5.2	-4.4	83.7
2 Herring sperm	42.2	19	33.1	68.0	5.2	-5.0	81.0
3 Human placenta	41.2	20	33.5	68.0	5.1	-5.2	101.8
4 Chicken blood	41.7	20	33.5	67.0	5.0	-5.2	113.0
5 Calf thymus	41.9	16	33.5	66.0	5.2	-5.2	98.7
6 <i>CL. perfringens</i>	31.0	31	32.2	61.0	5.4	-3.4	96.2
7 <i>E. coli</i>	50.0	33	34.3	73.0	5.0	-5.3	99.5
8 <i>M. lysodeikt</i>	72.0	36	36.0	81.0	4.4	-6.1	101.6
9 Phage T7	48.0	43	34.7	70.0	5.0	-5.9	101.3
10 Phage PM2	43.0	33	33.1	72.0	5.0	-5.4	95.8
11 Plasmid pzme 134	50.0	33	33.1	72.0	5.0	-5.4	97.2
12 poly d(A-T)	-	75	31.4	45.0	6.0	-1.7	98.7
13 poly d(G-C)	100.0	60	37.7	97.0	3.9	-7.7	101.8

<sup>a</sup> Average cooperative length in base pairs.

<sup>b</sup> Linear phosphate distance as obtained from projection on the helix axis.

- For long DNA sequences with relatively random distribution of nearest neighbors, the following simple equations can be used to predict  $T_m$  from the fraction of GC content,  $F_{GC}$ .

$$T_m (^{\circ}\text{C}) = 193.67 - (3.09 - F_{GC})(34.64 - 6.52 \log[\text{Na}^+])$$

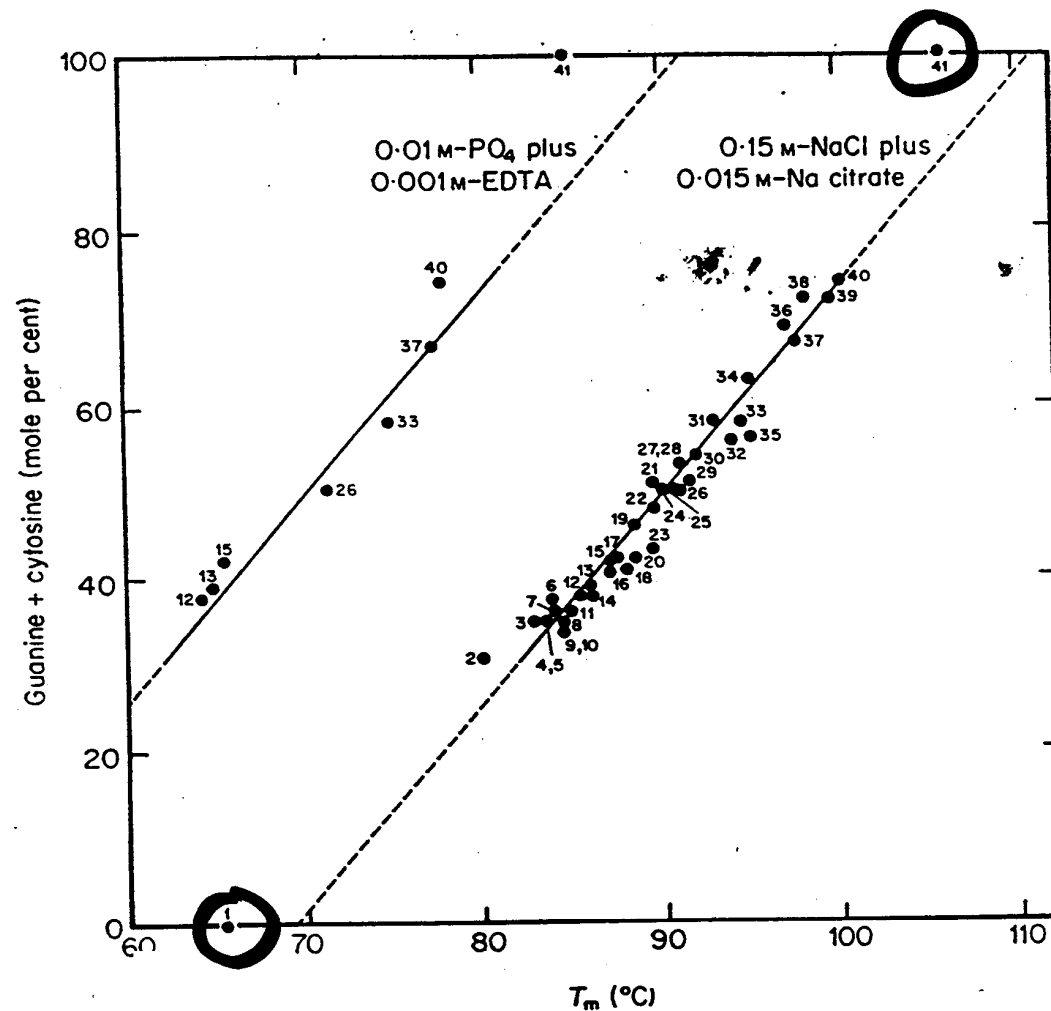
( $0.3 < F_{GC} < 0.7$ , and  $0.02 < [\text{Na}^+] < 0.4\text{M}$ )

or

$$T_m (^{\circ}\text{C}) = 81.5 + 41F_{GC} + 16.6 \log([\text{Na}^+]/(1.0 + 0.7[\text{Na}^+]) - 500/D - P$$

(D is the duplex length, and P is the percentage of mismatches)

$T_m$  estimate may be used to obtain %GC for DNA solution



**Figure 6-17.** Dependence of melting temperature  $T_m$  on guanine + cytosine (G + C) content of various samples of DNA obtained from different sources. DNA was dissolved in 0.15 M NaCl + 0.015 M Na-citrate, pH 7.0. Points 1 and 41, for poly(dA-dT) and poly(dG-dC), fall off the least-squares line which is described analytically by  $T_m = 69.3 + 0.41(\%C)$ . From (549).

# Factors to affect the stability of helix

- **Salt concentration:**

In solutions containing only Na<sup>+</sup>, increasing salt concentration up to about 1 M continuously increases helix stability. At salt concentration above 1 M, addition of salts lowers the T<sub>m</sub> of DNA;

- **Solvent effects:**

Addition of cosolvents to aqueous solutions of nucleic acids usually destabilizes the stability of helix. Typically, the T<sub>m</sub> of a double helix will be a linear function of cosolvent concentration. The cosolvent concentration required for 50% denaturation correlate well with enhancement of base solubility.

- **pH:**

Usually, at lower and higher pH values, stability is decreased; however, there are some exceptions.

Table 8.15  
Effects of Denaturants on Duplex Stability

Cosolvent	Molarity for 50% denaturation of T4 DNA at 73°C, 43 mM Ionic Strength		$\Delta t_m$ (°C) at 10 mol % for 18.7 $\mu$ M Oligo in 1 M NaCl	
	Predicted <sup>a</sup>	Observed <sup>b</sup>	A <sub>7</sub> U <sub>7</sub> <sup>c</sup>	(dGC) <sub>3</sub> <sup>d</sup>
<b>Alcohols</b>				
Methanol	3.9	3.5		6.6
Ethanol	1.3	1.2	8.3	11.1
1-Propanol	0.47	0.54	9.1	8.4
2-Propanol	0.64	0.90	8.4	
Ethyleneglycol	1.7	2.2	7.7	
Glycerol		1.8	7.9	
Cyclohexyl alcohol		0.22		
Phenol		0.08		
<b>Other Compounds</b>				
Pyridine		0.09		
1,4 Dioxane		0.64	18.7	
Formamide	1.5	1.9	14.8	12.0
<i>N, N</i> dimethylformamide	0.54	0.60	16.9	~22
Urea	1.1	1.0	17.8	13
Acetonitrile		1.2		
TritonX-100		> 10%		

<sup>a</sup>Herskovits and Harrington, (1972); Herskovits and Bowen, (1974).

<sup>b</sup>Levine et al., (1963).

<sup>c</sup>Hickey and Turner, (1985).

<sup>d</sup>Albergo and Turner, (1981).

# B to A transition

- A-DNA is a high-energy conformation of the double helix under physiological conditions;
- It can be stabilized by decreasing the water activity (adding alcohol to the solutions);
- It may be biologically significant.

# B to A transition: CD studies

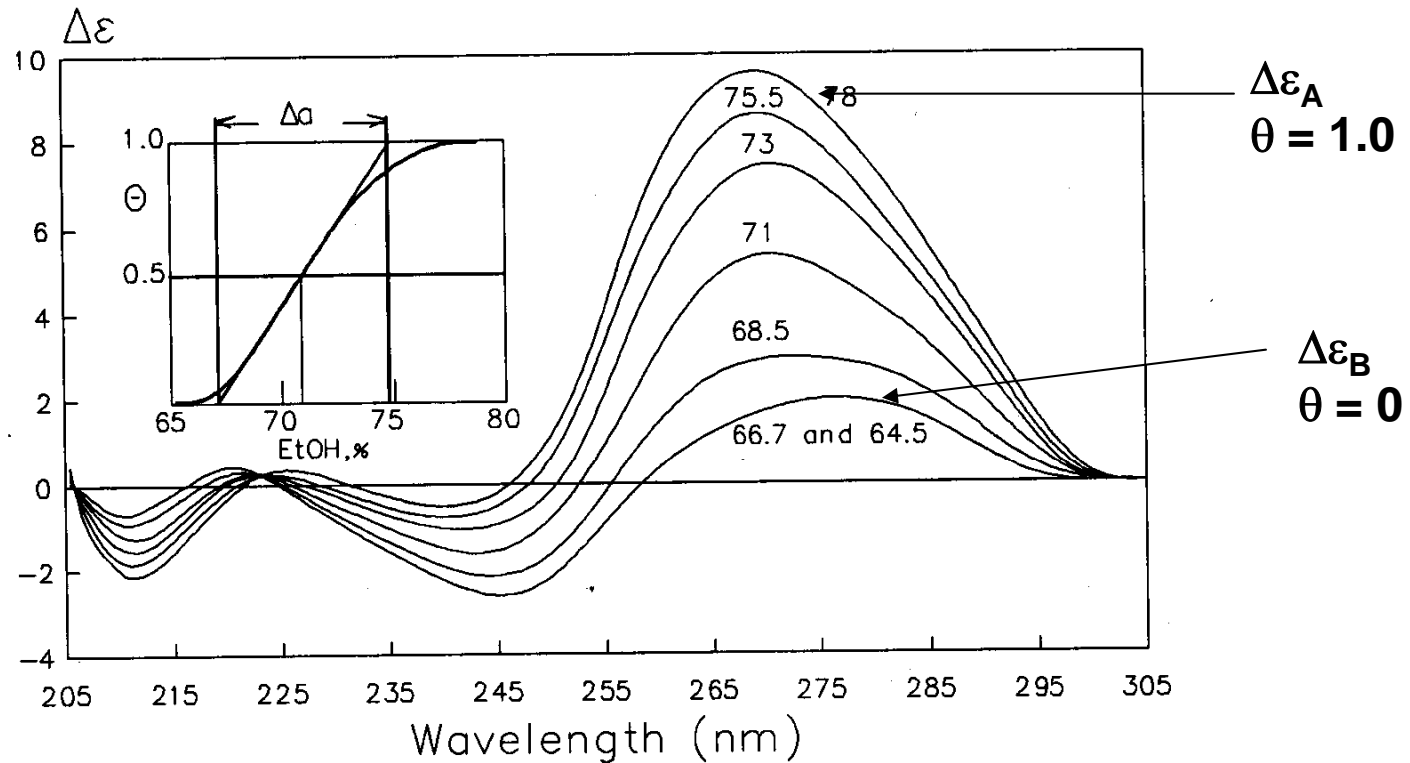


FIG. 1. Circular dichroism spectra of calf thymus DNA in water-ethanol solutions. The spectra are labeled with the volume percent of ethanol. (Inset) Curve of the B-A transition based on the change in the CD magnitude at 270 nm. (For conditions, see text.)

$\theta = (\Delta\varepsilon - \Delta\varepsilon_B) / (\Delta\varepsilon_A - \Delta\varepsilon_B)$ . We can analyze these data by a cooperative model, which like the one described for DNA melting.

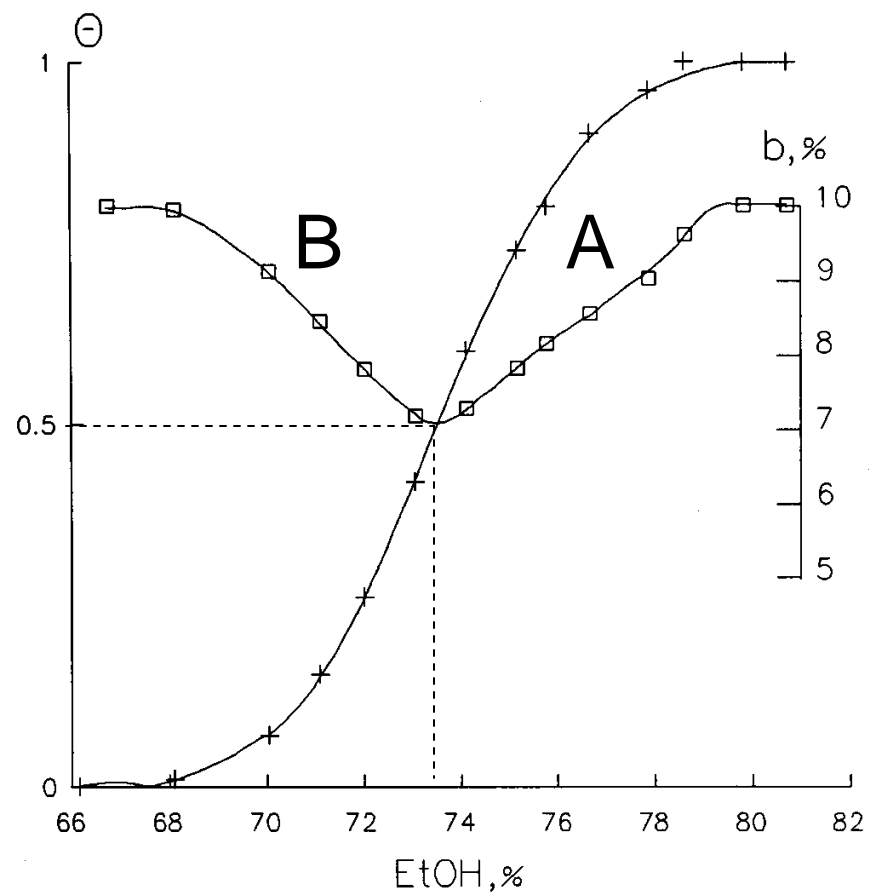
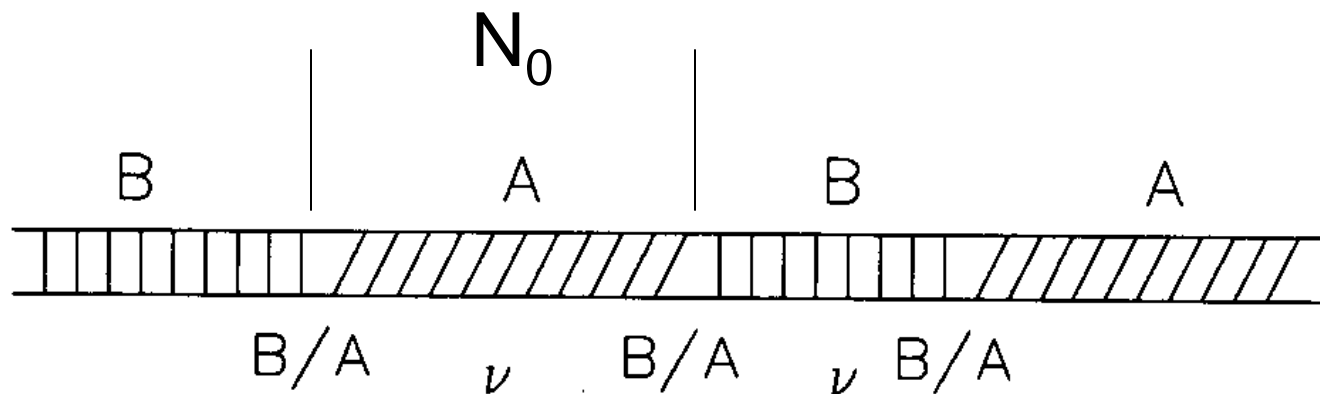


FIG. 6. The B-A transition of DNA as studied by CD (left ordinate) and by the method of DNA orientation in flow (V-shaped curve, right ordinate).



**FIG. 3. Scheme of DNA structure within the B–A transition interval (see text).**

$N_0$  is about 10 to 30 bp for B to A transition

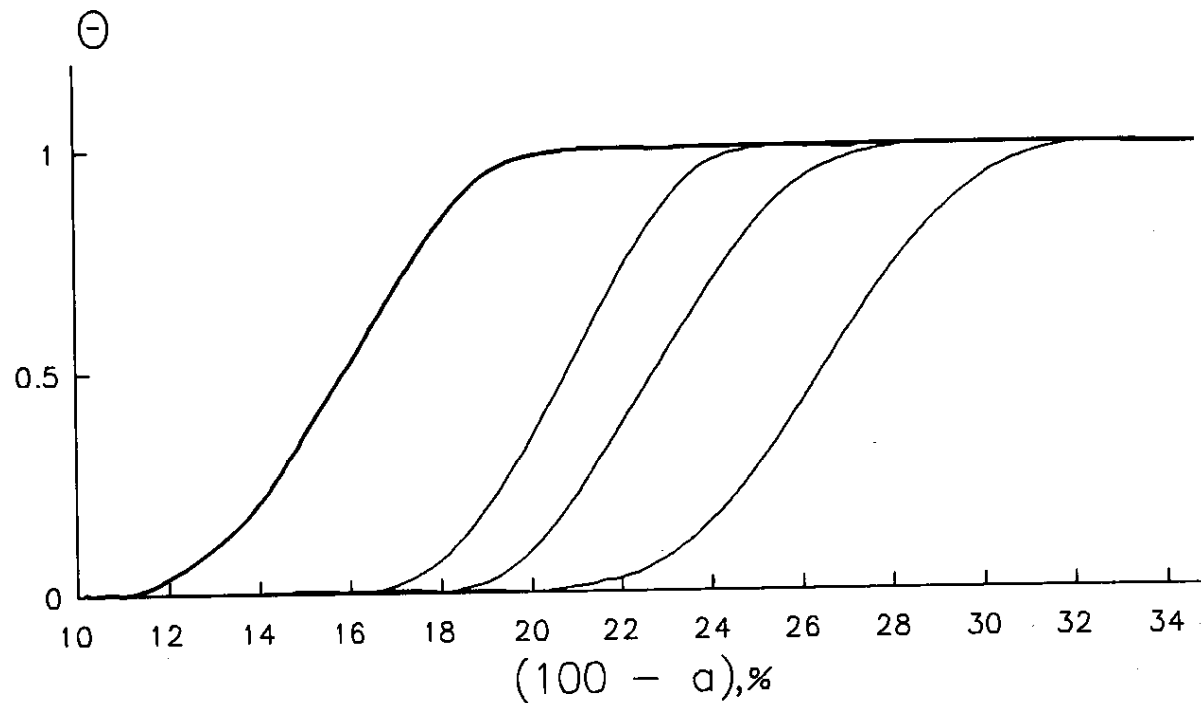


FIG. 4. B-A transition profiles of self-complementary decadeoxyduplexes. From left to right: CCCCCGGGGG, ATACCGGTAT, GCTACGTAGC, CCAAGCTTGG (only one strand is listed). Note that the first duplex (marked by a heavy line) has the lowest B-A transition point and has the highest number of CC dinucleotide steps (contacts).

**Note: since the length of oligos are smaller than N0, it is all or none transition. Sequence effect is obvious!**

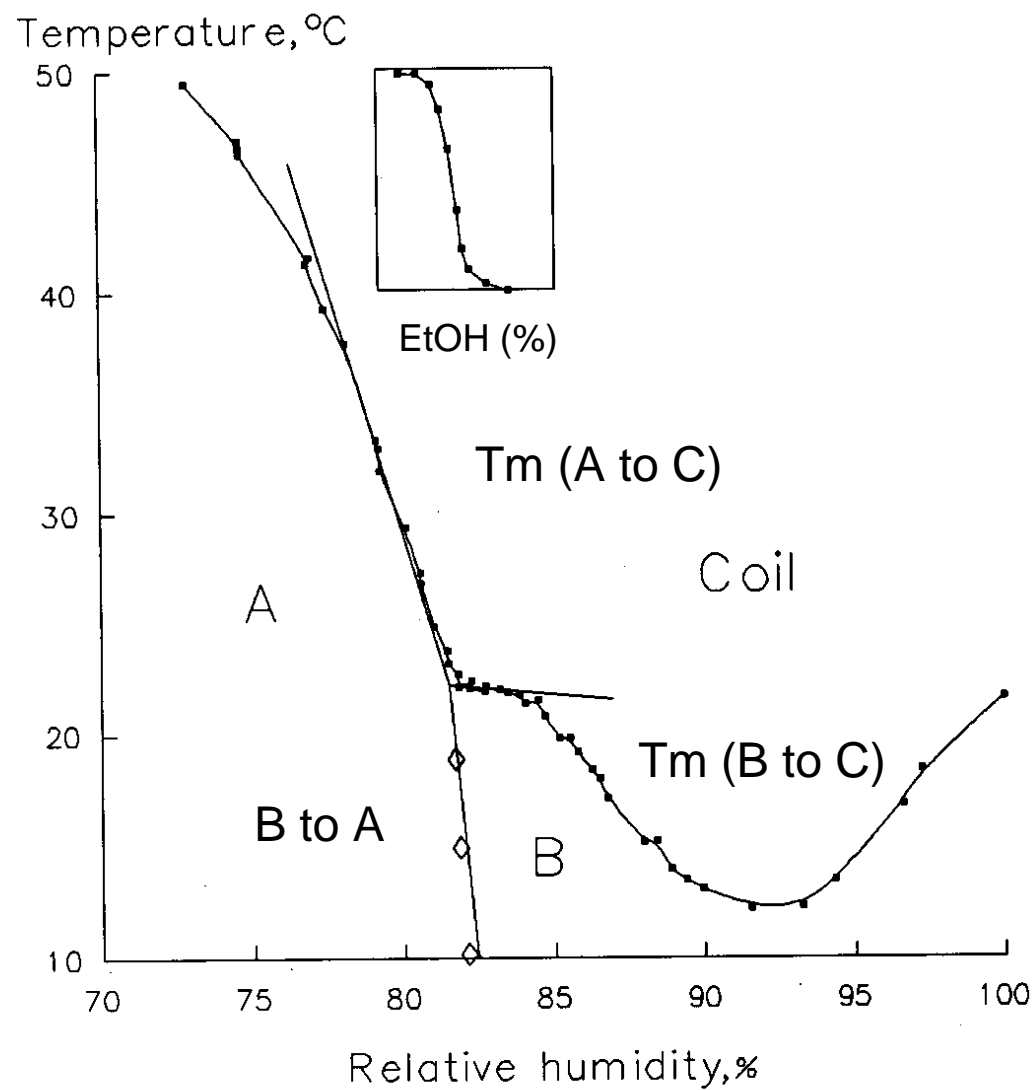


FIG. 5. Phase diagram for poly[d(A-T)]. (Inset) Corresponding B-A transition curve. Note that the B-A transition point, measured by CD, coincides with the break point in the helix-coil branch. For explanation, see text.

## B to Z transition

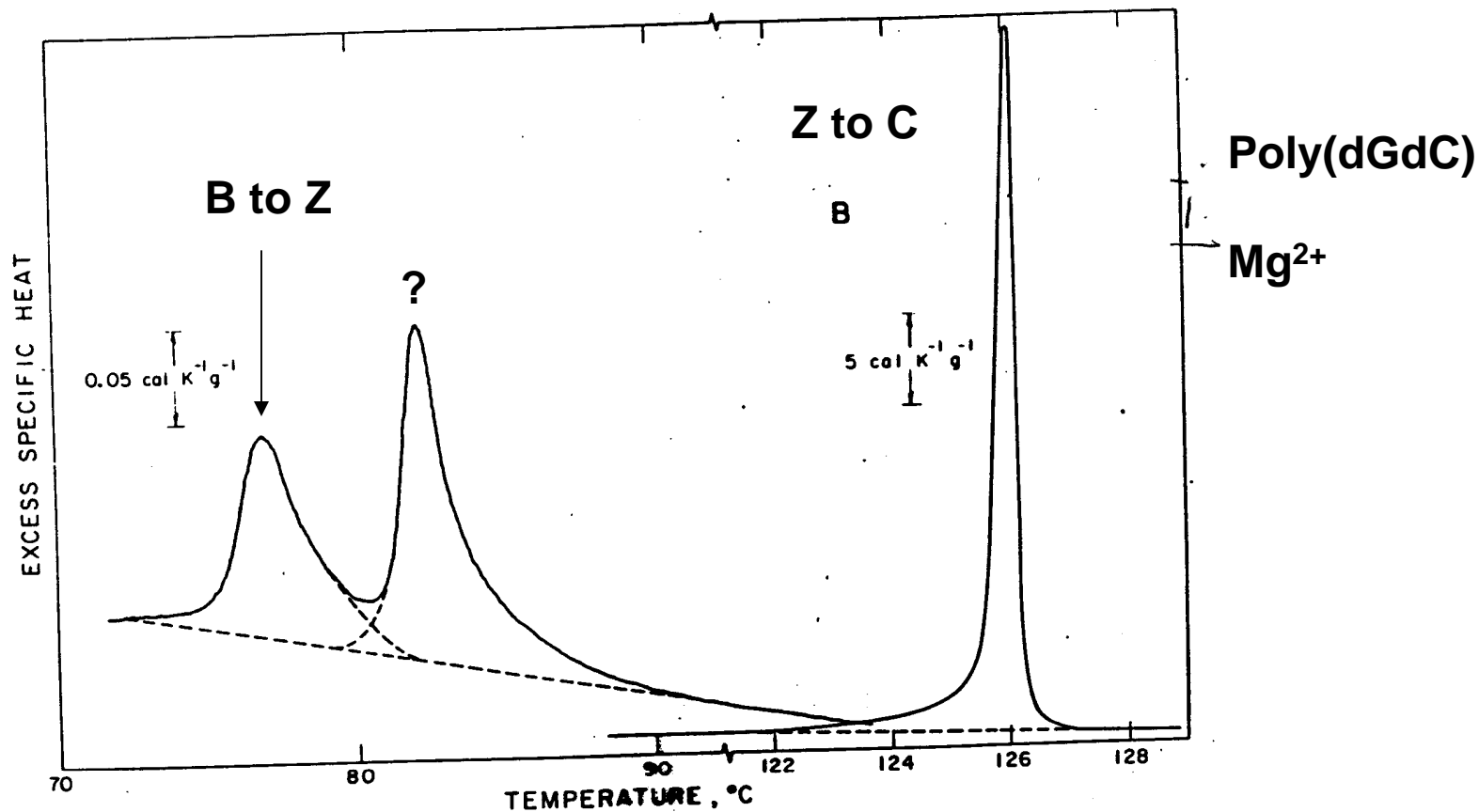


Fig. 1. Tracings of DSC curves obtained with poly(dGdC) at a concentration of 1.76 mM bp in 1 mM MgCl<sub>2</sub>, 1 mM Na cacodylate, pH 6.4. Scan rate 0.25 K min<sup>-1</sup>. (A) B to Z and Z to ψ(-) in 1 mM MgCl<sub>2</sub>, 1 mM Na cacodylate, pH 6.4. Scan rate 0.25 K min<sup>-1</sup>. (A) B to Z and Z to ψ(-) in 1 mM MgCl<sub>2</sub>, 1 mM Na cacodylate, pH 6.4. Scan rate 0.25 K min<sup>-1</sup>. The sloping baseline is of instrumental origin and is exaggerated by the high sensitivity employed. (B) The helix to coil transition, probably including the disaggregation of the ψ(-)-form.