Double Helix Stability and Base Composition

Helical formation can be monitored by observing the optical density of a solution. As discussed above, the disruption of base stacking alters the electronic interaction between the bases. As the electronic interaction decreases, it becomes easier for an electron to absorb a photon. Hence, denaturation of DNA leads to the "hyperchromic" effect, i.e., the increased absorption of light (Figure 19 & 20)

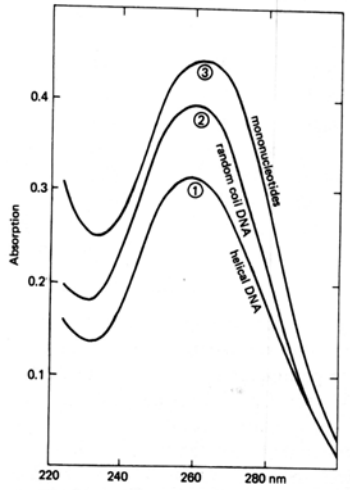


Figure 19 Ultraviolet absorption spectra of DNA in native double helical (1) and denatured random coil (2) states and spectrum of individual, monomeric, unstacked nucleotides of the same concentration as in native DNA (3). In denatured state (2), bases are still considerably stacked. From (185).

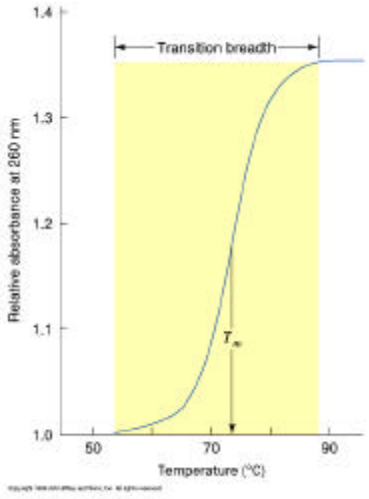


Figure 20

In general, helical stability is linearly related to fractional G+C base pair content in DNA. As G+C increases so does stability (Figure 21).

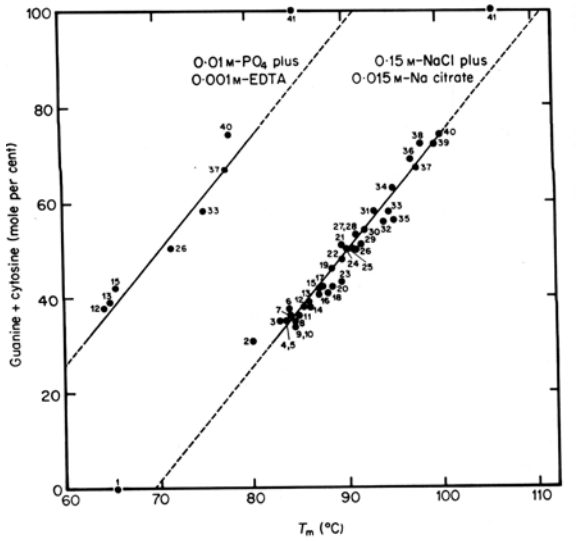


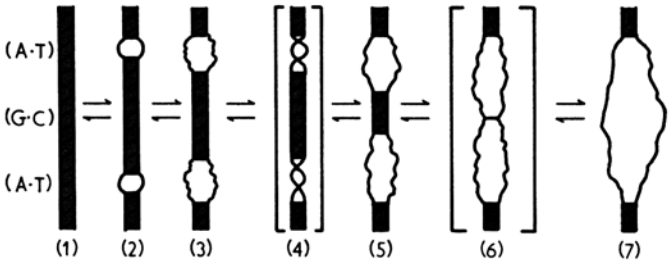
Figure 21 Dependence of melting temperature T_m on guanine + cytosine (G + C) content of various samples of DNA obtained from different sources. DNA was dissolved in 0.15 *M* NaCl + 0.015 *M* Na-citrate, pH 7.0. Points 1 and 41, for poly(dA-dT) and poly(dG-dC), fall off the least-squares line which is described analytically by T_m = 69.3 + 0.41 (%C). From (549).

An empirical formula for calculating the melting temperature of a particular helix is given as

$$T_m\ (^oC) = 69.3 + 41 * fG/C$$

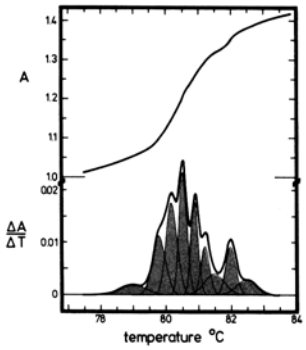
This expression quantifies the observed result that there is a linear relation between T_m and G+C content. This observation argues that the energetic contributions of the bases in the helix to its stability are independent and therefore additive--->this implies that stabilization energies are sequence independent. That is the base pairs are all contributing equally and independently a constant amount of stacking energy, independent of the neighbors.

This line of argument is, however, an oversimplification. In complex DNA, melting occurs in domains **(FIGURE 22 & 23).**



Scheme describing processes involved in DNA or RNA double-helix de- and renaturation. A–T-rich regions melt first, giving rise to states (2) and (3). In (4), additional base-pairs are opened and the twist is taken up in coil regions. From (550).

Figure 22



Melting profile of DNA (top) and its first derivative dA/dT (bottom). The latter curve is deconvoluted into nine individual peaks characterized by temperature, amplitude, and breadth. *A* indicates UV absorption at 260 nm; dA/dT or ΔA/ΔT are first derivatives with respect to temperature *T*. These curves are simulated; for some realistic data see Ref. (557).

Figure 23

Table 4

Prediction of DNA Double Helix Stability from Base Sequence m (555)]

Stability Matrix for Nearest-Neighbor Stacking in Base–Paired Dinucleotides in B-DNA Geometry[a]:

	3′			
5′	A	T	G	C
T	36.73	54.50	54.71	86.44
A	54.50	57.02	58.42	97.73
C	54.71	58.42	72.55	85.97
G	86.44	97.73	85.97	136.12

[a] Numbers give T_m values in °C at 19.5 m*M* Na^+.

T_m Values Predicted with This Matrix for a Collection of Synthetic DNA Polymers with Defined Sequence:

	T_m(°C)		
Polynucleotide	Experimental[a]	Calculated[b]	Difference[c]
Poly(dA-dT)·poly(dA-dT)	45.0	46.9	−1.9
Poly(dA-dA-dT)·poly(dA-dT-dT)	49.2	49.4	−0.2
Poly(dA)·poly(dT)	53.0	54.5	−1.5
Poly(dG-dA-dA)·poly(dT-dT-dC)	64.5	66.5	−2.0
Poly(dG-dT-dA)·poly(dT-dA-dC)	66.8	64.3	2.5
Poly(dA-dA-dC)·poly(dG-dT-dT)	70.2	69.0	1.2
Poly(dG-dA)·poly(dT-dC)	71.3	72.4	−1.1
Poly(dG-dA-dT)·poly(dA-dT-dC)	72.0	66.1	5.9
Poly(dG-dG-dA)·poly(dT-dC-dC)	76.3	76.9	−0.6
Poly(dG-dT)·poly(dA-dC)	77.4	76.2	1.2
Poly(dG)·poly(dC)	87.8	86.0	1.8
Poly(dG-dC)·poly(dG-dC)	99.2	104.3	−5.1

[a] Experimental melting temperatures at various ionic strengths are interpolated to 19.5 m*M* Na^+.
[b] Calculated from values in Table 6-9(A) and nearest-neighbor frequencies in each polymer.
[c] T_m (experimental) − T_m (calculated).

This realization gives rise to a calculated stability matrix, for stacked paired dinucleotides in B-DNA configuration **(Table 3, above)**. This stability matrix gives T_m values to the doublets under standard conditions. From these data, the melting of any DNA can be calculated. And the answers are surprisingly accurate **(Table 4)**.

If the observed and calculated T_m's are plotted against the stacking energies we saw before, a linear correlation is observed **(FIGURE 24)**. This indicates that stacking has a role in determining the T_m of DNA. Since stacking is a sequence dependent phenomenon then T_m is sequence dependent.

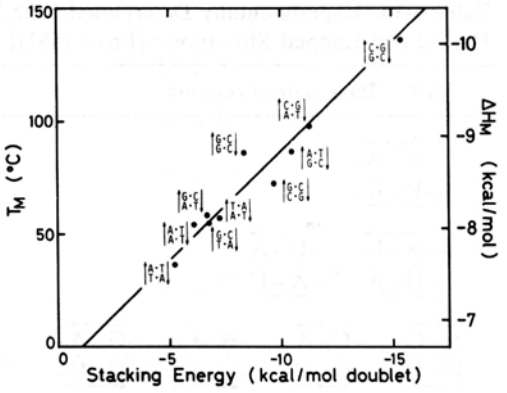


Figure 24

Helical Stability and Salt

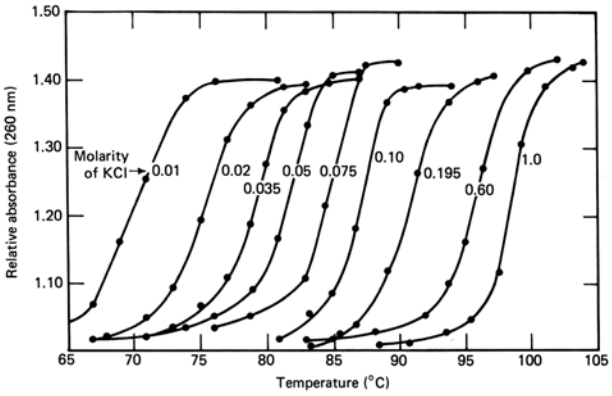


Figure 25

It has been long observed that multiple stranded polynucleotide helices are stabilized by increasing monovalent cation concentration (**Figure 25**). In fact the T_m of a given DNA is linearly dependent on the log of the monovalent cation concentration.

We will not spend a lot of time on the polyelectrolyte behavior of nucleic acids, but instead we will simplify the treatments and take an empirical and thermodynamic approach.

The DNA phosphate backbone is negatively charged. In salt solutions, cations are associated with it. When DNA is denatured fewer total cations are associated with the separated strands than with the nucleic acid helix in its native state. This is because the charge density on double stranded DNA is higher than single strand nucleic acids. This creates a larger electrostatic potential, which more effectively attracts counterions.

Thus, in the denaturation reaction, the mass action equation can be written.

$$DNA_{(Helix)} \cdot M_{rh} \text{ <----> } DNA_{(Coil)} \cdot M_{rc} + M_{(rh-rc)}$$

rh= # of ions bound/base pair in a helix
rc= # of ions bound/base in a coil
rh-rc= net gain in free cations due to denaturation

Therefore, the denaturation reaction equilibrium can be shifted by adjusting the cation concentration.

We have already discussed that effect of temperature on helix->coil transition. The two effects can be balanced at particular conditions. That is if the salt is raised, increases helix potential, can increase temperature to denature.

We have only discussed here the effect of monovalent cations on structure. The effects of divalent cations are much more complex due to their multiple interactions with the DNA phosphate backbone-each M^{2+} can potentially bind one or two DNA phosphates and the binding is likely to be cooperative. Hence, the T_m dependence on divalent cation concentration is decidedly non-linear.