

The Chemical Differences Between DNA and RNA Have Biological Significance

Two fundamental chemical differences distinguish DNA from RNA:

- 1. DNA contains 2-deoxyribose instead of ribose.
- 2. DNA contains thymine instead of uracil.

What are the consequences of these differences, and do they hold any significance in common? An argument can be made that, because of these differences, DNA is chemically more stable than RNA. The greater stability of DNA over RNA is consistent with the respective roles these macromolecules have assumed in heredity and information transfer.

Consider first why DNA contains thymine instead of uracil. The key observation is that cytosine deaminates to form uracil at a finite rate in vivo (Figure 10.25). Because C in one DNA strand pairs with G in the other strand, whereas U would pair with A, conversion of a C to a U could potentially result in a heritable change of a C: G pair to a U:A pair. Such a change in nucleotide sequence would constitute a *mutation* in the DNA. To prevent this C deamination from leading to permanent changes in nucleotide sequence, a cellular repair mechanism "proofreads" DNA, and when a U arising from C deamination is encountered, it is treated as inappropriate and is replaced by a C. If DNA normally contained U rather than T, this repair system could not readily distinguish U formed by C deamination from U correctly paired with A. However, the U in DNA is "5-methyl-U" or, as it is conventionally known, thymine (Figure 10.26). That is, the 5-methyl group on T labels it as if to say "this U belongs; do not replace it."

The other chemical difference between RNA and DNA is that the ribose 2'-OH group on each nucleotide in RNA is absent in DNA. Consequently, the ubiquitous 3'-O of polynucleotide backbones lacks a vicinal hydroxyl neighbor in DNA. This difference leads to a greater resistance of DNA to alkaline hydrolysis, examined in detail in the following section. To view it another way, RNA is less stable than DNA because its vicinal 2'-OH group makes the 3'-phosphodiester bond susceptible to nucleophilic cleavage (Figure 10.27). For just this reason, it is selectively advantageous for the heritable form of genetic information to be DNA rather than RNA.





RNA is relatively resistant to the effects of dilute acid, but gentle treatment of DNA with 1 m*M* HCl leads to hydrolysis of purine glycosidic bonds and the loss of purine bases from the DNA. The glycosidic bonds between pyrimidine bases and 2'-deoxyribose are not affected, and in this case, the polynucleotide's sugar-phosphate backbone remains intact. The purine-free polynucleotide product is called **apurinic acid**.

DNA is not susceptible to alkaline hydrolysis. On the other hand, RNA is alkali labile and is readily hydrolyzed by hydroxide ions (Figure 10.27). DNA has no 2'-OH; therefore, DNA is alkali stable.











Functional groups of purine and pyrimidine bases

• Ring nitrogens, carbonyl groups, and exocyclic amino groups.

• Hydrogen bonds involving the amino and carbonyl groups are the second important mode of interaction between bases in nucleic acid molecules.

• Hydrogen bonds between bases permit a complementary association of two strands of nucleic acid.





11.3 Can the Secondary Structure of DNA Be Denatured and Renatured?

Thermal Denaturation of DNA Can Be Observed by Changes in UV Absorbance

When duplex DNA molecules are subjected to conditions of <u>pH</u>, temperature, or ionic <u>strength</u> that disrupt base-pairing interactions, the strands are no longer held together. That is, the double helix is **denatured**, and the strands separate as individual random coils. If temperature is the denaturing agent, the double helix is said to *melt*. The course of this dissociation can be followed spectrophotometrically because the relative absorbance of the DNA solution at 260 nm increases as much as 40% as the bases unstack. This absorbance increase, or **hyperchromic shift**, is due to the fact that the aromatic



bases in DNA interact via their π -electron clouds when stacked together in the double helix. Because the UV absorbance of the bases is a consequence of π -electron transitions, and because the potential for these transitions is diminished when the bases stack, the bases in duplex DNA absorb less 260-nm radiation than expected for their numbers. Unstacking alleviates this suppression of UV absorbance. The rise in absorbance coincides with strand separation, and the midpoint of the absorbance increase is termed the **melting temperature**, T_m (Figure 11.20). DNAs differ in their T_m values because they differ in relative G + C content. The higher the G + C content of a DNA, the higher its melting temperature because G:C pairs have higher base stacking energies than A:T pairs. Also, T_m is dependent on the ionic strength of the solution; the lower the ionic strength, the lower the melting temperature. Because cations suppress the electrostatic repulsion between the negatively charged phosphate groups in the complementary strands of the double helix, the double-stranded form of DNA is more stable in dilute salt solutions. DNA in pure water melts even at room temperature.

pH Extremes or Strong H-Bonding Solutes also Denature DNA Duplexes

At pH values greater than 10, the bases of DNA become deprotonated, which destroys their base-pairing potential, thus denaturing the DNA duplex. Extensive protonation of the bases below pH 2.3 also disrupts base pairing. Alkali is the preferred denaturant because, unlike acid, it does not hydrolyze the glycosidic bonds linking purine bases to the sugar-phosphate backbone. Small solutes that readily form H bonds can also denature duplex DNA at temperatures below $T_{\rm m}$. If present in sufficiently high concentrations, such small solutes will form H bonds with the bases, thereby disrupting H-bonding interactions between the base pairs. Examples include formamide and urea.





					A form	B form	Z form	
		R		Helical sense Diameter	Right handed ~26 Å	Right handed ~20 Å	Left handed ~18 Å	
				Base pairs per helical	2011	2011	1011	
	1000	and the second		turn	11	10.5	12	
	1000	2000 P	S. 49 1	Helix rise per base pair	2.6 A	3.4 A	3.7 A	
4		1.	Sec. 4	base the normal to the	20°	6°	7°	
And a second		3		Sugar pucker conformation	C-3' endo	C-2' endo	C-2' endo for pyrimidines; C-3' endo for purines	
	r i		- 2	Glycosyl bond conformation	Anti	Anti	Anti for pyrimidine syn for purines	
	forms	Bform	Zform	FIGURE 8–19 of ture shown here phosphate atom in blue. Blue is i ters. The table si	FIGURE 8–19 Comparison of A, B, and Z forms of DNA. Each a ture shown here has 36 base pairs. The bases are shown in gray phosphate atoms in yellow, and the riboses and phosphate oxy in blue. Blue is the color used to represent DNA strands in later - ters. The table summarizes some properties of the three forms of			











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