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The Double Helix in Retrospect: A Critique of Available Evidence

Sosale Chandrasekhar[1*]

[1]Department of Organic Chemistry, Indian Institute of Science, Bangalore 560 012, India.

Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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Opinion Article

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ABSTRACT

This short review commentary deals with the evidence for the DNA double helix and raises the intriguing question: does it exist to any significant extent in the solution phase? Well past the sixtieth year of its discovery via X-ray diffraction, the DNA double helix has now acquired the status of a sacred truth, with a large area of modern science revolving around it. This paper presents the pros and cons of the double helix from a physico-chemical perspective, although with critical inputs from chemical biology as appropriate.

Keywords: DNA melting; duplex; Gibbs free energy; helicase; RNA; single strand.

1. GENERAL OVERVIEW OF THE DNA DOUBLE HELIX

The DNA double helix is central to modern biology, perhaps even a sacrosanct principle of science [1-4]! Indeed, the double helix serves to bridge the gulf between classical Mendelian genetics and the modern era of 'designer genes'. Yet, despite this conceptual inheritance (sic), molecular DNA must

**Corresponding author: E-mail: sosale@orgchem.iisc.ernet.in;*

conform to the laws of physico-chemical science.

The double helical structure of DNA, possessing as it does an eerie symmetry, is beautiful to behold. It rests on early crystallographic work, the culmination of a brilliant piece of scientific investigation which is as intriguing as a le Carré thriller [4]! Interestingly, however, despite its current status as a bed-rock, the double helix is apparently not beyond the reach of a devil's advocate, given the complexity of the molecule and the reactions it undergoes. Indeed, a particularly intriguing question concerns the extent of the double helical form in solution – assumed as self-evidently total so far.

Such doubts would be trivial – even cynical – in the case of smaller (and lesser) molecules. However, DNA is far too critically important to be treated dismissively and peremptorily, and indeed far too complex to be buried in blasé assumptions! This paper thus undertakes the onerous task of a careful and dispassionate consideration of the evidence, garnered by some of the most astute scientific investigators over half a century and more.

2. AN A PRIORI ANALYSIS OF THE PROS AND CONS OF THE DOUBLE HELIX

2.1 Thermodynamic Considerations

The physico-chemical basis of the double helical form of DNA may be analyzed fundamentally, a thermodynamic approach leading to revealing insights (Fig. 1 and Eqs. 1-6) [5,6]. Thus, the formation of the double-stranded form (*ds*-DNA, **3**) from the corresponding complementary single strands (*ss*-DNA, **1**) would – by implied assumption – be accompanied by a loss of both enthalpy and entropy (*cf.* $\Delta G_{3\text{-}1}$). On the other hand, *ss*-DNA would be highly solvated in the aqueous medium (*cf.* **1**), so desolvation (to form **2**) prior to duplex formation (**3**) would be accompanied by gains in both enthalpy and entropy (*cf.* $\Delta G_{2\text{-}1}$). The relative extents of these losses and gains would determine the overall stability of *ds*-DNA relative to *ss*-DNA (*cf.* $\Delta G_{3\text{-}1}$).

$$G_n = H_n + TS_n \quad (1)$$

$$\Delta G_n = \Delta H_n + T\Delta S_n \quad (2)$$

$$\Delta G_{2\text{-}1} = G_2 - G_1 \quad (3)$$

$$\Delta G_{3\text{-}2} = G_3 - G_2 \quad (4)$$

$$\Delta G_{3\text{-}1} = G_3 - G_1 \quad (5)$$

Eqs. 1-5 refer to the Gibbs free energy (G), enthalpy (H) and entropy (S) contents of *ss*-DNA (**1** and **2**) and *ds*-DNA (**3**). (These are hydrated at the bases in **1** but not in **2** and **3;** hydration elsewhere in the molecule is assumed to be similar in **1-3,** hence ignored.) The subscripts (n = 1-3) indicate the three states (**1, 2** and **3**), the corresponding changes being ΔG, ΔH and ΔS. Notably, the duplex is stabilized by hydrogen bonding and base stacking interactions, and destabilized by electrostatic repulsion between the negatively charged sugar-phosphate backbones (but weakly, as these are mutually remote); all these are essentially enthalpic effects, but the duplex would also be destabilized entropically relative to the corresponding single strands. (The *ss* form would be also destabilized by the putative hydrophobic effect of the bases, which would be largely relieved upon duplex formation.)

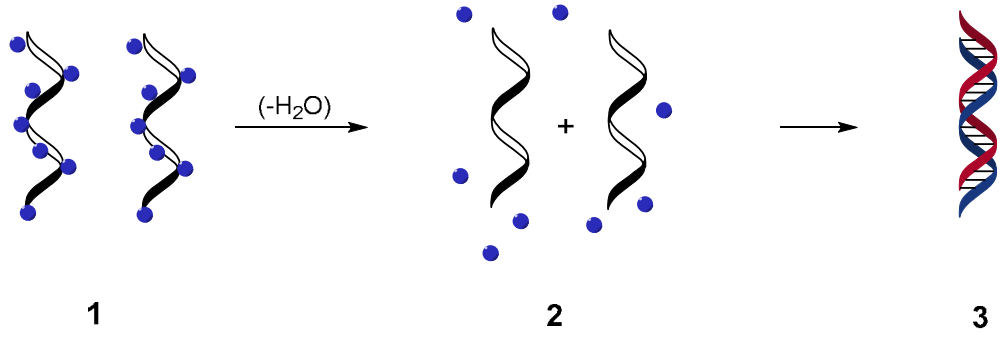

Fig. 1. Cartoon representation of DNA duplex formation: 1 is *ss*-DNA that is hydrated at the bases; 2 is *ss*-DNA with its bases stripped of water; 3 is *ds*-DNA. The blue spheres represent water molecules, bound to the bases in 1, and free in 2

Thus, the stability of *ds*-DNA over *ss*-DNA depends critically on ($\Delta G_{3\text{-}1}$) being substantially negative. This implies (Eq. 6) that the apparent gain in Gibbs free energy upon the desolvation of *ss*-DNA ($\Delta G_{2\text{-}1}$) is overwhelmed by the loss of Gibbs free energy upon duplex formation ($\Delta G_{3\text{-}2}$): clearly, assumed by current dogma as inviolable.

$$\Delta G_{3\text{-}1} = (\Delta G_{2\text{-}1} + \Delta G_{3\text{-}2}) << 0 \quad (6)$$

However, it is not easy to assess – even qualitatively – the above losses and gains. Thus, is the gain in (essentially) solvent entropy upon desolvation of the *ss* form ($\Delta S_{2\text{-}1}$) greater or less than the loss of entropy upon duplex formation ($\Delta S_{3\text{-}2}$)? Is the enthalpy gain upon the desolvation ($\Delta H_{2\text{-}1}$) greater or less than the enthalpy loss upon duplex formation ($\Delta H_{3\text{-}2}$)? (There is also the possibility of enthalpy-entropy compensation in these steps.)

2.2 Crystallographic Caveats

The existence of crystalline *ds*-DNA is, of course, what is to be expected from considerations of symmetry and stability. (In other words, the stable *crystalline* form of DNA would be *ds*-DNA.) Thus, it is possible that *ds*-DNA crystallizes out of a mixture of forms in solution, even if *ds*-DNA were to be a minuscule component of the mixture, dragging the equilibrium (between *ss* and *ds* forms) along in the process. This enduring caveat of X-ray crystallography, understandably acquires particular piquancy in the case of DNA structure!

3. EXPERIMENTAL EVIDENCE FOR THE DOUBLE HELIX IN SOLUTION

3.1 General Considerations

Current molecular biology, on one hand, and belief in the existence of *ds*-DNA in solution, on other, apparently share a symbiotic relationship, reinforced by the ideas of base specificity and complementarity. In particular, the replication of DNA occurs upon a template strand of *ss*-DNA, thus bolstering the belief that this leads to *ds*-DNA. Furthermore, the idea of a compact symmetrical double helical form is not only aesthetically appealing, but also represents (however inaccurately) a “Mendelian vestige” – that of shared inheritance, *i.e.* one gene from each parent for every phenotypical characteristic.

None of these considerations *per se*, however, proves or disproves the existence of *ds*-DNA in solution. Thus, DNA replication could occur upon a template strand without prejudice to the possibility that the *ss* form exists – even exclusively – in solution. The template strand would then be akin to a cofactor for DNA polymerase [7]. (In other words, base pairing occurs only at the active site of the polymerase, with the strands separating on either side of the active site.) One balks at the idea that complementary *ss* forms could thus ignore each other in solution, but why not if thermodynamic considerations deem it so?

Thus, the existence of complementary strands could reflect the need for a template at the active site of DNA polymerase, eventually resulting in genetic information being stored in two complementary sets. In other words, if *ss*-DNA can survive on its own, so be it. Although it is intuitively appealing to imagine that in *ds*-DNA the complementary strands somehow protect each other (say, against mutations), this *per se* does not prove that *ds*-DNA predominates in solution.

3.2 DNA Melting and Related Studies

3.2.1 DNA melting

DNA melting is an intriguing phenomenon which apparently implicates *ds*-DNA [8,9]. Essentially, the ‘melting’ involves heating a solution of DNA while following the change in its ultraviolet absorbance. It is observed that the intensity of the absorption band at ~ 260 nm increases with the temperature, the resulting plot resembling a sigmoid. This is interpreted in terms of the gradual collapse of the duplex structure of *ds*-DNA, with the high temperature plateau region representing complete strand separation to form *ss*-DNA. Accordingly, the mid-point of the sigmoid is considered to represent a 1:1 mixture of *ds*-DNA and *ss*-DNA, the ‘melting temperature’ (T_m) at the mid-point being characteristic of the sample of DNA as defined by its base composition and sequence. (The observed changes can also be reversed, albeit slowly, upon cooling.)

This analysis, however, clearly assumes that the initial sample is *ds*-DNA. In fact, all that is being observed is a reversible change, which most likely involves breaking and reforming hydrogen bonds. A particular problem is that the above analysis assumes a continuous and rapidly attained equilibrium between *ds* and *ss* forms at each temperature: however, it is known that the (putative) reverse ‘annealing’ process is relatively

slow, so reversibility *en route* cannot be assumed.

An alternative interpretation to the inter-conversion between *ds*-DNA and *ss*-DNA, however heretically, would involve only *ss*-DNA. The structure of *ss*-DNA would expectedly be self-coiled *via* internal base-pairing, akin to that of ribonucleic acid (RNA) [1-3]. Thus, the 'melting' phenomenon would represent the uncoiling of *ss*-DNA leading to various linear forms consisting of greatly reduced hydrogen bonded base pairs (Fig. 2). (In fact, such behaviour is indeed known in the case of RNA – famously single stranded [10,11]!)

"Annealing" would then refer to the recoiling of the extended linear forms. This also explains the van't Hoff plots obtained from DNA melting experiments [8]: The two-state model would still apply, but to an equilibrium between the coiled and extended *ss* forms, rather than between *ds* and *ss* forms. It is also easier to accept the notion of reversibility of the uncoiling of *ss*-DNA, as this would be a fast unimolecular process. The relative slowness of the 'annealing' process would arise from the fact than many *ss* coiled forms are possible, with the intermediate states, however, involving structurally-localized rapid equilibria (Fig. 2).

In fact, an intriguing problem with the current view of DNA melting is noteworthy: the melting of particularly long duplexes would require prohibitively large inputs of energy. Thus, assuming (conservatively) an average base-pairing strength of ~ 5 kcals/mole, even a moderately long 100-mer duplex would require ~ 500 kcals/mole for complete strand separation. Then, a free energy of activation of at least this magnitude is also indicated. This implies an impossibly slow process, certainly ruled out entirely in the case of naturally occurring DNA! (The above arguments, *in toto,* apply equally to the 'polymerase chain reaction' technique, which involves DNA melting as a key component [1,3]).

3.2.2 Related studies

There have been several other elegant and rigorous studies that have been reported on the kinetics and thermodynamics of duplex formation [12-14]. However, these appear for the most part to deal with relatively short oligonucleotides, and the validity of extending the results to the case of naturally occurring DNA is unclear. Shorter oligonucleotide chains cannot form the U-shaped folded structures that are accessible to longer (single stranded) chains, so the shorter chains are predisposed to (intermolecular) duplex formation.

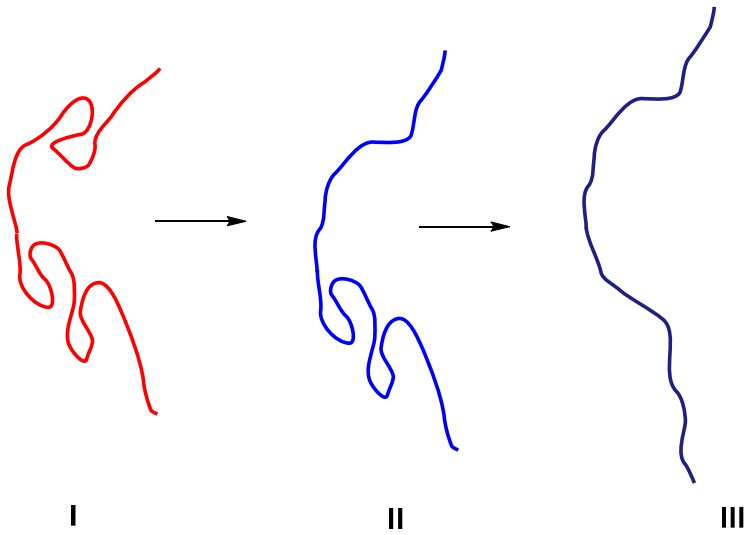

Fig. 2. Cartoon representation of the uncoiling of *ss*-DNA, possibly during DNA melting. I represents the fully coiled form, II the partly coiled form and III the fully extended form. The transition between I and II, as also between II and III, is relatively fast and reversible; however, the transition from III to I would be relatively slow. Hence, the overall transformation would be slowly reversed ('annealing'), although intermediate states may well attain rapid equilibrium among themselves. (The hydrogen bonds stabilizing the 'hairpin loops' in I and II are not shown)

An ingenious strategy involving effective molarity (EM) values for different inter-strand cross-linking reactions [14], generally revealed low EM's (0.8-25) even in the case of moderately long oligonucleotides (19-35 units). (EM's are a quantitative measure of the proximity effect, and apparently indicate the possibility of duplex formation.) However, it should be noted that an EM > 1 can also arise in the case of two complementary single strands, in which the transition state for the cross-linking reaction is stabilized by base pairing. In fact, when the cross-linking reaction was reversible, the thermodynamic EM's were lower than the kinetic EM's: this possibly indicates that duplex formation is temporary and localized around the reaction centre (*i.e.* the transition state).

In recent decades, advanced nuclear magnetic resonance (NMR) techniques have been applied to the problem of nucleic acid structure and dynamics [15]. Although these ingenious studies have provided remarkable insights, particularly into the nature of the hydrogen bonding interactions involved, they do not appear to shed light on the extent of the duplex form in solution. Indeed, the identification of hydrogen-bonded motifs does not imply that these necessarily arise from intermolecular interactions, except in the case of shorter oligonucleotides (*vide supra*).

3.3 Helicases and Their Significance

These are a class of ATP-dependent enzymes that are believed to catalyze the unwinding of *ds*-DNA, leading to both the uncoiling of the double helix and partial strand separation [16]. This process, clearly, must precede certain key reactions of *ds*-DNA, *e.g.* replication, transcription, etc. However, a possibility that needs to be considered is that helicases act upon minor amount of *ds*-DNA present in solution, for a maximal and efficient utilization of the total DNA present. (This would also be an evolutionary justification for the existence of the helicases.) Thus, the mere existence of the helicases does not prove that all of the solution state DNA is *ds*-DNA. These caveats gain credence from the fact that helicases specific to RNA are indeed also known [16], and catalyze the uncoiling of local duplex structures in RNA. Clearly, a similar function in *ss*-DNA cannot entirely be ruled out!

It is also noteworthy that, although the helicases could substantially lower the free energy of activation for the unwinding of the duplex, the thermodynamic barrier (discussed above) remains. As noted, this is prohibitively high, thus implying correspondingly enormous quantities of ATP to drive the process to reasonable completion.

3.4 The Case of RNA

An enduring question, perhaps obliquely relevant to the present discussion, concerns the structure of RNA in its varied forms. This is almost always single stranded [1-3], raising the question: Why not double stranded? Conversely, if RNA is single stranded, why not DNA too? Structurally, it is possible that the C_2 hydroxyl in ribose would sterically hinder duplex formation in the case of RNA, although the extent of this putative interaction appears unknown. (These structural considerations relate to the larger question of why DNA and RNA have been chosen for their distinct biological functions, noting indeed that natural RNA occurs without a complementary strand!)

In fact, evolutionary theory posits the existence of a prebiotic RNA world [17]. Interestingly, this raises the question whether duplex formation in DNA is necessary to stabilize it, if single stranded RNA could survive the particularly harsh conditions of the primordial world!

4. CONCLUSIONS

Available evidence is apparently inconclusive about the stable form of DNA – whether single stranded or duplex – in solution. Practically all studies conducted so far have been based on the assumption that the early crystallographic duplex structure may be extended to the solution phase. Thus, the results of DNA melting and related studies are apparently ambiguous, and may equally be interpreted in terms of the uncoiling of single-stranded DNA. In like vein, the helicases may also act upon localised duplex structures in single-stranded DNA (as is known in the case of RNA).

In fact, fundamental thermodynamic considerations indicate that the duplex structure may be unviable. This is because of the prohibitively high energetic cost of separating a putative duplex form of DNA, prior to the plethora of reactions critical to survival (replication, translation, etc.). Thus, intriguingly, the co-existence of complementary strands in solution could be "artefactual", deriving from the need for a template strand in the action of DNA

polymerase (hence does not prove the duplex structure *per se*).

Current experimental evidence, in fact, does not appear to distinguish between the *ds* and *ss* forms; the existence of partial duplexes may also be envisaged, so that the solution state could (in principle) consist of all three states in equilibrium.

Clearly, further studies are indicated, current evidence being too finely balanced to resolve the dilemma – although perhaps leaning toward single-stranded DNA (in this author's modest opinion)!

COMPETING INTERESTS

Author has declared that no competing interests exist.

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