

Reviewer's Comments:

I still have the question of dsDNA formation in solution.

For sure, NMR cannot study large DNA fragment, but if shorter fragment can form a stable dsDNA, then why question the ability of longer complementary DNAs to form an even more stable duplex?

Another crucial evidence that DNA form stable dsDNA is to see how PCR works. Right now every biotechnology lab. uses PCR to amplify a certain dsDNA fragment. Does the author know why PCR needs to be carried out in several different temperatures? This is simply due to the reason that dsDNA needs to be dissociated into ssDNA to enable primers to find its binding partner. After that, the temperature has to be cooled down to form partial dsDNA for the synthesis of second DNA strand. Then temperature needs to be risen again to melt the dsDNA for another primer to bind to restart the synthesis of the third cycle. Thus the requirement of different temperatures in PCR means that DNA exists in ssDNA and dsDNA forms at different temperatures alternatively. Since everybody is working on PCR and is very happy about this technology, I am sure everybody will agree that dsDNA stably exist in solution.

Author's Feedback:

This is a useful point. However, the heating-cooling cycles employed in the PCR can be explained on the basis of coiled ss-DNA that needs to be straightened out to remove the hairpin loops, before the primer can find its target and bind to it. This annealing clearly would be favored at a slightly lower temperature.

The point emphatically made in the paper is that the current view of DNA melting is thermo-dynamically unviable, as the energy needed for putative strand separation is far too high for the process to be experimentally feasible. There is simply no way around this argument. Ironing out loops in ss-DNA would be less demanding thermodynamically, as discussed at length under DNA-melting in the paper.

About shorter fragments forming duplexes: this is because they cannot bend around easily (if at all, in the case of particularly short ones) to form U-shaped hairpin loops, and are thus constrained to form intermolecular base pairs. This is why studies employing shorter fragments lead to ambiguous results, as discussed in the paper under 'Related studies'.

A discussion of PCR is beyond the scope of this paper, and in any case can be treated as an extension of the DNA melting discussion: as melting is such an important part of PCR, the ambiguities raised concerning DNA melting apply equally to PCR as well, by implication! All the same, I have included a brief summarizing statement on PCR at the end of the DNA melting section.

Although this referee is free to critique the paper, I feel he is not at liberty to attack the author by assuming his ignorance, as indicated by referee's choice of words: 'does the author know...?', 'the author is ignorant..' (previous review). This is one of the hazards of single blind reviewing, as the author is at the mercy of an anonymous reviewer, although the reviewer knows the author!).