Conformations of DNA Hairpin Loops under Crowded Conditions

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Using UV Vis absorption spectroscopy on a SPECORD® PLUS, we have monitored the conformation of various ssDNA hairpin loops in crowded fluids that mimic intracellular conditions. As a result, we have found that macromolecular crowding significantly alters the steady-state fraction of closed ssDNA hairpins.

Motivation

Hairpin loops of oligonucleotides are of great interest since hairpin-like configurations can form in any biological process that involves (single-stranded) DNA/RNA, e.g. during gene duplication or transcription. Artificially manufactured hairpin loops basically consist of a single-stranded piece of DNA that can be dissected into a stem and a loop (*see Figure1*). In the stem, complementary bases provide a means for transient association via hydrogen bonds whereas the loop acts as a polymeric spacer between the stem regions. Due to thermal energy, a hairpin stochastically undergoes a transition between the open and the closed state. Hence, at low temperatures the majority of hairpins can be expected to be in a closed configuration whereas an open configuration is preferred at sufficiently high temperatures. So far, the conformation of DNA hairpin loops has been extensively studied in a variety of aqueous solutions [1]. However, given that the intracellular concentration of macromolecules is in the range of 400 g/l, it is of great interest to study the DNA hairpin configuration in artificially crowded fluids that mimic intracellular conditions. Indeed, theoretical predictions and fluorescence experiments have indicated that macromolecular crowding leads to an enhanced fraction of closed DNA hairpins at physiological temperatures [2]. To gain deeper insights into this, we have used the SPECORD® PLUS (*Figure 2*).

Materials and Methods

DNA constructs with five cognate bases in the stem region (CCCAA-X-TTGGG) and a poly-T configuration in the loop (X=21T, 30T, or 50T) were purchased from metabion (Martinsried, Germany). As crowding agents we have used polyethylene glycol (10 kDa; Sigma Aldrich) and sucrose (Roth). Crowders and DNA were dissolved in TE buffer (1 mM EDTA, 100 mM NaCl, 10 mM Tris at pH 7.5). In order to determine the DNA's configuration, we exploited DNA hyperchromicity, i.e. in the open state DNA shows an enhanced absorption in the wavelength range 250-270 nm. For quantitative results, we recorded melting curves of our samples using a SPECORD® PLUS equipped with temperature-regulated sample holders. Thanks to the dual-chamber option of the SPECORD® PLUS, we could record in parallel a sample fluid with DNA and the same fluid without DNA ('control'). Hence, we directly obtained the difference in UV absorption between sample and control. Measurements were conducted in accordance with the settings reported in application note UV_PLUS_08_12_d |06/2012|AK. Owing to the very exact and fast temperature regulation, it was possible to start our experiments near freezing point and heat up sequentially to 90°C. The availability of low temperatures enabled us to also measure DNA hairpins at high loop lengths, e.g. constructs with 50 thymine bases. Besides the effective and fast cooling, we also appreciated the precise heating. Having increased the temperature in steps of one degree, a waiting time of 30 s guaranteed a temperature uncertainty of less than 0.3°C within the sample. For simplicity, we compared the absorbance at 20°C with that obtained in the saturation regime of the melting curve. This ratio reports on the fraction of open DNA hairpins at room temperature.

Results and Discussion

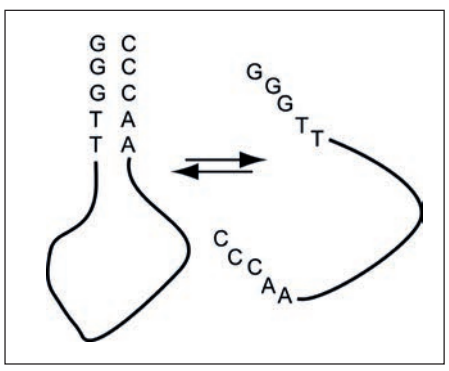

Figure 1: Scheme of a single-stranded DNA hairpin loop.

Conformational changes of three DNA strands in sucrose and PEG solutions (10% and 30% weight per volume) were evaluated as described above (*Figure 3*). As a result, we found that the probability for DNA hairpins to be in the open state slightly increases with loop length for each condition. Furthermore, in sucrose solution the open probability is largely independent of the additive's concentration (*Figure 3*, grey bars) whereas we observed significantly lower probabilities for the open configuration in PEG-crowded fluids (*Figure 3*, red bars). The tendency for closed hairpins increased with the concentration of PEG, hence confirming the hypothesis that crowded fluids feature a significantly increased probability of the associated/closed state of the DNA hairpin. These data highlight that crowded fluids may not only affect the opening-closing kinetics by slowing down association and dissociation. Rather, the steady-state fraction of closed hairpins is significantly enhanced.

A ssDNA hairpin can be dissected into a loop region (shown as full line) that connects two complementary sequences of bases that form the stem region (explicit bases are shown). Due to thermal energy, the hairpin stochastically switches between an open and a closed state.

Figure 2: SPECORD® PLUS

The fraction of open DNA hairpin loops, i.e. the probability of being in the open state, increases for increasing loop lengths irrespective of the additive (sucrose, PEG) in the solution. Yet, a significant drop in the fraction of open hairpins is observed in PEG-crowded solutions (red bars) as compared to sucrose solutions (grey bars). Notably, the probability of being in the open state is basically independent of the amount of sucrose but it significantly decreases with increasing PEG concentrations.

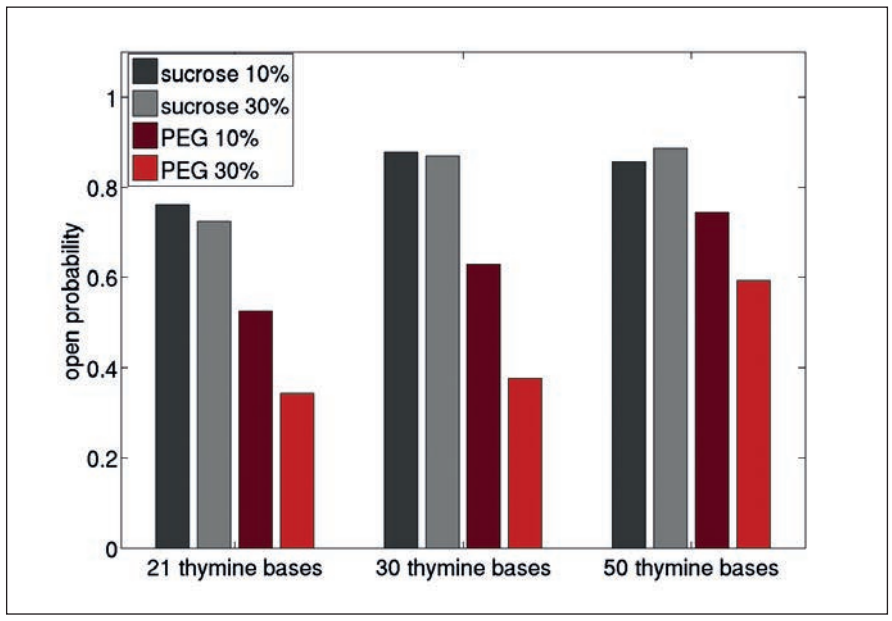

Figure 3: Open probability of DNA hairpin loops.

Acknowledgements

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References

[1] G. Bonnet, O. Krichevsky, and A. Libchaber, Proc. Natl. Acad. Sci. USA 95, 8602 (1998).

[2] O. Stiehl, K. Weidner-Hertrampf, and M. Weiss, New J. Phys. 15, 113010 (2013).

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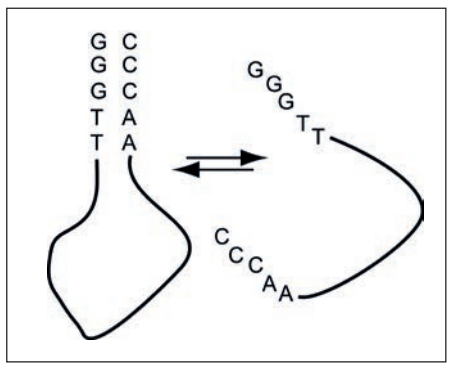

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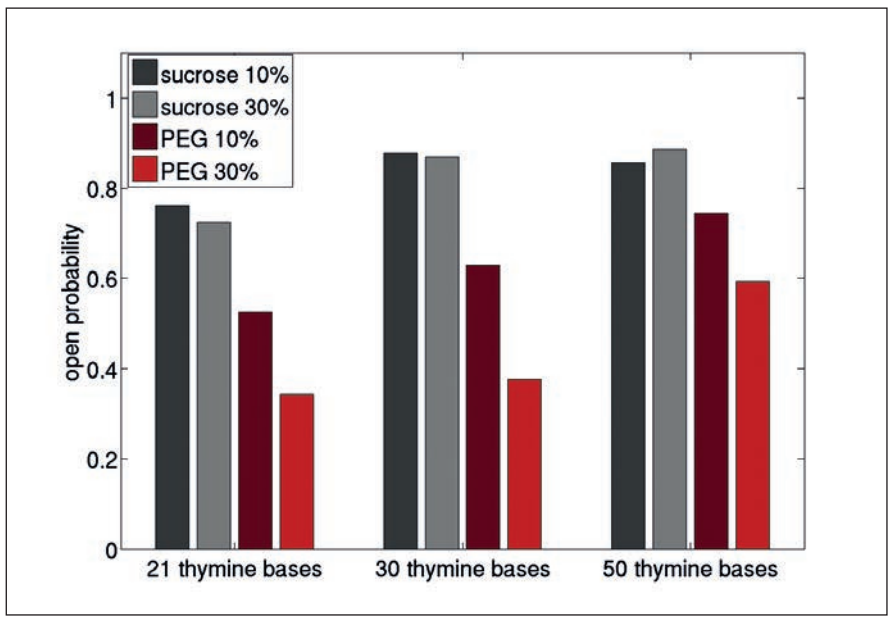

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