Chemical Physics 369 (2010) 91–95

Contents lists available at ScienceDirect

Chemical Physics

journal homepage: www.elsevier.com/locate/chemphys

A RNA-based nanodevice recording temperature over time

Siegfried Höfinger [a,b,*], Francesco Zerbetto [a]

[a] Dipartimento di Chimica "G. Ciamician", Università di Bologna, Via F. Selmi 2, 40126 Bologna, Italy
[b] Department of Physics, Michigan Technological University, 1400 Townsend Drive, Houghton, MI 49331-1295, USA

ARTICLE INFO

Article history:
Received 17 November 2009
In final form 9 March 2010
Available online 15 March 2010

Keywords:
RNA
Thermometer
Nanodevice
Hairpin
Unfolding

ABSTRACT

Nucleic acids provide a wealth of interesting properties that can find important applications in nanotechnology. In this article we describe a concept of how to use RNA for temperature measurements. In particular the principal components of a nanodevice are outlined that works on the basis of RNA secondary structure rearrangement. The major mode of operation is a hairpin-coil transition occurring at different temperatures for different types of short RNA oligonucleotides. Coupling these events to a detection system based on specific RNA hybridization provides the framework for a nanodevice capable of temperature records as a function of time. The analysis is carried out with the help of a statistical mechanics package that has been specifically designed to study RNA secondary structure. The procedure yields an optimized list of eight RNA sequences operational in the range from −10 to 60 °C. The data can form the basis of a new technology of potential interest to many fields of process and quality control.

1. Introduction

All living systems are confronted with the problem of temperature control. Temperature sensing and corresponding signal transduction are of vital importance to all kingdoms of life. In *rhizobia* the corresponding temperature-responsive element has been narrowed down to RNA [1,2]. In particular, the ROSE element was identified as responsible for temperature sensing. ROSE is composed of four RNA stem loops from which stem loop IV seems to be most directly linked to temperature control. RNA secondary structure provides the mechanical basis for this type of signal transduction.

Due to their elementary role in the early evolution of life, nucleic acids in general and RNAs in particular are increasingly becoming important building blocks in various problems of current nanotechnology. This is mainly caused by the combination of a very robust architecture together with the enormous potential resulting from sequence variability. A recent review summarizing the structural potential of DNA and RNA in current nanotechnology has been given by Aldaye et al. [3].

In the present article we want to investigate the use of RNAs for the purpose of temperature recording. Short RNA oligonucleotides are proposed to form the building blocks of a nanodevice designed for temperature sensing. Learning from nature we want to employ short RNA sequences that form individual helical hairpins (similar to stem loop IV in the ROSE element). Each of the RNA oligonucleotides shall have a characteristic transition temperature at which the hydrogen bonds that stabilize the hairpin structure will thermally break. As a result of this breakup, a single-stranded RNA conformer in random-coil conformation will form. The accompanied exposure of specific RNA sequences can directly be used for detection and characterization of individual unfolding events and hence identify in a unique way specific hairpin-coil transition temperatures. A similar model system has been successfully employed in hybridization studies with 5′,3′-labeled DNA oligonucleotides termed *Molecular Beacons* [4].

In this work we make use of the statistical mechanics framework provided with the *DINAMelt* toolkit and the related *UNAFold* package [5]. Given the impressive computational performance of these programs they can be used in reverse sense, meaning to apply them to a series of random sequences until a certain property is detected by chance.

2. Materials and methods

2.1. Statistical mechanics based RNA modeling framework

All computations were carried out with the *DINAMelt* package [5]. A typical run of a sequence similar to the one shown in Fig. 1 terminates within approximately 10 s using standard computer hardware. Full exhaustive searches are too computationally intensive, because even a short decamer generates $4^{10} = 1.1 \times 10^6$ sequences, which roughly translates into 182 CPU days. Therefore the following enrichment protocol was applied,

* Corresponding author at: Dipartimento di Chimica "G. Ciamician", Università di Bologna, Via F. Selmi 2, 40126 Bologna, Italy.
E-mail addresses: siegfried.hoefinger@unibo.it (S. Höfinger), francesco.zerbetto@unibo.it (F. Zerbetto).

0301-0104/$ - see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.chemphys.2010.03.007

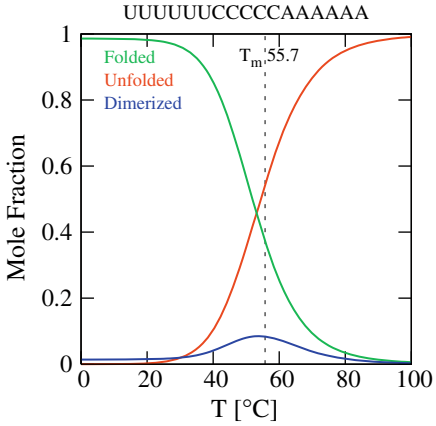

Fig. 1. Concentration plot obtained from DINAMelt analysis [5] for the RNA sample sequence UUUUUUCCCCCAAAAAA. At the critical temperature the folded form (green line) is mainly lost and the unfolded form (red line) becomes the major structural motif in the ensemble. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

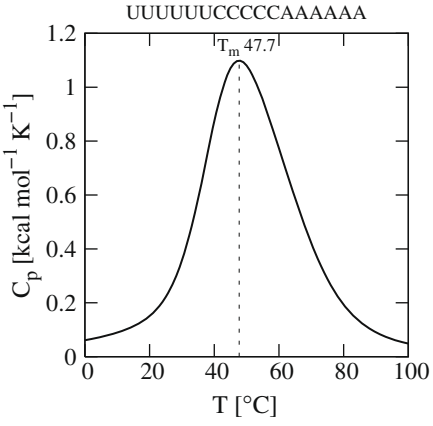

Fig. 2. Specific heat plot obtained from DINAMelt analysis [5] carried out for the RNA sample sequence UUUUUUCCCCCAAAAAA. A phase transition is indicated at the position of the peak.

- Define target temperature;
- Compute random sequence of 10–20 nucleotides;
- Check that the random sequence had not already been considered in previous attempts;
- Run *hybrid2.pl* from *DINAMelt*;
- Extract the critical temperature and store results;
- Check whether the extracted critical temperature is closer to the target temperature than any one found in a hitlist of 25 items and replace sequences in case;
- Repeat the above; 12,500 attempts are usually sufficient to obtain a hitlist of 25 candidates that all show thermo-response to within ±1 °C of the defined target temperature.

2.2. Molecular dynamics simulation of RNA unfolding at 20 °C

An experimentally determined (but otherwise unrelated) RNA hairpin structure was downloaded from the pdb data bank [6] (pdb code 2RPT). This RNA structure was used as a template for the targeted RNA sequence GUAAAGAAAGAUA predicted to be sensitive to thermal unfolding at 20 °C (see Supplementary Fig. V). Nucleotides G_2, C_3, C_4, C_5, G_6 and C_7 of the original 2RPT-pdb structure were mapped to G_1, U_2, A_3, A_4, A_5 and G_6 of the intended target sequence. The original 2RPT-pdb structure was discarded beyond nucleotide C_7 except for nucleotides G_{14}, C_{15}, C_{16}, G_{17}, G_{18}, C_{19} and C_{20} that were again mapped onto A_7, A_8, A_9, G_{10}, A_{11}, U_{12} and A_{13} of the intended target sequence. H-atoms were discarded and base planes defined by three atoms, the N-atom of the purin/pyrimidine bases connecting to the ribose unit together with the two adjacent C-atoms inside the purin/pyrimidine base planes. The resulting fragmental pdb structure was read into *XLeap* of the AMBER package [7] and all missing atoms added automatically. The *ff99* force field was employed. An initial minimization performed in vacuum (5000 steps, cut off radius of 18 Å, Coulomb type electrostatics without Ewald sums, all bonds allowed to adjusted except those that were explicitly restrained) was followed by a vacuum equilibration to 300 K (50,000 steps, time step 1 fs, otherwise identical conditions). Four residues (nucleotides 1, 2, 11 and 12) involved in forming the essential H-bonds stabilizing the hairpin structure (see Fig. V(d) of the Supplementary material) were restrained to the model-geometry, hence maintained close to the experimental structure (force constant 1.5 kcal/mol Å^2). The resulting vacuum RNA hairpin structure was again read into *XLeap*, 12 Na^+ counter ions were added, and the system was solvated in explicit TIP3P water [8] (10 Å solvent layer beyond solute boundaries leading to a simulation cell of orthorhombic geometry). The solvated system was minimized (5000 steps, cut off radius of 12 Å, standard Ewald summation with positional restraints on residues 1, 2, 11 and 12) and then heated to the target temperature of 293 K in 25,000 steps of equilibration MD using a Berendsen thermostat [9] within the NTV ensemble (default Ewald summation, time step of 2 fs, cut off radius of 10 Å, positional restraints and all bonds involving H-atoms kept static). This initial equilibration MD was followed by another short run of equilibration MD of 10 ps to release all restraints (otherwise identical conditions). The resulting equilibrated RNA structure in explicit water was then simulated for a production interval of 5 ns at unrestrained conditions. Snapshot structures were saved every 5000 steps and analyzed with program *ptraj* from the AMBER package (all individual frames rms-fitted against the initial structure, water and counter ions stripped off, the center of the initial 13 RNA residues shifted into the origin and four distances calculated, the latter regarding all H-bonds involved in the hairpin structure, i.e. those formed between residues G_1 and U_{12} and between U_2 and A_{11}). All individual snapshots were graphically processed and combined into an mpeg movie (see Movie 1 of the Supplementary material).

3. Results and discussion

3.1. Default DINAMelt [5] operation delivers critical temperatures that can be used to classify the thermal sensitivity of RNA sequences

An arbitrary RNA sequence, UUUUUUCCCCCAAAAAA, is defined and analyzed with the *DINAMelt* package in default mode. Results are summarized in Figs. 1–4. The concentration plot given in Fig. 1 shows how the concentration of the dominant hairpin structure (green[1] line) changes with increasing temperature. A sharp drop in concentration of the hairpin form accompanied by a sudden increase of the unfolded form (red line) reveals a critical transition temperature of 55 °C approximately. Fig. 2 shows a plot of the corresponding specific heat, C_p, with critical temperature of about 48 °C indicative of a phase transition. Similarly, the characteristic inversion point in the UV-absorption spectrum [10] shown in Fig. 3 hints at a critical transition temperature of about 49 °C. A structural hint based on the statistical mechanics evaluation performed with program *UNAFold* [5] is shown in Fig. 4 (see also Supplementary Fig. I(b)). In addition to the analysis focussing on single

[1] For interpretation of color in Figs. 1, 4, 5, the reader is referred to the web version of this article.