Biophysical Chemistry xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Biophysical Chemistry

journal homepage: http://www.elsevier.com/locate/biophyschem

Exploring the effects of temperature and pressure on the structure and stability of a small RNA hairpin

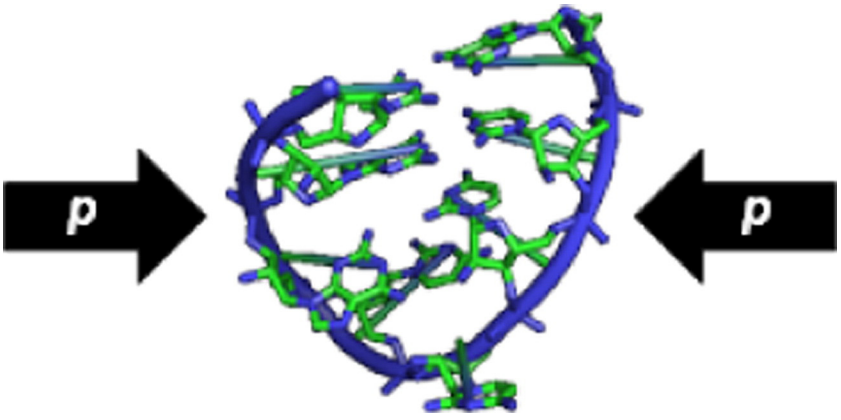
Caroline Schuabb, Salome Pataraia, Melanie Berghaus, Roland Winter *

TU Dortmund University, Department of Chemistry and Chemical Biology, Physical Chemistry I, Biophysical Chemistry, D-44221 Dortmund, Germany

HIGHLIGHTS

- The sRNAh structure is not fully unfolded even at 90 °C.
- Pressure up to 400 MPa induces small conformational perturbations, only.
- Cy3/Cy5 labeling of the sRNAh structure changes its stability.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 21 September 2016
Received in revised form 26 October 2016
Accepted 26 October 2016
Available online xxxx

Keywords:
Small RNA hairpin
High pressure
Temperature-pressure stability
FTIR
UV/Vis
FRET
SAXS

ABSTRACT

RNAs perform multiple vital roles within cells, including catalyzing biological reactions and expression of proteins. Small RNA hairpins (sRNAh) are the smallest functional entities of nucleic acids and are involved in various important biological functions such as ligand binding and tertiary folding initiation of proteins. We investigated the conformational and free energy landscape of the sRNAh gcUUCGgc over a wide range of temperatures and pressures using fluorescence resonance energy transfer, Fourier-transform infrared and UV/Vis spectroscopy as well as small-angle X-ray scattering on the unlabeled and/or fluorescently labeled sRNAh. The sRNAh shows a broad melting profile with continuous increase of unpaired conformations up to about 60 °C. However, the sRNAh structure might not be fully unfolded at temperatures as high as 90 °C and still comprise various partially unfolded compact conformations. Pressure up to 400 MPa has a small effect on the base pairing and base stacking interactions of the sRNAh, indicating small conformational perturbations, only, which might originate from minor changes in packing and hydration of the RNA molecule upon compression. Pressurization at 70 °C, i.e. at a temperature above the melting transition, has no significant effect on the conformational ensemble of the sRNAh, i.e., it does not promote formation of new native stem connections after thermal denaturation. Finally, we noticed that Cy3/Cy5 labeling of the sRNAh changes, probably via stacking interactions between the fluorescent dyes and the nucleotide rings, the stability of the sRNAh, thereby rendering FRET analysis of the conformational dynamics of such small RNA structure inappropriate.

* Corresponding author.
E-mail address: roland.winter@tu-dortmund.de (R. Winter).

http://dx.doi.org/10.1016/j.bpc.2016.10.006
0301-4622/

1. Introduction

RNA molecules are known to serve not only as carriers of genetic information, but also of catalytically active functional units. These discoveries have contributed significantly to the RNA world theory [1,2]. Hence, folding of the three dimensional structure of RNA molecules is of vast importance in regulating essential biological processes. RNA hairpins are common secondary structure elements in the complex 3D structure of RNA molecules that can serve as nucleation sites for RNA folding, ligand binding and tertiary folding initiation [3–9]. Moreover, RNA hairpins are integral components of ribozymes and riboswitches. Revealing the free energy and conformational landscape of these structures, and exploring the forces controlling their stability are also highly important to understand the thermal and pressure stability of these molecules in thermophilic and piezophilic organisms, because the adaptation mechanisms of these organisms thriving under particular harsh conditions are still largely unanswered.

The small RNA hairpin (sRNAh) structure is formed when a polynucleotide single strand folds back on itself to form intramolecular Watson-Crick base pairs, resulting in a helical stem capped by a loop (Fig. 1). The hairpin is the predominant secondary structure of RNAs, and its amount varies depending on the RNA type and function. The amount of nucleotide residues at the hairpin loop also varies depending on the RNA molecule. For example, tRNA hairpin loops contain seven nucleotides while ribosomal hairpin loops contain four nucleotides [10]. It has been shown that 70% of all tetraloops in rRNA are either UNCG or GNRA (N is related to nucleotide and R is related to purines), and they vary between organisms, from UUCG to GCAA for the same hairpin in closely related organisms [11]. Also, these RNA hairpins have significant thermodynamic stability due to the presence of several noncanonical interactions [12,13]. Due to their importance, simplicity and size, small hairpins serve as prototype for folding dynamics and structural stability studies of polynucleotides, also in theoretical studies [13–19].

A molecular dynamics study of Chakraborty et al. [13] showed that the energy landscape of small tetraloop hairpins, such as UUCG and GCAA hairpin loops, can be quite complex and that two-state kinetic profiles cannot accurately describe their folding mechanism. They also showed that these structures are highly thermostable, so that even at temperatures as high as 1000 K in their simulations, extended-chain conformations are hardly populated compared to more compact conformational states.

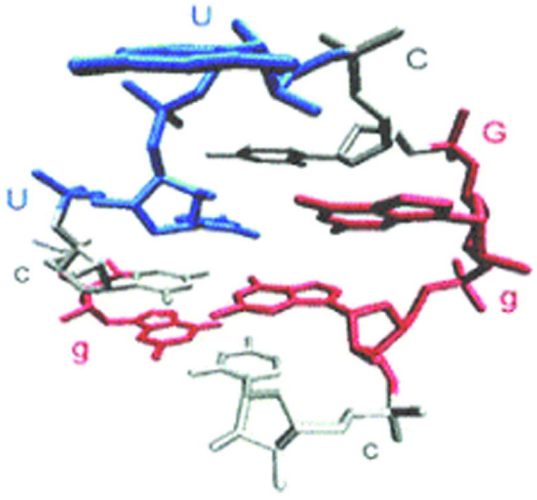


Fig. 1. Schematic representation of the stem-loop structure of the sRNAh gcUUCGgc [14]. The bases written in uppercase are the unpaired bases found in the loop region, and those written in lowercase comprise the stem. Protein Data Bank (PDB): 1F7Y. In the labeled sRNAh, the donor fluorophore Cy3 and the acceptor fluorophore Cy5 are attached to the 5′ and 3′ ends of the hairpin, respectively.

The folding/unfolding equilibrium thermodynamics of the RNA tetraloop gcUUCGgc has been studied theoretically by Garcia & Paschek [16]. In this work, a calculation of the free energy landscape as a function of temperature and pressure was performed, resulting in a temperature-pressure stability phase diagram of the small RNA hairpin, which is accompanied by population of multiple conformational states. Recent molecular dynamics work by Miner et al. [19] characterized the thermodynamics and folding free energy landscape of the RNA tetraloop gcGCAAgc in detail. They showed that the hairpin structure is primarily dominated by right-handed A-RNA stems with diverse loop conformations. In addition, a left-handed *Z*-RNA type stem, and a compact purine triplet have been identified. Each of these structural configurations presented a different temperature and pressure stability. Under high hydrostatic pressure conditions, the A-RNA conformation has been shown to be stabilized and its population is therefore increased. On the other hand, the Z-RNA conformation is destabilized and its population decreases at high hydrostatic pressure (HHP). Changes in pressure also modulated the interaction of the RNA molecule with the solvent and ions.

In this study we set out to conduct experiments to analyze conformational changes of the RNA tetraloop gcUUCGgc over a wide range of temperatures and pressures using fluorescence, UV–Vis and FTIR spectroscopies as well as small-angle X-ray scattering (SAXS) experiments, and compare the results with theoretical predictions.

2. Materials and methods

2.1. RNA nucleotide and sample preparation

The small RNA hairpin (sRNAh) molecule used in this study has the 5′-gcUUCGgc-3′ sequence. This sequence creates a loop with a double base paired stem between G—C nucleotides. The sRNAh used in the FRET studies has two fluorescent dyes attached, Cyanine 3 phosphoramidite (Cy3) and Cyanine 5 phosphoramidite (Cy5) to the 5′ and 3′ ends of the sequence, respectively. Their chemical nature is built from two indole rings that are connected by a polymethine chain. Cy3 serves as the donor fluorophore and Cy5 as the acceptor in the FRET assay. The small RNA hairpins (sRNAh non-labeled 5′<!–[/INS]-GCUUCGGC-3′, and sRNAh labeled 5′-Cy3-GCUUCGGC-Cy5-3′) were synthetized by IBA Life Solutions for Life Science GmbH (Göttingen, Germany), and were received as lyophilized powder. The sRNAh was suspended in nuclease free water to obtain a 0.1 mmol μL^{-1} solution and stored at −80 °C until use. For the various techniques applied, different sRNAh concentrations were used. The samples were lyophilized to remove H_2O, and then suspended in pure 50 mM TRIS-HCl buffer + 0.1 mM ethylenediaminetetraacetic acid (EDTA), pH 7.5. The experiments were carried out multiple times using new preparations, and also different batches of RNA were used. No concentration dependent changes have been observed in the concentration range covered by the various techniques.

2.2. Fluorescence spectroscopy (FRET-based assay)

The Förster resonance energy transfer (FRET) signal between the two fluorescent dyes Cy3 and Cy5 was recorded with a K2 fluorescence spectrometer from ISS, Inc., Champaign, IL, USA. The spectrometer uses a xenon arc lamp as light source. The Cy3 fluorescent dye (donor) was excited at 550 nm, and the emission of the Cy3 was recorded starting from 560 to 690 nm. The FRET signal between Cy3 and Cy5 was observed between 640 and 690 nm (Figs. SI 1 and 3). Cy3 and Cy5 are the most widely used fluorescent sulfoindocyanines for covalently labeling nucleic acids due to their remarkable stability against photobleaching. The Förster distance of the Cy3-Cy5 pair is $R_0 \approx 6$ nm. In all experiments, the temperature was controlled with a circulating water bath to an accuracy of 0.1 °C, and it was given sufficient time to equilibrate the sample chamber. In case of the pressure dependent

Please cite this article as: C. Schuabb, et al., Exploring the effects of temperature and pressure on the structure and stability of a small RNA hairpin, Biophys. Chem. (2016), http://dx.doi.org/10.1016/j.bpc.2016.10.006