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SCIENCE DIRECT
Journal of Structural Biology 153 (2006) 207–222
Journal of Structural Biology
www.elsevier.com/locate/yjsbi

On the structural features of hairpin triloops in rRNA: From nucleotide to global conformational change upon ligand binding

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Received 4 August 2005; received in revised form 1 December 2005; accepted 2 December 2005
Available online 27 December 2005

Abstract

RNA structure can be viewed as both a construct composed of various structural motifs and a flexible polymer that is substantially influenced by its environment. In this light, the present paper represents an attempt to reconcile the two standpoints. By using the 3D structures both of four (16S and 23S) portions of unbound 50S, H50S, and T30S ribosomal subunits and of 38 large ribonucleoligand complexes as the starting point, the behavior, which is induced by ligand binding, of 73 hairpin triloops with closing g-c and c-g base pairs was investigated using root-mean-square deviation (RMSD) approach and pseudotorsional (η, θ) convention at the nucleotide-by-nucleotide level. Triloops were annotated in accordance with a recent proposal of geometric nomenclature. A simple measure for the determination of the strain of a triloop is introduced. It is believed that a possible classification of the interior triloops, based on the 2D η–θ unique path, will aid to conceive their local behavior upon ligand binding. All rRNA residues in contact with ligands as well as regions of considerable conformational changes upon complex formation were identified. The analysis offers the answer to: how proximal to and how far from the actual ligand-binding sites the structural changes occur?

Keywords: rRNA; Triloops; Ligand binding; Nucleotide and global conformational changes

1. Introduction

A variety of RNA molecules have important biological functions in cells, including protein synthesis and targeting, many forms of RNA processing and splicing, RNA editing and modification, and chromosome end maintenance. To decipher the biology of a cell, a need to know the identity of all encoded RNAs, the molecules with which they interact and the molecular structures of these complexes, is of vital importance. Hence, knowledge of the molecular structures of biological macromolecules is a prerequisite for the understanding of their functions and interactions (Doudna, 2000).

Various recurrent motifs such as the U-turn (Quigley and Rich, 1976), the E-loop (Varani et al., 1989; Wimberly et al., 1993), the GNRA tetraloop (Jucker and Pardi, 1995), the GNRA-like pentaloop (Robertson et al., 2005), the A-minor motif (Nissen et al., 2001), the kink-turn (Klein et al., 2001), the SRP motif (Gundelfinger et al., 1984; Keenan et al., 2001), and the T-loop/lone pair triloop motif (Lee et al., 2003; Nagaswamy and Fox, 2002) have been established as constitutive parts of large RNAs. The modularity of RNA or its ability to use known structural motifs to design self-assembling building blocks has been recognized (Chworos et al., 2004). The existing RNA motifs and a number of novel structural elements may serve as common modules in biological RNA (Popenda et al., 2004; Vallazza et al., 2004). Therefore, RNA structure, in general, and motif structure, in particular, are inclined to change by interacting with surrounding agents such as proteins, other RNAs, metals or other ligands (Holbrook, 2005). A more general effort toward defining the motifs in terms of their three-dimensional (3D) structures has been undertaken (Klostermann et al., 2002, 2004).

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doi:10.1016/j.jsb.2005.12.001

The interference of RNA secondary structure and tertiary interaction motifs such as trinucleotide repeats with human disease has been reported as an emerging direction in RNA structural biology (Barciszewska et al., 2005; Jasinska et al., 2003; Michlewski and Krzyzosiak, 2004; Philips et al., 1998; Singer, 1998; Sobczak et al., 2003). The interaction motifs of hairpin loops have been recognized as possible RNA targets for the binding of proteins (Darnell et al., 2005). Proteins, sometimes, act as "mortars" fitting the space between the RNA domains (Major and Griffey, 2001). The RNA–protein interactions can be considered as being a consequence of induced fit between flexible loops (Hainzl et al., 2005). Whereas triloops are common in a variety of naturally occurring RNAs, they may possess some particularly useful structural characteristics (Davis et al., 1993). Several solution structures of hairpin triloops in various RNAs have been reported in the literature (Kim and Tinoco, 2001; Leeper et al., 2003). We herein attempt to learn on the behavior of 73 interior triloops (mainly in the 2.4–3.5 Å resolution range) that is induced by complex formation. Besides, in the 2.5–3 Å resolution range, sugar puckers and torsion angles are unknown, and it is difficult to discuss most of the known recurrent motifs, such as sharp turns, U-turn, etc. (Davis et al., 2004). The pseudotorsion analysis given below might therefore be a good way to bypass these difficulties.

The descriptions and comparisons of RNA molecular structures can be based upon several distinguishable representations including Cartesian coordinates (Reijmers et al., 2001), torsion angles (Hershkovitz et al., 2003), pseudotorsion angles (Duarte and Pyle, 1998), and RMSDs (Gendron et al., 2001). Although differences between compared structures are detectable by means of each of these methods, a reduced representation of RNA conformational space using pseudotorsions of two virtual bonds of individual nucleotides is more likely to register conformational peculiarities with a higher sensitivity (Duarte et al., 2003). To provide more insight into conformational organization at a nucleotide level, a specially chosen strategy composed of RMSD procedure and pseudotorsional convention was employed throughout this work.

2. Methodology

2.1. Databases

The investigated structures were denoted by their Protein Data Bank (PDB) (Berman et al., 2000) and Nucleic Acid Database (NDB) (Berman et al., 1992) codes (Tables 1 and 2). The Structural Classification of RNA (SCOR) database (Klostermann et al., 2002, 2004) classification of internal triloops, based on the number of bases in the main stack, was initially used in the present study.

2.2. Computational methods

The calculations pertaining to the RMSD comparison of triloops and closing base pairs were performed using the Swiss-PdbViewer program (Guex and Peitsch, 1997). The RMSD procedure involving all atoms was done at the nucleotide-by-nucleotide level.

The standard RNA backbone torsion angles (α, β, γ, δ, ε, and ζ) of single nucleotides can be sequentially combined to provide a meaningful description of RNA conformation. A variety of combinations of standard backbone torsion angles frequently describe the same nucleotide morphology, due to compensatory changes that cause vanishing effects at a polynucleotide level. To overcome the difficulties, it is possible to simplify the conformational space of individual nucleotides by defining two virtual bonds extending from P to C4′ and from C4′ to P of the adjacent nucleotide (Olson, 1976). Two pseudotorsions around these virtual bonds, η ($C4'_{i-1}$–P_i–$C4'_i$–P_{i+1}) and θ (P_i–$C4'_i$–P_{i+1}–$C4'_{i+1}$), determine conformational features of a given nucleotide, i (Duarte and Pyle, 1998). Conceivable as a sort of the Ramachandran plot (Malathi and Yathindra, 1982), a path-annotated η–θ plot shows qualitative correlations with discrete nucleotide conformations. The η–θ values were computed using the Algorithmic Method for Identifying Groupings of Overall Structure (AMIGOS) program (Duarte and Pyle, 1998). The RNA worm method (Duarte et al., 2003) was used for RNA structure-based comparisons. As an RNA worm quantitatively defines a particular RNA structure, it is possible to compare subtle structural differences between molecules by way of comparing their worm representations. A definition and several recommendations for the determination of the difference in η–θ values are given in the footnotes of Table 1. The Probing RNA structures to Identify Motifs and Overall Structural changes (PRIMOS) software package (Duarte et al., 2003) was employed to create RNA worm files and to perform the necessary comparisons. The sequence position of RNA residues in contact with ligands was determined by the program ENTANGLE (Allers and Shamoo, 2001) in all of the considered complexes but in the 1njn, 1njo, and 1p9x structures, which were analyzed by the LPC software (Sobolev et al., 1999). The two-dimensional (2D) graphs (Figs. 1, 2, and 5) were generated using SigmaPlot 5.0 (SPSS). Fig. 4 only was generated by PyMol (DeLano, 2004).

The annotation of a RNA structure is the process of extracting residue conformations and inter-residue relations using the accumulated knowledge on allowed conformations. The annotation of hairpin triloops and closing base pairs was performed using the MC-Annotate (Gendron et al., 2001) program. All the annotated structures, available as the Supplementary material, were schematically described using a recent proposal of geometric nomenclature (Leontis and Westhof, 2001, 2003). The annotation symbols used in this paper are: =, GC cis canonical Watson–Crick basepair; ●, GU wobble base-