Structural studies of RNA involved in the pathogenesis of neurodegenerative diseases

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Abstract

Many human diseases, especially neurodegenerative and neuromuscular diseases, are caused by the expansion of nucleotide repeats known as microsatellites in various genes. Transcripts generated from these genes, due to the presence of repeated nucleotide sequences, can form different structures. Studying RNA structures associated with neurodegenerative diseases is an important step in understanding the mechanisms behind these diseases and may contribute to the discovery of potential new therapies.

I set two goals for myself. My first task was to develop a protocol to produce RNA hairpins, stabilization of RNA in the form of hairpin for crystallization and to determine the three-dimensional structure of RNA hairpins. The second task involved determination of the three-dimensional structure of RNA complex containing the G_2C_4 sequence with a synthetic molecule ANP77. Based on this structure, I wanted to assess the therapeutic potential of the ANP77 molecule and whether it could be used to stabilize RNA structures.

For the first task, I developed a protocol to produce RNA hairpins for crystallization studies. Short RNAs (up to 10 nucleotides in length) can be efficiently produced using solid-phase chemical synthesis. However, RNAs longer than 40 nucleotides should be synthesized *via in vitro* transcription using ribozymes or modified DNA templates. I designed a series of constructs related to neurodegenerative diseases for which I wanted to determine the crystal structure. Constructs containing CUG trinucleotide repeats (associated with the development of myotonic dystrophy) were circularized and I aimed to increase their crystallization potential through the interaction of RNA structures with the U1a RNA binding domain protein.

For the second task, I successfully obtained crystal structures for the G_2C_4 construct with and without the ANP77 molecule. I described the fold of RNA, RNA-ANP77 interactions and interactions between molecules in the crystal lattice. Using differential scanning calorimetry in the pH range of 5,2–7,0, I determined the level of stabilization of the G_2C_4 structure by the ANP77 molecule.

The research presented in this work reveals the potential of RNA containing G_2C_4 sequence to form triplexes with triplets of nucleotides C-C-C and C-G-C. This is an important contribution to our understanding of the mechanisms associated with neurodegenerative diseases described in the study.