Structural Changes in Therapeutic RNAs

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Summary: Therapeutic RNAs have been strongly influencing the pharmaceutical market, which has been marked by capturing its part in last decade. Wide range of biological activities, production easiness and relative cheapness result in a powerful background for its mass production and implication in pharmacology. Their structure is known to be directly linked to the conformation they posses at given conditions. Conformational changes that may occur in response to various factors are believed to cause serious functional activity changes.

Keywords: biological action; conformation; small RNAs

Introduction

Small RNAs have been increasingly attracting scientists for a last decade. Their participation in genome regulation expression was shown along with involvement in apoptosis, oncogenesis, and inflammatory processes.[1] It is worth mentioning, that even tiny conformational changes in lmwRNAs structure may affect their biological action in many possible ways – activity level alteration, affinity rate modulation etc. Sure enough, microenvironment strongly contributes to such shifts. There is no clear understanding of such dependence. Many efforts are currently being put to solve this phenomenon.

Experimental Part

3 commercial RNA preparations were used in this study: 1–"RNA" – heterogeneous fraction of yeast RNA 25 ± 2 nucleotides in length, possibly containing short double-stranded fragments, 2–intravenous form of "RNA" – "3% RNA", 3–highly purified homogeneous form of "RNA", with addition of mannitol, in capsule form – "Nuclex".[2]

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Spectrophotometric melting was performed on Specord UV-VIS equipment (Germany). Sample RNA solution optical density was recorded as a function of gradually rising temperature (from 30°C to 70°C; 2.5°C per minute). Samples were stored in 1 cm long quartz cuvettes.

Dynamic light scattering (DLS) experiments were carried out with the use of Zetasizer Nano system (UK) at 633 nm. Sample solutions were stored in 1 cm long cuvette.

In both cases of either spectrophotometric melting or DLS experiments the sample concentration equaled 1 mg/ml of water solution.

Results and Discussion

Obtained experimental data indicated, that "RNA" sample's melting point equaled 45.5°C. We believe that such effect could have occurred due to presence of double stranded regions. Other sample, on the other hand, displayed hypochromic effect under conditions of gradually rising temperature (see Figure 1), which might be an evidence for complex RNA sample organization. We suggest that such significant differences occurred because of biopolymer microenvironment. In this respect, "Nuclex" sample contained mannitol in large quantities. Rich in hydroxyl groups,

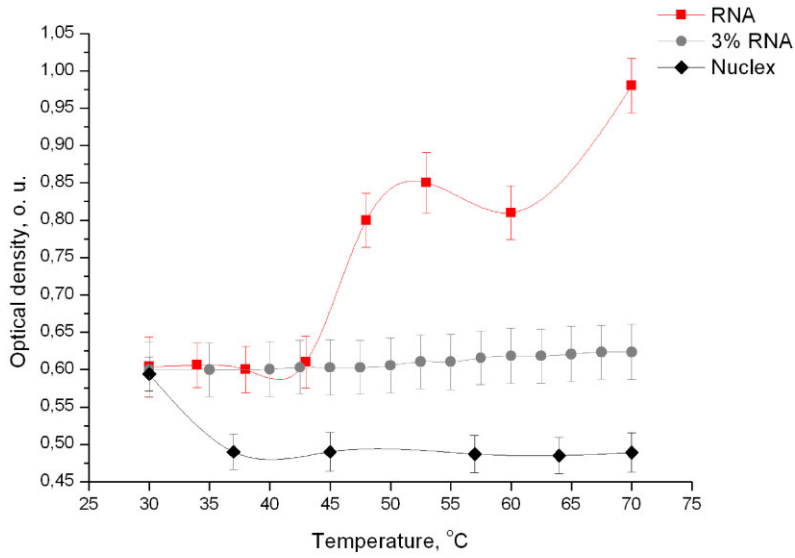

Figure 1.
Dependence of optical density on temperature.

this alcohol was capable of changing solvent polarity, which could have caused polymer dehydration and, in turn, altered surface-bound charge. We suppose that sample molecules formed more compact and structured composition – supramolecular associates, which were mainly held together by electrostatic interactions.

Light scattering experiments actually proved this suggestion. These structures tended to be unstable and to destabilize upon temperature raise. We managed to

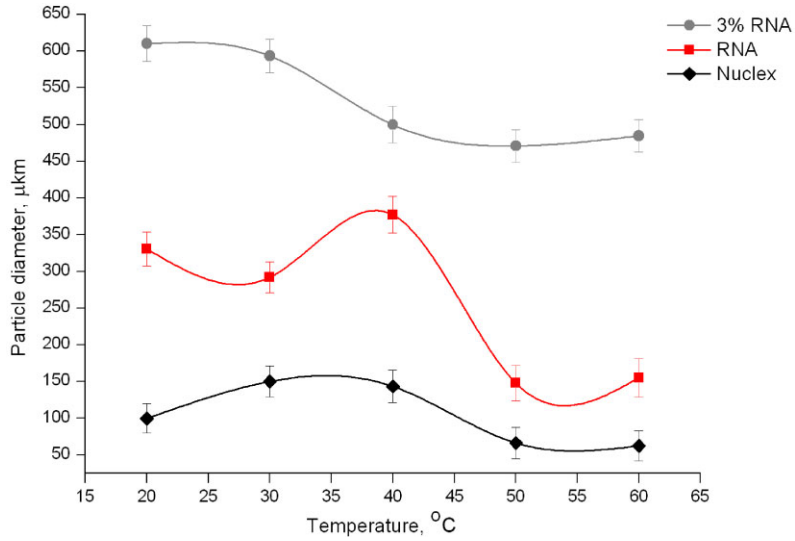

Figure 2.
Dependence of particle diameter on temperature.

indicate different-sized particles within studied drug samples, which was an unexpected outcome due to their suspected homogeneity (see Figure 2). Gradual temperature raise led to particle diameter decrease, which can be a result of supramolecular associates thermal destruction, presented by particle species of different size.

We assume that RNA sample microenvironment may not only have an impact on biopolymer structure, but also is capable of affecting its biological and therapeutic characteristics: surface-bound charge decrease, for instance, may cause enhanced cell permeability and/or higher nuclease resistance.

Conclusion

According to our results, particle diameter value strongly correlates with particular sample behavior under the conditions of rising temperature, which, as mentioned above, is a striking outcome, since the studied samples were of the same origin.

[1] Z. Tkachuk, **2004**, Compound, composition and method for treatment of inflammatory and inflammatory-related disorders. United States Patent # US 6,737,271.
[2] Z. Tkachuk, **2012**, Multiantiviruses compound, composition and method for treatment of virus disease. US patent publication # 0232129A1.

