Water Soluble Nanoparticles from PEG-Based Cationic Hyperbranched Polymer and RNA That Protect RNA from Enzymatic Degradation

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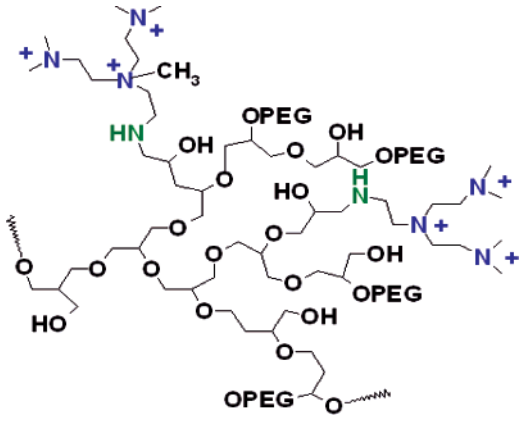
Recent advances in understanding biological systems have proven that RNA is not merely the carrier of genetic information, but also a key molecule in regulation of gene expression and other crucial metabolic processes. Therefore, it is being considered as an ideal therapeutic candidate both for metabolic and genetic disorders. However, research involving RNA molecules faces a practical limitation since RNA is highly labile. We have developed a novel method to protect RNA from cleavage by complexing it with a hyperbranched cationic polymer. It was found that total cellular RNA isolated from yeast spontaneously interacts with the positively charged polymer to form a spherical nanoparticle morphology. This interaction protects the RNA against enzymatic degradation. This methodology can be easily adapted for long-term storage of RNA, long distance transfer of RNA, and genetic engineering using RNA as a building block.

In the post-genomic era, genetic engineering has opened up new challenges and opportunities for development of medicine and biomedical research. However, genetic engineering technology is limited to the DNA level, primarily due to practical limitations of handling RNA, a labile molecule, which is difficult to study and manipulate, especially when it comes from an in vivo source.[1] Considerable research, such as addition of inhibitors in DNA solutions, entrapping in liposome, or forming nanaoparticles, etc., has been done to devise methods to protect nucleic acids from cleavage.[2−4] These methods are successful in protecting DNA to a considerable extent, but have not attained similar success in protecting RNA from degradation.[5,6] Recent advances in understanding of the role of RNA molecules have established that RNA is not merely a carrier of genetic information but also an important molecule that plays a central role in regulation of gene expression and other crucial metabolic processes, indicating its potential as a therapeutic agent both for metabolic and genetic disorders.[7] Therefore, efforts are required to develop methodologies to make RNA molecules stable enough to carry out experimental studies and manipulation. It will be an added advantage if such methods include versatility and potential of nanoparticles, since in that case, it might be possible to deliver multiple therapeutic agents to the cell at the same time. Utilizing RNA, Khaled et al. has shown the self-assembly of RNA nanoparticles of different shapes and sizes from pRNA of bacteriophage phi-29, which were resistant to environmental stresses.[8,9] They were also able to engineer these nanoparticles for delivery of multiple therapeutic agents.[10,11] However, better ways to give any RNA sequence a nanoparticle shape and protect it from degradation by enzymes in the body during its delivery process need to be found. In this communication, we report a concept of using a cationic hyperbranched polymer to complex with RNA, resulting in water soluble nanoparticles, which then protect RNA strands from enzymatic cleavage.

Scheme 1. Schematic Representation of the Cationic Hyperbranched Polymers Used in This Study[a]



[a] All of the amines were quaternized as described in the Experimental Section in the Supporting Information.

An amino-modified poly(ethylene glycol) (PEG) based hyperbranched polymer (HP; Scheme 1) was synthesized by ring opening anionic polymerization of glycidol and MPEG epoxide initiated from tris(hydroxymethyl) propane using potassium methylate (see Supporting Information). Aminated PG-PEG polymers were obtained by post modification of PG-PEG by mesytylation, amination with tris(2-aminoethyl)amine, and methylation. The synthesized polymer was characterized by ^{1}HNMR and conductometric titration for the determination of amine content. The tertiary amine groups in PG-PEG amine were quaternized using ethyl bromide. Due to the presence of quaternized amino groups at the surface (Scheme 1), this

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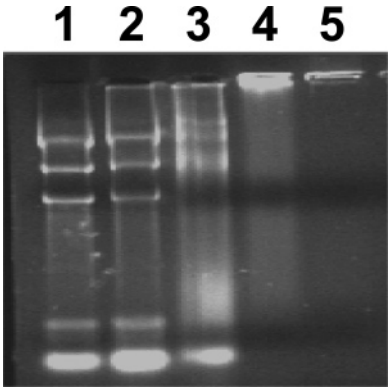

Figure 1. Electrophoretic mobility shift assay for HP/RNA complexes; lane 1: native RNA, lane 2: HP/RNA complex at $Z_{+/-} = 1$, lane 3: HP/RNA complex at $Z_{+/-} = 3$, lane 4: HP/RNA complex at $Z_{+/-} = 5$, and lane 5: HP/RNA complex at $Z_{+/-} = 7$. Complexes were prepared simply by mixing aqueous solutions of HP and RNA at different $Z_{+/-}$ (see Supporting Information).

polymer is positively charged and is able to interact with negatively charged RNA molecules.

Electrophoretic mobility shift assay (EMSA) was performed to visualize the interaction of RNA and HP. In brief, complexes of HP and RNA were obtained by mixing aqueous solutions of HP and RNA at different positive units of HP vs negative units of RNA or charge ratios ($Z_{+/-}$). Denaturing agarose gel electrophoresis was subsequently performed. A representative gel image is presented in Figure 1. Although free RNA or incompletely neutralized RNA migrated in the electric field toward the anode, full retardation occurred at and above the $Z_{+/-} = 3$. This clearly demonstrates that the cationic segments of the HP interact electrostatically with the negative charges of phosphate groups of RNA, thereby resulting in the formation of stable complexes. This leads to retardation in the migration rate of RNA molecules due to the larger size of the complex and charge neutralization.

Atomic force microscopy was then used to determine morphological changes in the shape of RNA after interaction with HP. Figure 2 shows graphs elaborating the size and morphology of complexes formed at different $Z_{+/-}$ values. An irregular elongated shape and a broad size distribution were observed in the images, where $Z_{+/-}$ is lower than 3. EMSA results (Figure 1) showed that at these charge ratios the HP could only partially bind. However, as the extent of complexation is increased by raising the charge ratio ($Z_{+/-} > 3$), where the entire RNA gets bound to the HP, the morphology of the HP/RNA complex changes into spherical nanoparticles. The particles are highly monodispersed, and sizes are around 60 ± 10 nm. These particles have the minimum possible size for a RNA/polycation conjugate based on the partial specific volumes of the constituent components. Subsequent turbidity measurements at 550 nm and size measurements showed that turbidity of the solution and size of the particles remained unchanged. These observations revealed that the systems are quite stable and remained soluble over a period of time.

The stability of this spherical nanoparticle-shaped RNA was further studied from the viewpoint of nuclease resistance. Addition of RNase to native RNA solution immediately increases the absorbance at 260 nm due to the fragmentation of RNA, but no substantial increase in the absorbance was observed for the nanoparticles, where $Z_{+/-}$ was 3 (Figure 3). At lower $Z_{+/-}$, the rate of increment of absorbance was less than that of the native RNA revealing the RNA was partially protected by the polymer. High nuclease resistance ability was

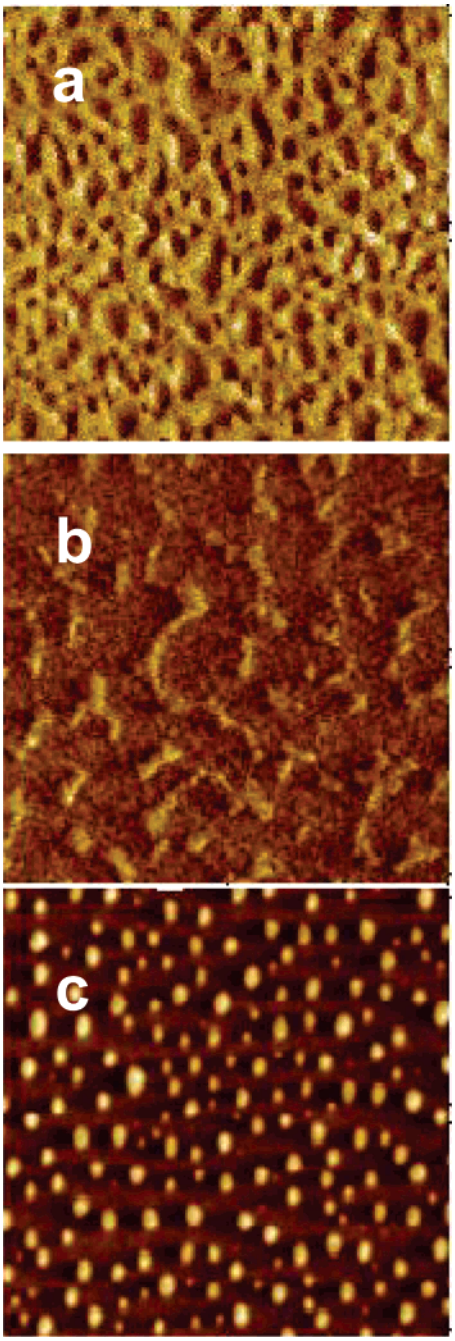

Figure 2. Atomic force microscopy images (3000 nm × 3000 nm) of (a) native RNA, (b) HP/RNA complex at $Z_{+/-} = 1$, and (c) HP/RNA complex at $Z_{+/-} = 3$.

observed for the spherical nanoparticle shaped RNA of $Z_{+/-} = 3$. This indicates the stable and inert nature of RNA when it is fully complexed with the HP, implying that the complex formation hinders the availability of RNA for nuclease action.

In summary, small stable water-soluble RNA nanoparticles which are resistant to nuclease action can be made from the novel cationic HP. Our results clearly show that RNA could be easily adsorbed onto the positively charged surface of the cationic HP and protect RNA strands from nuclease action. Ingenious methods can be designed to release RNA from HP/RNA complex for further use. For example, a solvent system which preferentially solublizes HP but not RNA can be developed. An ion exchange system or buffers with different pH may also serve the purpose. By careful and smart design of hyperbranched cationic polymers, it is quite possible to syn-

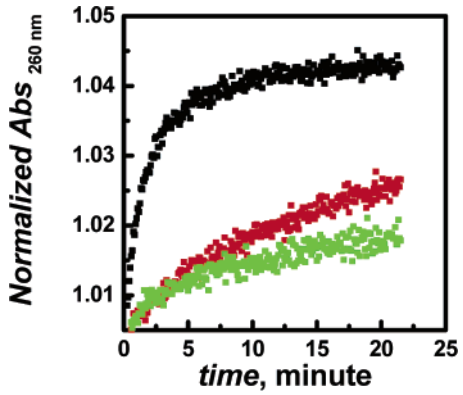

Figure 3. RNase digestion assays for native RNA (black), HP/RNA complexes at $Z_{+/-}$ 3 (red) and 5 (green). 10 μg/mL of RNase was added at each time and absorbance was measured at 260 nm.

thesize efficient positively charged surfaces which will be useful in RNA separation, purification, manipulation, detection, storage, long distance transportation, and also delivery inside cell. It might also be possible to administer many drug candidates synergistically using such systems, if the nanoparticles are formed in properly planned environment. Efforts in this direction are already in progress in our laboratory.

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Supporting Information Available. Details of the experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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