PAMAM dendrimers interact with biological RNA molecules

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Polycationic dendrimer-based gene transfer is one of the most important biological applications of dendrimers, which involves electrostatic interactions between the positively charged dendrimer end groups and the negatively charged phosphate groups in DNA. Such features could also be used to bind biologically important RNA molecules, which are emerging as both important therapeutic targets and therapeutic agents. With a view to using dendrimers to target RNA as well as to deliver RNA agents, we have evaluated a series of new polycationic dendrimers with different cores and different end groups for their interaction with the *Candida* ribozymes. The amine terminating dendrimers bind strongly to the ribozyme and efficiently inhibit the activity of the ribozyme. The inhibition effects increase with the increasing dendrimer generation, which reflects the cooperative amplification processes at work, depending on the size and three-dimensional structure of the dendrimers. The possibility of closely controlling the size, shape and surface chemistry of dendrimers gives us a unique opportunity of creating a repertoire of structure-, size- and shape-tailored dendrimers binding to various RNA molecules as required. Our findings open new perspectives for using polycationic dendrimers for both RNA targeting and RNA delivery.

**Keywords:** Dendrimer, RNA, RNA/dendrimer complex, Polycationic dendrimer, ribozyme
PAMAM dendrimers are safe and efficient systems for gene delivery

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Dendrimers are perfectly structured molecules with large numbers of cascade-branched units emitting from a central core. [1] Polycationic dendrimer-based gene transfer is one of the most important applications of dendrimers, which involves electrostatic interactions between the positively charged amine end groups in dendrimer and the negatively charged phosphate groups in DNA. [2] Such features could also be used to bind biologically important RNA molecules, [3] although very few efforts have been made so far on these lines. Since RNA is emerging as both important therapeutic targets and therapeutic agents, [4] we would like to study interactions between dendrimers and RNAs with a view to using dendrimers to target RNA as well as to deliver RNA agents.

PAMAM dendrimers with triethanolamine and tri(ethylene)glycol as core and various amines as end groups have been developed previously in our laboratory (Scheme 1). They are referred here as GA and GG. Both GA and GG are flexible dendrimers, and GG has the possibility to link a biological ligand for specific targeting purpose. [5, 7] At present work, we have studied the interaction between these dendrimers and the Candida ribozyme [6], a model RNA system which provides a unique opportunity for correlating RNA binding and activity.

From generation 2 to 4, both types of dendrimers with various amine end groups were found to have strong inhibitory effects on the catalytic activity of the Candida ribozymes, and the inhibitory efficiency of the dendrimers increased with the generations (Table 1). Meanwhile, the dendrimers with ester end groups had no inhibitory effects at all (data not shown). No inhibition was observed with small cationic ammoniums such as NH₄Cl or NMe₄Cl (up to 1 mM, data not shown). The strong inhibitory effects of the dendrimers can therefore be attributed to local
cooperative interactions between the amine end groups of the dendrimers surface and the ribozymes. Furthermore, no significant differences in inhibition were observed among dendrimers of the same generation with different amine end groups such as -NH$_2$, -NMe$_2$, and -NMe$_3^+$ (Table 1). This suggests that the electrostatic forces were the main interactions between the dendrimers and the RNA ribozymes.

Scheme 1 Dendrimers' structures referred here to as Ga$_n$-NH$_2$, Ga$_n$-NMe$_2$, and Ga$_n$-NMe$_3^+$, and Gg$_n$-NH$_2$, Gg$_n$-NMe$_2$, and Gg$_n$-NMe$_3^+$ respectively (n: the generation number of dendrimer).

Table 1 Inhibitory effect of dendrimers (IC$_{50}$ values) on the self-splicing of Candida ribozyme.

<table>
<thead>
<tr>
<th>Types of Dendrimer</th>
<th>Self-splicing of Ca.LSU/μM</th>
<th>Types of Dendrimer</th>
<th>Self-splicing of Ca.LSU/μM</th>
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<tbody>
<tr>
<td>Gan dendrimers</td>
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<td>Ggn dendrimers</td>
<td></td>
</tr>
<tr>
<td>G$_2$</td>
<td>0.54</td>
<td>G$_2$</td>
<td>2.23</td>
</tr>
<tr>
<td>G$_3$</td>
<td>0.28</td>
<td>G$_3$</td>
<td>0.64</td>
</tr>
<tr>
<td>G$_4$</td>
<td>0.15</td>
<td>G$_4$</td>
<td>0.29</td>
</tr>
</tbody>
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We demonstrated further that the dendrimers formed stable complexes with the RNA ribozyme by gel retardation in native agarose gel (Fig. 1A). Dendrimers with various amine end groups were able to completely prevent the mobility of the ribozyme in the gels (Fig. 1A), when the charge ration N/P is over 2.5. No gel retardation was observed with dendritic molecules having ester end groups, even at the charge ratio of 40 (data not shown). In addition, the gel retardation of the ribozyme depends on the generation of dendrimers involved: the higher the generation of the dendrimers, the stronger the interactions between the dendrimer and the RNA, and therefore the greater the gel retardation of the RNA became (Fig. 1A). All these
results correlate well with those obtained in the ribozyme activity assays, which show that the inhibitory effects of the dendrimers on the splicing activity of the ribozymes increased with the generations, due to the increasingly strong interactions occurring between the dendrimers and the ribozymes (Table 1).

![Image](image_url)

**Fig. 1** A) Gel retardation of RNA in agarose gel with dendrimer G\textsubscript{gen}-NMe\textsubscript{2} at N/P ratios ranging from 1:10 to 20:1. B) CD spectra of Gg4-NH\textsubscript{2} dendrimer at various N/P ratios. The spectrum of individual complexes showed a charge ratio-dependent decrease in the intensity of the 270 nm CD bands: at a charge ratio of N/P 1/10, the intensity of the CD peak was only slightly reduced; at N/P 1/1, it was dramatically reduced; and at N/P ratios above 2.5/1, the CD band completely disappeared.

We further used CD spectroscopy to characterize the structure of ribozyme within the RNA/dendrimer complexes (Fig. 1B). Complexation of the ribozyme Ca.L-11 by dendrimers reduces the intensity of the 270 nm band, suggesting that the RNA/dendrimer complex altered the active form of the ribozyme conformation. This correlates well with the results obtained for complex formation between dendrimers and RNA, which show that the RNA/dendrimer complex is more stable with the increasing N/P ratio, due to the charge compensation and the strong interactions occurring between dendrimer and RNA.

Take all the above results together; we have proposed a binding model for the RNA/dendrimer polyplex, which have the dendrimers interwoven with RNA molecules via electrostatic interactions (Fig. 2). Since RNA molecules have diverse structures and sizes and can perform a wide range of important biological activities, structure-controlled, size-tailored dendrimers should provide useful means of
regulating many biological processes involving RNA, such as protein synthesis, mRNA splicing, RNA interference and RNA delivery. Further studies using these dendrimers to interact with RNA molecules for both RNA delivery and RNA targeting purposes are currently under way at our laboratories.

Fig. 2 Proposed mechanism of interaction between dendrimers and RNA.

References:
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