Polycationic dendrimers interact with RNA molecules: polyamine dendrimers inhibit the catalytic activity of *Candida* ribozymes†

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Various polyamine dendrimers with a triethanolamine core inhibit the activity of the *Candida* ribozyme by forming RNA–dendrimer complexes *via* electrostatic interactions.

Dendrimers are spherical molecules with large numbers of cascade-branched units emerging from a central core, resulting in dense end groups at the surface.[1] The biochemical and biomedical applications of dendrimers have been increasing sharply in the last two decades.[2] One of the most active research fields has been that of dendrimer-based gene transfer, which involves polycationic dendrimers such as poly(amidoamine) (PAMAM) and poly(propyleneimine) (PPI).[3] These dendrimers, along with cationic amphiphiles, polymers and block copolymers, are promising non-viral gene delivery vectors.[4–7] Both PAMAM and PPI dendrimers have primary amine end groups which participate in DNA binding processes. Such features of these dendrimers could be used to bind to biologically important RNA molecules,[8] although very few efforts have been made so far on these lines. In our ongoing project focusing on RNA targeting and the development of RNA therapeutic agents, we are therefore studying the interactions between polycationic dendrimers and RNA molecules with a view to using dendrimers to target RNA as well as to deliver RNA agents.

RNA molecules adopt various structures correlating with diverse cellular functions such as protein synthesis, post-transcriptional RNA processing, transcriptional regulation and retroviral replication. Among the various RNAs, ribozymes[9] provide a unique opportunity for correlating RNA structure and function. Valuable information about ligand binding can be obtained by analyzing the inhibitory effects of ligands on the ribozyme activity. In the present study, a *Candida* ribozyme, Ca.LSU, was used as a model RNA. Ca.LSU is a self-splicing group I intron from the 26S rRNA of the opportunistic fungal pathogen *Candida albicans*.[10] The self-splicing process involves two consecutive transesterification reactions that start when the 5′ splice site is attacked by a guanosine or a guanosine nucleotide, resulting in the excision of the intron and ligation of the flanking exons. Here we report on a new set of cationic poly(amidoamine) dendrimers with various amine end groups (Fig. 1(A)) and assess their interactions with the *Candida* ribozymes. The strong interactions occurring between these dendrimers and the ribozymes were reflected in the strong inhibition of the ribozyme activity observed. Binding of the dendrimers to the RNA was studied by performing gel mobility-shift assays, where the binding ability was found to be clearly correlated with the inhibitory effects of the dendrimers.

Partially degraded dendrimers were reported to have more flexible structures and therefore to interact more efficiently with DNA.[11] In order to develop flexible dendrimers for RNA binding, we have chosen triethanolamine as the dendrimer core (Fig. 1(A)). The structure of triethanolamine allows us to have dendrimers with the branching units starting away from the center amine with a distance of 10 successive bonds. It is therefore expected that these dendrimers will have less densely packed branching units and end groups than the commercial available PAMAM dendrimers with NH_3 or ethylenediamine as core, the branching units starting immediately at the center amine of the core. The dendrimers with triethanolamine core were prepared using the conventional procedure for synthesizing PAMAM dendrimers[12] having various amine end groups $–NH_2$, $–NMe_2$ and $–NMe_3^+$, which are referred to here as $\mathbf{G_n–NH_2}$, $\mathbf{G_n–NMe_2}$ and $\mathbf{G_n–NMe_3^+}$, respectively (n: the generation number of dendrimer).[13]

RNA-binding was determined by assessing the inhibitory effects of these dendrimers on both the *trans*-cleavage activity of the *Candida* ribozyme Ca.L-11[14] and the self-splicing activity of the *Candida* ribozyme Ca.LSU.[15] Ca.L-11 is a truncated version of Ca.LSU, which catalyzes the cleavage of the RNA oligonucleotide GCCUCUAAAAA into GCCUCU and AAAAA, while the self-splicing of Ca.LSU gives intron and exon products. From generation two to four, these dendrimers with various amine end groups were found to have strong inhibitory effects on both the *trans*-cleavage (Fig. 1(B)) and the self-splicing processes (Fig. 1(C)) in the *Candida* ribozymes, and the inhibitory efficiency of the dendrimers increased with the generations (Table 1).[12] Meanwhile, the dendrimers with ester end groups had no inhibitory effects at all (data not shown). Nor was any inhibition observed with small cationic ammonium ions such as with NH_4Cl or NMe_4Cl (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 and the ribozymes. The two- to three-fold increase in the inhibitory capacity of the dendrimers from one generation to the next correlated well with the two-fold increase in the amine end groups, resulting in stronger interactions and thus in stronger inhibition of the ribozyme activity. Furthermore, no significant differences in inhibition were observed among dendrimers of the same generation with different end groups such as $–NH_2$, $–NMe_2$ and $–NMe_3^+$. Since quaternary ammoniums can participate only in electrostatic interactions, while primary and tertiary amines are able to form H-bonds as well, we could therefore conclude that the interactions between these dendrimers and the *Candida* ribozymes are mainly

† Electronic supplementary information (ESI) available: Details of dendrimer synthesis, ribozyme activity assay and gel-mobility-shift assay. See http://www.rsc.org/suppdata/cc/b4/b414241a/

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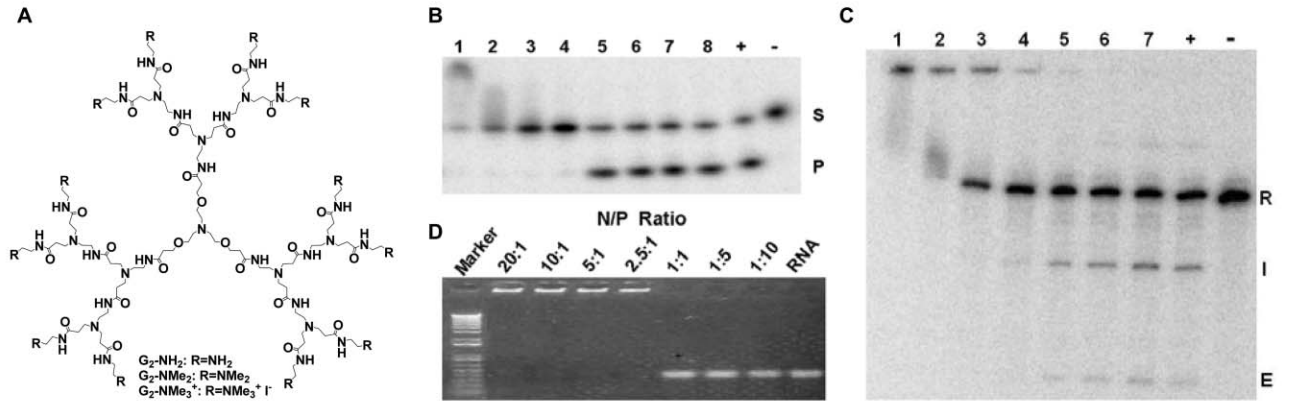

Fig. 1 (A) Dendrimer structures. (B) Inhibitory effects of $\mathbf{G_3}$–$\mathbf{NMe_3^+}$ on the *trans*-cleavage of Ca.L-11 using radiolabeled GCCUCUAAAAA as the substrate. S: substrate; P: product; lane (−): substrate; lane (+): substrate + Ca.L-11; lanes 1–8: substrate + Ca.L-11 + dendrimer (dendrimer concentration, from left to right: 9.40, 4.70, 2.30, 1.20, 0.56, 0.29, 0.15, 0.073 μM). (C) Inhibitory effects of $\mathbf{G_4}$–$\mathbf{NMe_3^+}$ on the self-splicing of Ca.LSU. R: Ca.LSU; I: intron; E: exon; lane (−): Ca.LSU at 4 °C; lane (+): Ca.LSU at 37 °C; lane 1–7: Ca.LSU + dendrimer (dendrimer concentration, from left to right: 1.20, 0.59, 0.29, 0.15, 0.073, 0.037, 0.018 μM). (D) Gel retardation of RNA in agarose gel with dendrimer $\mathbf{G_4}$–$\mathbf{NMe_2}$ at N/P charge ratios ranging from 1:10 to 20:1.

Table 1 Inhibitory effects of dendrimers (IC_{50} values) on the *trans*-cleavage of Ca.L-11 and the self-splicing of Ca.LSU

	trans-Cleavage/μM			Self-splicing/μM		
Generation	$-NH_2$	$-NMe_2$	$-NMe_3^+$	$-NH_2$	$-NMe_2$	$-NMe_3^+$
$\mathbf{G_2}$	2.17	3.52	1.52	0.54	1.25	0.35
$\mathbf{G_3}$	0.61	1.22	0.82	0.28	0.56	0.18
$\mathbf{G_4}$	0.29	0.31	0.38	0.15	0.22	0.057

electrostatic ones, not the H-bonds. However, end groups such as $-NH_2$ and $-NMe_2$ might form H-bonds with the branching units inside the dendrimers, generating back-folding effects and making the end groups of the NH_2- and NMe_2-terminating dendrimers less accessible for RNA binding. This might be the reason why slightly stronger inhibition was observed with the NMe_3^+-terminating dendrimers.

The strong inhibition exhibited by the dendrimers on ribozyme catalysis suggests that these dendrimers bind to the RNA molecules, which is consistent with the smearing and the retardation of both the short RNA oligonucleotide (Fig. 1(B)) and the *Candida* ribozyme Ca.LSU (Fig. 1(C)) observed in the polyacrylamide gel at high dendrimer concentrations. This RNA binding was further confirmed by the retarded RNA migration observed in native agarose gel (Fig. 1(D)). Dendrimers from generation 2 to 4 with various amine end groups were able to completely prevent the mobility of the ribozyme in the gels at charge ratios N/P $>$ 1. No gel retardation was observed with dendrimers having ester end groups, even at charge ratios where the dendrimer was in more than 40-fold excess (data not shown). These findings provide additional evidence that strong binding occurred between RNA molecules and the dendrimers having cationic amine end groups. The gel retardation of the ribozyme depends on the generation of dendrimers involved (data not shown): 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. This correlates well with the results obtained in the ribozyme activity assays, which show that the inhibitory effects of the dendrimers on the activity of the ribozymes increased with the generations, due to the increasingly strong interactions occurring between the dendrimers and the ribozymes (Table 1). The considerable gel retardation observed with the high-generation dendrimers also interfered with the ribozyme activity measurements. This is why we did not assess the inhibitory effects of dendrimers of generations higher than four on these *Candida* ribozymes.

In conclusion, a series of polycationic dendrimers with various amine end groups have been characterized in terms of their interactions with the *Candida* ribozymes. These dendrimers efficiently inhibited the catalytic activity of the ribozyme by forming stable RNA–dendrimer complexes *via* electrostatic interactions. Since RNA molecules have diverse structures and sizes and can perform a wide range of important biological functions, 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 in our laboratories.

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