Leadzyme RNA catalysis

Eric Westhof and Thomas Hermann

The structure of a lead-dependent ribozyme reveals binding sites of divalent ions. One of them mimics the binding of catalytic lead and allows modeling of the cleavage reaction.

Since the discovery of catalytic RNA by Sidney Altman and Thomas Cech in the early 1980s, our knowledge of RNA structure, folding and catalysis had progressed dramatically. For many years, the L-shaped fold of transfer RNA had been the archetype of RNA structure. However, in the last five years, we have witnessed the determination of complete or fragmentary catalytic RNAs such as the hammerhead ribozymes[1–3], the 160-nucleotide P4-P6 domain of a group I intron[4] and, recently, a 247-nucleotide fully active group I ribozyme[5] and a 72-nucleotide hepatitis delta virus ribozyme[6]. Despite the structural richness of the newly acquired information, the description of the precise chemistry underlying the catalytic steps is still lacking. This issue of *Nature Structural Biology* presents the crystal structure of the leadzyme[7], a small catalytic RNA molecule that self-cleaves at a specific phosphodiester bond in the presence of lead ions. The new structure, albeit at a moderate 2.7 Å resolution and although architecturally not as intricate as that of the hammerhead or the hepatitis delta virus ribozymes, contains new features and, most importantly, lends itself to a mechanistic model for catalysis.

Historically, the first artificial ribozyme discovered was yeast $tRNA^{Phe}$ which, in presence of lead ions, gives rise to two fragments of definite lengths[8]. Since lead is a poison in biological systems, the observation did not unravel a new biological paradigm, but it was later extensively studied with the hope of better understanding biological RNA catalysis[9]. In 1992, Pan and Uhlenbeck[10], using an *in vitro* selection, isolated a small catalytic RNA that required lead ions for catalysis, thereafter called the 'leadzyme'.

The leadzyme contains an asymmetric internal loop between two helical sections. There are four and two nucleotides in either side of the loop, with the cleavage site occurring between the first and second residues of the longer segment (Fig. 1*a*). In the two independent molecules of the crystal, the two-residue segment is in stacking continuity with the helical parts while, in the four-residue segment, the three nucleotides 3' to the cleavage site flip out and form a stacked GAG triplet almost perpendicular to the base pairs of the helices. The only contact within the internal loop is a single hydrogen bond between O2 of C23 and N6 of A45 (a very similar A–U base pair with one hydrogen bond tops the GA tandem of sheared base pairs in the hammerhead ribozyme). This latter observation contrasts with NMR results[11] which suggested the formation of a protonated C–A^+ base-pair with two hydrogen bonds. Although the overall topology of the fold as deduced by NMR and crystallography is the same, large differences can be observed in the GAG triplet of the large segment of the asymmetrical internal loop: in the crystal, it is flipped out and, in the NMR structure, it is folded in (Fig. 1*b*). The flexibility of this region is marked also in the crystal where the differences between the two independent molecules are most pronounced in that segment. In the crystal packing, this local flexibility is exploited since the molecules are linked through two striking, small parallel helices involving purine–purine base pairs of the GAG triplet. Although at least two crystal structures of small parallel helices exist[12–13], this is the first time a three base pair parallel helix has been observed (Fig. 2). In both helices, the G–G pairs involve N1H and O6 but, in one, both bases are *anti* and, in the other, *syn*; the A–A pairs involve, in one case, the Watson-Crick sites N1 and HN6 and in the other, the Hoogsteen sites N7 and HN6. Thus, like most crystal structures, the structure of the leadzyme contains structural and stereochemical features of interest. Now, what about catalysis?

The crystal structure of the leadzyme displays a wealth of new metal ion binding sites for three different types of ions: Mg^{2+}, Ba^{2+} and Pb^{2+} . Most of the binding sites are localized in the deep groove of the RNA close to the Hoogsteen region and one site near G42 is alternatively occupied by Mg^{2+} and Pb^{2+} . These two ions possess rather different physico-chemical proper-

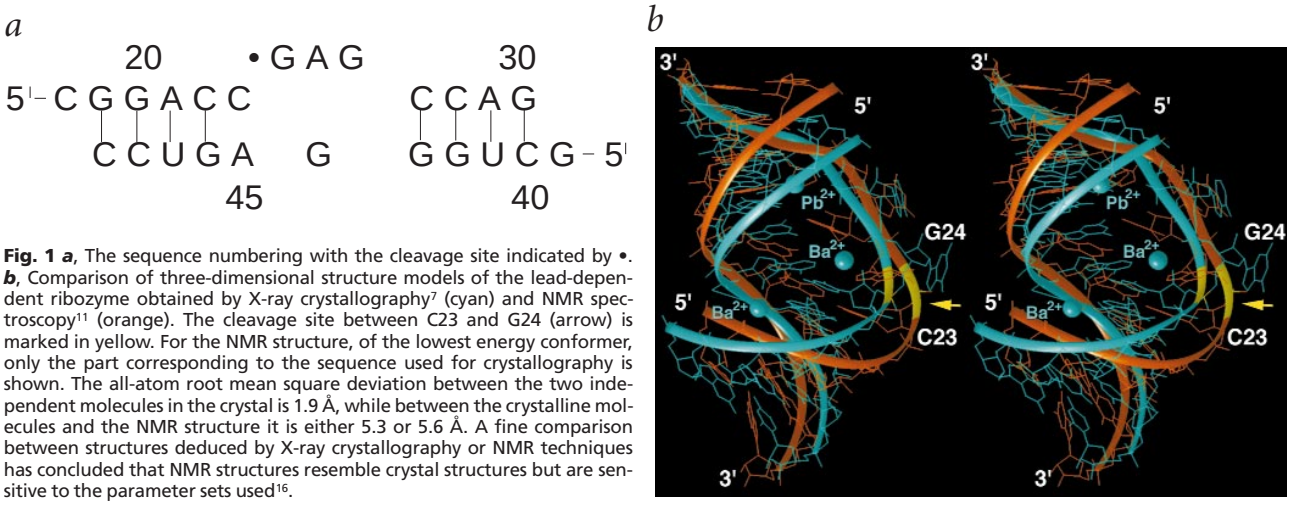

Fig. 1 ***a***, The sequence numbering with the cleavage site indicated by •. ***b***, Comparison of three-dimensional structure models of the lead-dependent ribozyme obtained by X-ray crystallography[7] (cyan) and NMR spectroscopy[11] (orange). The cleavage site between C23 and G24 (arrow) is marked in yellow. For the NMR structure, of the lowest energy conformer, only the part corresponding to the sequence used for crystallography is shown. The all-atom root mean square deviation between the two independent molecules in the crystal is 1.9 Å, while between the crystalline molecules and the NMR structure it is either 5.3 or 5.6 Å. A fine comparison between structures deduced by X-ray crystallography or NMR techniques has concluded that NMR structures resemble crystal structures but are sensitive to the parameter sets used[16].

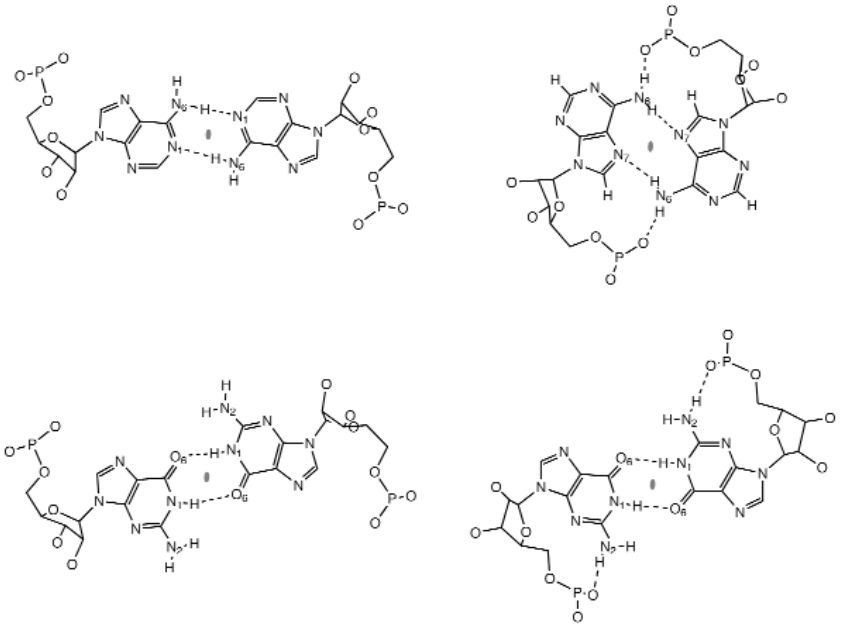

Fig. 2 The pairs forming two small three base pair parallel helices between the independent molecules of leadzyme in the crystal[7]. At the left, the A–A and G–G pairs adopted by A25 and G24 of molecule 1. At the right, those adopted by A25 and G26 of molecule 2. The two-fold symmetry axes are marked by the dots. The third base pair is a sheared G–G pair (between G24 in molecule 2) while the status of the pairing (between G26) could not be resolved for molecule 1. The two pairings at the right were shown to occur both theoretically and experimentally in repetitive GA sequences[17].

ties and adaptive changes in their respective environments are expected (Fig. 3). One site, occupied by a Ba^{2+} ion and only found in molecule 2, is of particular interest because of its closeness and position to the cleavable phosphate. The authors propose that during catalysis this barium site is occupied by a lead ion that activates the 2'-hydroxyl group, facilitated by conformational changes in the sugar-phosphate backbone to ensure in-line attack. This is, therefore, a mechanistic model utilizing a single metal ion. However, two key observations are not easily understood in light of this mechanism. First, the adenine to which the lead ion is proposed to bind (at the N1 position) can be replaced by several other residue types without severely impairing activity[14]. Secondly, it has been shown clearly that the addition of a second ion (especially belonging to the rare earth class, such as Nd^{3+}) in a 1:1 ratio with lead produces a very significant increase in cleavage yield[15]. In short, the present crystal structure will not close the heated discussions and subtle controversies about RNA catalysis but, importantly, this structure of the leadzyme allows a description of a minimal set of structural features for RNA catalysis and bears out the explicit role of sugar-phosphate backbone flexibility in catalytic RNAs.

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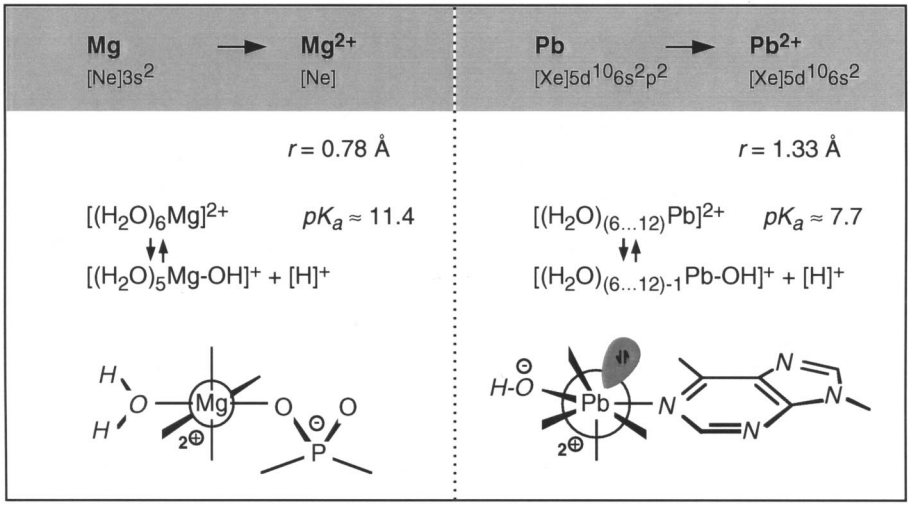

Fig. 3 Some differences between magnesium and lead ions. While Mg^{2+} ions have a closed electron shell and form predominantly six-coordinated octahedral complexes (bottom, left), Pb^{2+} ions have a xenon core extended by filled 5d orbitals and, in the valence shell, a lone pair of electrons[18]. For Pb^{2+} a range of coordination numbers extending up to 12, with 6- and 8-coordination most frequent, can be observed. The geometries of Pb^{2+} complexes are complicated by the lone pair which may reside in the spherically symmetric orbital, being stereochemically inactive, or may exhibit stereochemical activity in a hybrid orbital (bottom, right).