J. Biochem. **133**, 309–315 (2003)
DOI: 10.1093/jb/mvg042

5S rRNA Is a Leadzyme. A Molecular Basis for Lead Toxicity

Miroslawa Z. Barciszewska[1], Eliza Wyszko[1], Rolf Bald[2], Volker A. Erdmann[2] and Jan Barciszewski[*,1]

[1]Institute of Bioorganic Chemistry of the Polish Academy of Sciences, Noskowskiego 12, 61704 Poznan, Poland; and [2]Institute of Biochemistry of Free University, Thielallee 63, 14195 Berlin, Germany

Received September 25, 2002; accepted December 26, 2002

This paper reports that the D-loop sequence of cellular mammalian ribosomal 5S RNAs is a natural leadzyme that specifically binds and cleaves *in trans* other RNA molecules in the presence of lead. The D-loops of these 5S rRNAs are similar in sequence to the active site of the leadzyme derived from $tRNA^{Phe}$, which cleaves a single bond *in cis*. We have devised a 12 nt model substrate based on the leadzyme sequence cleaved *in trans* by a 12 nt RNA molecule containing of the D-loop sequence. The model reaction occurs only at the appropriate concentration of lead and enzyme/substrate stoichiometry. The native 5S rRNA carries the same cleavage activity, although with different optimal lead concentration and stoichiometry. On the other hand, the isolated D-loop does not serve as a substrate when incubated with an RNA molecule with the potential to base pair with it and form the same internal loop (the bubble) present in the leadzyme-substrate complex. We show that the leadzyme cuts C-G, but not G-G or U-G linkages. The 5S rRNA leadzyme appears to have the shortest asymmetric pentanucleotide purine-rich loop flanked by two short double stranded RNAs. The leadzyme activity of native 5S rRNA may be an important aspect of lead toxicity in living cells. Because the leadzyme motif has been found in natural RNA species, its activity can be expressed *in vivo* even at a very low lead concentrations, of lead leading to the inactivation of other cellular RNAs. This might be one of the ways in which lead poisoning manifests itself at the molecular level. Lead toxicity is based not only on its binding to calcium and zinc binding proteins (such as Zn-fingers) and random hydrolysis of nucleic acids, but also, and most importantly, on the induction of the hydrolytic properties of RNA (RNA catalysis).

Key words: lead cleavage, leadzyme, ribosomal RNA, RNA catalysis, 5S rRNA.

Ribosomal 5S RNA is the smallest ribosomal RNA (rRNA) localized in the central protuberance of the large subunit of the ribosome. A 120-nucleotide-long RNA with a molecular mass of 40,000 Da is found in virtually all ribosomes with the exception of the mitochondria of some fungi, higher animals and most protists (*1*, *2*). The 5S rRNA is highly conserved throughout evolution, and phylogenetic analysis has provided a general model of its secondary structure. It consists of five helices (I–V), two hairpin loops (C and D), two internal loops (B and E) and a hinge region (A) organized into the three helix junctions (*3*, *4*). From nucleotide sequence analysis and biochemical data, it has been proposed that the D loop is composed of four nucleotides and shows a tetra loop structure of the GNRA type (*3–6*). However, the cleavages induced by RNase H and α-sarcin suggest that the D loop could be longer than 4 nucleotides (*7–9*). We have analyzed the nucleotide sequences of all known eukaryotic 5S rRNAs and showed that the D loop of mammalian 5S rRNAs is homologous to the lead-dependent ribozyme that has been conserved through *in vitro* selection (Fig. 1). The lead-dependent ribozyme is one of the smallest catalytic RNAs (*10–13*). It cleaves a selected phosphodiester bond in the presence of Pb^{2+} leaving a 3′-monophosphate product, whereas the classical RNAzymes require magnesium, manganese or calcium ions (*13*). Leadzyme was first isolated by an *in vitro* selection approach using a library of $tRNA^{Phe}$ sequences obtained by random mutagenesis (*14*, *15*). It is a short double helical RNA, with a cleavage site located within an asymmetric purine-rich internal loop flanked on both sides by a stable RNA helix (*16*). Indeed, the crystal structure of the leadzyme reveals an RNA duplex with a central internal loop containing three-bulging nucleotides (*17*, *18*). Although the lead that binds to the crystallized RNA is rather distant from the attacking nucleophile at the cleavage site, Wedekind and McKey postulated that a second lead ion might bind at a site where barium had been observed, which is sufficient for a bond cleavage (*17*, *53*).

We prepared a 5S rRNA-oligonucleotide complex with the potential to form only a 5 nucleotide bubble (but not 6 as in Ref. *10*) flanked by 4 and 5 base RNA pairs on both ends. The 5S rRNA leadzyme diverges from the leadzyme consensus sequence and displays a slightly lower activity than was expected for a fully *in vitro* evolved leadzyme as identified by the Uhlenbeck laboratory (*10*). The cleavage site occurs on the RNA molecule *in trans*. The data show that such a complex is formed, binds lead and acquires a specific catalytic (hydrolytic) activity. We have proved that the cleavage is due to leadzyme rather than to lead

[*]To whom correspondence should be addressed. Tel: +48-61-8528503, Fax: +48-61-8520532, E-mail: jan.barciszewski@ibch.poznan.pl

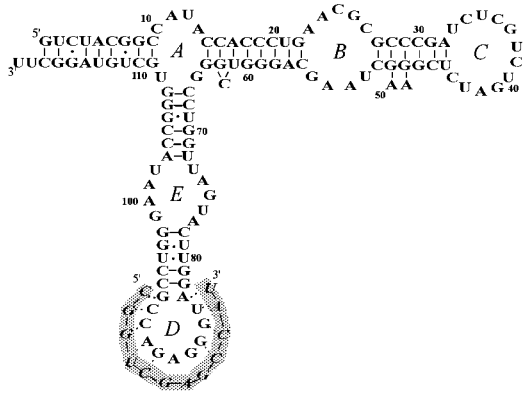

Fig. 1. **The secondary structure of mammalia 5S rRNA.** The nucleotide sequence of loop D is highly conserved and characteristic. It can form a catalytic RNA, which in the presence of lead ion cleaves oligoribonucleotides *in trans* (dashed).

itself as it was shown before (*54*). The observed activity of leadzyme immediately implies that the D loop of the 5S rRNA forms two double stranded RNAs. The finding of the 5S rRNA leadzyme motif in many other natural RNAs (*19*) strongly suggests that leadzyme can function *in vivo*. That reaction offers a clue to a possible understanding of lead toxicity to cells and, especially to the protein synthesis machinery (*54*).

MATERIALS AND METHODS

5S rRNA from calf liver was isolated and purified as described previously (*20*).

RNA synthesis was performed on a PCR-Mate EP model 391 DNA synthesizer (Applied Biosystems) with 2′-0-triisopropylsilyl-protected phosphoramidite synthons. After deprotection, the oligoribonucleotides were desalted on a Qiagen Tip 500 column as described earlier (*21*). This method was used for the preparation of oligonucleotides L1–L7: L1 (substrate, 11 mer)-(5′) GGACGAGCCAG; L2 (leadzyme, 10 mer)-(5′) CUGGGAGUCC; L3 (stem and loop. D of 5S rRNA, 21 mer)-(5′) CUUGGAUGGGAGACCGCCUGG; L4 (leadzyme for D loop, 10 mer)-(5′) CGGUGACCAU; L5 (substrate for leadzyme, 12 mer)-(5′) CGGUCGAGCCAU; L6 (substrate, 29 mer)-(5′) GGCAGAUCUGAGCCUGGGAGCUCUCUGCC; L7 (leadzyme, 14 mer)-(5′) CCCAGGGAAGAUCU.

5S rRNA (5 μg) was labeled at the 5′ end with [γ ^{32}P] ATP and 10 U T4 polynucleotide kinase (Promega) or at the 3′end with [5′-^{32}P]pCp and T4 ligase and separated in a 15% polyacrylamide gel with 7M urea. The radioactive band was cut out, and the RNA was eluted with buffer and precipitated with ethanol (*20*, *22*, *23*).

Oligoribonucleotides were labeled by the same method, but for their purification a 24% polyacrylamide gel with 7M urea was used.

The lead ribozyme cleavage reaction was carried out in 15 mM MOPS, pH 7.5, buffer at 25°C.

The labeled substrate and leadzyme were mixed and heated together up to 60°C for 2 min, cooled slowly (1°C / min) and incubated at 27°C for 30 min. The reaction was initiated by the addition of Pb^{2+}, heated for 30 min, and stopped by adding an equal volume of loading buffer (25 mM sodium citrate pH 5, 1 mM EDTA, 7M urea, 0.1% xylene cyanol, and 0.1% bromophenol blue).

The reaction products were analyzed in 24% polyacrylamide gels with 7 M urea, pH 8.3. The limited hydrolysis of 5S rRNA or oligonucleotides with T1 RNase under denaturing conditions was done in 20 mM sodium acetate buffer, pH 4.5, containing 7 M urea and 1 mM EDTA for 20 min. at 55°C, exactly as described in Ref. *20*.

Alkaline hydrolysis was carried out in 50 mM NaOH and 1 mM EDTA for 35 seconds in the case of 5S rRNA and 18 minutes in the case of the oligonucleotides. The products of these reactions were separated in 20 or 24% polyacrylamide gels with 7 M urea in 0.09 M Tris/borate buffer, pH 8.3 (*22*).

For quantitative analysis, the radioactivity of ^{32}P on the gels was counted in a Typhoon 8600 Imager with ImageQuant software (Molecular Dynamics). The rate constants were calculated by plotting the natural log of the amount of noncleaved substrates *vs*. time (5–120 min).

RESULTS

A Model Leadzyme—To select the conditions for the leadzyme reaction, we started with two oligonucleotides: L1 (5′)GGACGAGCCAG(3′), 11 mer and L2 (3′)CCUGAGGGUC(5′), 10 mer, identical to those already published (*24*). However, the leadzyme that they form is different from that described by the Uhlenbeck laboratory (*10*). L1 and L2 create a structure with an asymmetric internal loop of five-purine nucleotides surrounded by two short double RNA helices. The loop is closed with two G-C base pairs, one at each site (Fig. 2A). In the presence of lead, the cleavage is specific and takes place at a C-G phosphodiester bond on the longer portion of the loop (*10*, *24*), (Fig. 2B). The reaction is Pb^{2+}-dependent and sensitive to the L1 and L2 stoichiometry. The highest efficiency of hydrolysis was observed at an L2/L1 ratio of 1.3 in the presence of 25 μM Pb^{2+} ion. At a higher Pb^{2+} concentration of (50 μM) or at a lower L2/L1 ratio (0.5), the yield was reduced. It is known that Pb^{2+} at higher concentrations causes non-specific (random) degradation of RNA single strands, but at a much slower rate (Fig. 2). Therefore, random hydrolysis is negligible (*13*, *24*, *25*). The major hydrolysis site at C4 of L1 is accompanied by a faster migrating satellite band that can be interpreted as the cyclic phosphate derivative (Fig. 2B) observed before by the Uhlenbeck laboratory (*10*, *11*). The autoradiogram also shows a minor cleavage site at a C-A bond which does not depend on Pb^{2+} concentration (Fig. 2). It is known that C-A bonds in RNA are very labile (*20*, *23*).

Leadzyme Activity of 5S rRNA—Our model leadzyme (L3) has a nucleotide sequence identical to that of the stem IV and loop D of calf liver 5S rRNA (*3*). As the substrate we selected oligonucleotide L5. A putative secondary structure of the L3-L5 complex is shown in Fig. 3. A specific lead-dependent ribozyme hydrolysis proceeds at the C-G bond of the L5 strand. This was proved by the strongest cut within the internal loop at a L3/L5 ratio of 0.2 and 100 μM Pb^{2+}. An additional cleavage site observed on L5 at a C-A bond in the double stranded portion is due to non specific hydrolysis of RNA (Fig. 3). In the next experiment, we used native 5S rRNA from calf

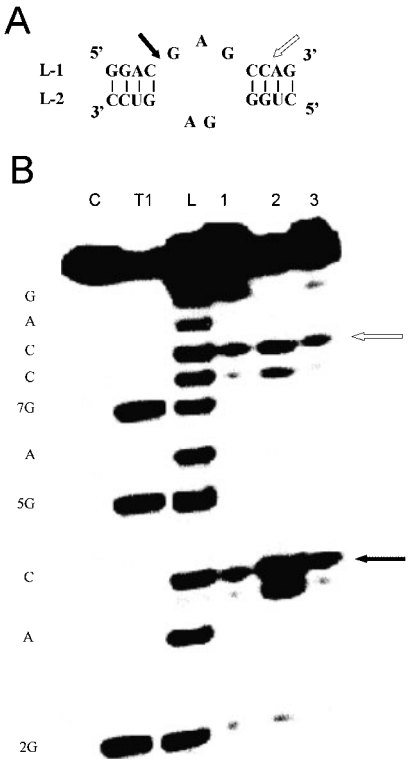

Fig. 2. **(A) Secondary structure of Pb-zyme. (B) Autoradiogram of the analysis in a denaturing 24% polyacrylamide gel containing 7 M urea of the specific cleavage of [5′-^{32}P labeled L1 substrate by the L2 leadzyme (A) at 27°C for 25 min.** Lanes: C, control; T1, L1 limited hydrolysis with RNase T_1; L, ladder, alkaline hydrolysis; 1–3, 0, 75 mM (1, 2) and 2 mM (3) of the L1 substrate (30,000 cpm) hydrolyzed with 1 mM of the L2 ribozyme in 15 mM NaMOPS (pH 7.5) buffer containing 50 mM (1) and 25 mM (2, 3) Pb^{2+}; black arrow, primary cut; empty arrow, secondary cut. The sequence of L1 is in the margin of the gel.

liver (as leadzyme) to cleave L5 labeled at the 5′ end (Fig. 4). The cleavage pattern observed is very similar to that of the model reaction (Fig. 3). The hydrolysis of the L5 strand proves that loop D of 5S rRNA as well as L3 create a RNA duplex with a small internal loop that specifically binds Pb^{2+} ion. About 50% of the substrate is cleaved by the 5S rRNA D-loop leadzyme at 27°C, pH 7.5, and 100 or 200 μM Pb^{+2} after 30 min at a 5S rRNA/L5 ratio of two (Fig. 4). It seems that Pb^{2+} concentrations above 100 μM do not effect the reaction rate so much. In the experiment using full length 5S rRNA, the optimal ratio of 5S rRNA/L5 as well as the Pb^{2+} concentration are slightly higher than in the model reaction, probably due to structural constraints imposed by the conformation of the entire 5S rRNA molecule. This is also a reason why the yield of hydrolysis observed at a fixed time for 5S rRNA is lower than for the model leadzyme. The specific cleavage site was confirmed using L5 substrate labeled at the 3′ and 5′ ends. The formation of a cyclic phosphate was also observed as double (satellite) bands (Fig. 4). The calculated rate constants k_{obs} for the L3/L5 and 5SrRNA/L5 reactions were 6.9×10^{-3} and 1.9×10^{-3} min^{-1}, respectively. These data clearly show that native 5S rRNA is a natural leadzyme.

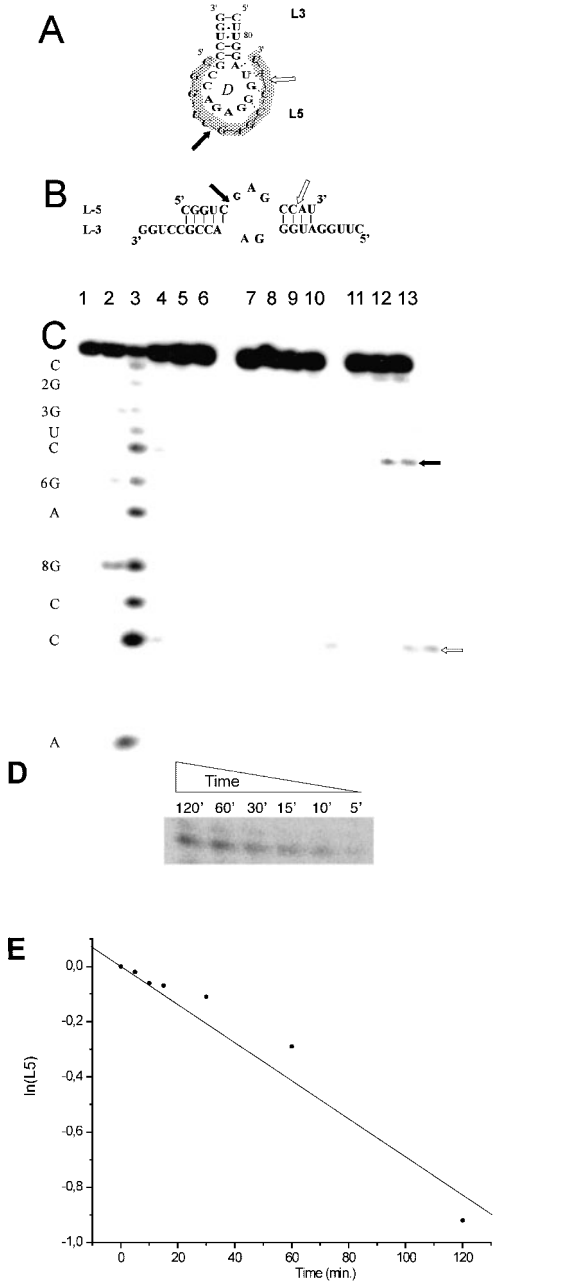

Fig. 3. **Autoradiogram of a denaturing 24% polyacrylamide gel (C) containing 7 M urea, showing the cleavage of the L5 substrate labeled at the 3′ end by the L3 leadzyme at 27°C during 30 min.** (A) L3 (analog of loop D of 5S rRNA showed as a hairpin)/L5 complex; (B) L3/L5 complex presented in the form of an RNA double helix; (C) polyacrylamide gel. Lanes: 1, control, incubation of L5/L3 complex without Pb^{2+}; 2, T_1- limited hydrolysis of L5 with a T_1 RNase specific for guanosine; 3, alkaline hydrolysis of L5 RNA; 4–6, 0.5 mM (30,000 cpm) L5 and 0.1, 0.5, and 2 mM of L3 ribozyme, respectively, in 15 mM NaMOPS (pH 7.5) buffer containing 50 mM Pb^{2+}; 7–10, 0.5 mM of L5 and 2 mM of L3 ribozyme in 15 mM NaMOPS (pH 7.5) buffer containing 5, 25, 50, 100 mM Pb^{2+}, respectively; 11–13, 0.5 mM of L5 and 0.1 mM of L3 ribozyme in 15 mM NaMOPS (pH 7.5) buffer containing 50, 100, 200 mM Pb^{2+}, respectively; black arrow, primary cut; empty arrow, secondary cut. (D) Autoradiogram of 20% denaturing polyacrylamide gel showing the cleavage L5 substrate by L3 ribozyme (lane 13 in C) in 15 mM NaMOPS (pH 7.5) buffer containing 100 mM Pb^{+2} at 25°C for 5–120 min. (E) Determination of the cleavage rate by plotting the natural log of amounts of noncleaved L5 substrate by L3 ribozyme as a function of time.

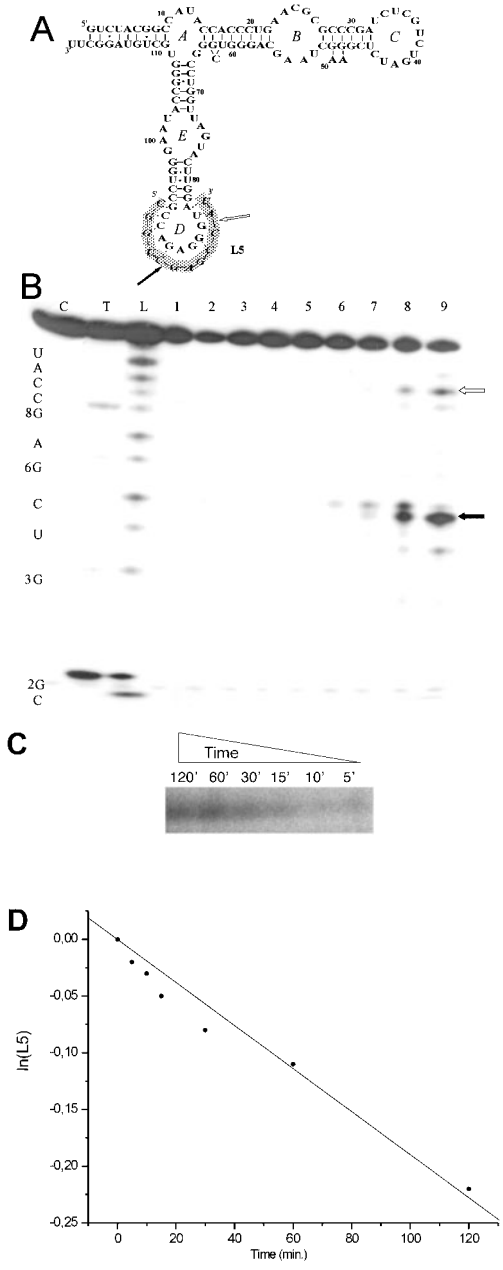

Fig. 4. **Autoradiogram of a denaturing 24% polyacrylamide gel (B) containing 7 M urea, analysis of the cleavage pattern of labeled of L5 substrate by 5S rRNA leadzyme at 27°C for 30 min.** (A) Secondary structural model of calf liver 5S rRNA with the L5 oligonucleotide complexed to loop D. (B) Polyacrylamide gel of 5′-^{32}P–labeled L5. Lanes: C, control incubation of L5; T, L5 treated with RNase T_1; L, ladder obtained by alkaline hydrolysis of L5; 1 and 2, incubation of L5 in 15 mM NaMOPS (pH 7.5) buffer containing 5 and 25 mM Pb^{2+}, respectively; 3, control incubation of L5/5S rRNA complex in 15 mM NaMOPS (pH 7.5); 4–9, 0.125 mM L5 and 0.250 mM 5S rRNA incubation in 15 mM NaMOPS (pH 7.5) buffer containing 5, 10, 25, 50, 100, 200 mM Pb^{2+}, respectively; closed and open arrows as above. (C) Autoradiogram of 20% denaturing polyacrylamide gel showing the cleavage of L5 substrate by 5SrRNA ribozyme (lane 9 B) in 15 mM NaMOPS (pH 7.5) buffer containing 100 mM Pb^{+2} during 5–120 min respectively at 25°C. (D) Determination of the rate cleavage by plotting the natural log of the amounts of noncleaved L5 substrate by 5S rRNA ribozyme as a function of time.

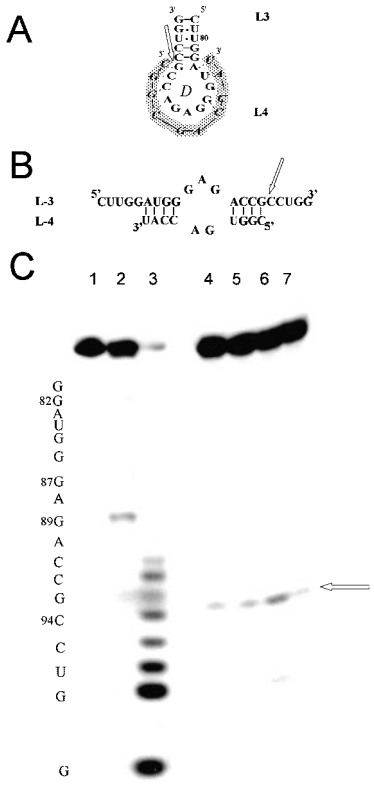

Fig. 5. **Autoradiogram of a denaturing 20% polyacrylamide gel (C) containing 7 M urea, showing the cleavage of the 3′-^{32}P–labeled L3 substrate by the L4 leadzyme at 27°C for 30 min.** (A and B) Different presentations of the secondary structure of the L3/L4 complex as a hairpin and double helix, respectively. Lanes: 1, control incubation of L3/L4 complex without Pb^{2+}; 2, L3 hydrolysis with RNase T_1; 3, alkaline hydrolysis of L3; 4–7, cleavage reaction of 0.5 mM of L3 substrate by 0.1, 0.25, 0.5, 1 mM of L4 leadzyme in 15 mM NaMOPS (pH 7.5) buffer containing 50 mM Pb^{2+}. Nucleotide numbering is identical to that in 5S rRNA. There are no primary cuts.

Exploring the Mechanism of Leadzyme—To understand the mechanism of leadzyme formation and its specificity, different oligonucleotides to be hydrolyzed in trans were prepared. Oligonucleotide L4 has the sequence required for Pb^{2+} binding and the potential to cleave in trans both the oligo L3 and the entire 5S rRNA at a predetermined site (G86). Although the putative structure of the internal bubble is identical to those of previously engineered leadzymes, neither L3-L4 (Fig. 5) nor 5S rRNA-L4 was active, and the G-G bond was not split (Fig. 5). This experiment clearly identifies the role of the base pair closing the asymmetric loop in leadzyme catalysis. In order to prove this, we carried out the reaction of an HIV-1 TAR RNA element (L6) for which a hairpin structure has already been proposed (*26*). Using specific cleavage with hammerhead ribozyme, it has also been shown that it can exist in equilibrium with the double stranded form (Fig. 6), (*27*). To cleave the TAR RNA element, we prepared a leadzyme (L7) that binds TAR RNA specifically as well as lead ion. Although the identical bubble was formed, the U-G bond was not cleaved (Fig. 7). These results suggest that the internal loop alone is not sufficient for leadzyme, and that the two G-C base pairs closing the asymmetric loop are also required for leadzyme activity.

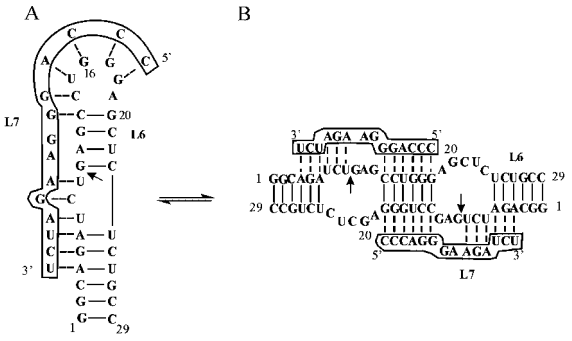

Fig. 6. **Secondary structures of HIV TAR RNA (L6) showed as a hairpin (A) or RNA duplex (B) with leadzyme L7 (in frame).** Arrows indicate putative leadzyme cleavage sites.

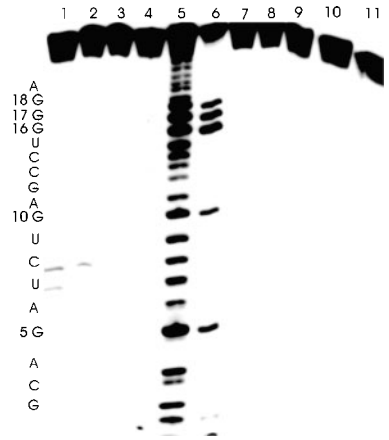

Fig. 7. **The autoradiogram of 20% polyacrylamide gel electrophoresis with 7 M urea showing the cleavage reaction pattern of 2.5 mM (30,000 cpm) [5′-^{32}P]TAR RNA labeled with leadzyme.** The reaction was carried out in 15 mM NaMOPS buffer, pH 7.0, for 20 min at 25°C with various concentrations of Pb^{2+} and ribozyme. Lanes: 1–4, 2.5 mM TAR RNA with different concentrations of ribozyme and Pb^{2+}; 1, 0.025 mM ribozyme/25 mM Pb^{2+}; 2, 0.025 mM ribozyme/50 mM Pb^{2+}; 3, 0.0025 mM ribozyme/25 mM Pb^{2+}; 4, 0.0025 mM ribozyme/50 mM Pb^{2+}; 5, alkaline ladder; 6, limited hydrolysis of TAR RNA with RNase T1; 7, TAR RNA with 0.25 mM leadzyme; 7,11, TAR RNA in water; 9,8, TAR RNA with 50 and 25 mM Pb^{2+}, respectively; 10, TAR RNA in cleavage buffer. There are no primary cuts.

DISCUSSION

The 5S rRNA is an attractive molecule for exploring the fundamental issues of RNA conformation and RNA-protein interaction. This is due to its relatively small size of 120 nt (ca 80 for tRNA), ease of preparation from natural sources, the presence of very few modified nucleotides, and a rich array of non-canonical base pairs (*28*). The main questions about 5S rRNA are its tertiary structure and its relation to function. Recently, almost 40 years after its discovery, the three dimensional structure of 5S rRNA within 50 S ribosomal subunits has been solved (*29*, *30*). However, the structure of free 5S rRNA involved in many processes outside ribosomes (*31*) is unknown. Having folded 5S rRNA in the ribosome context, one can ask about a structure-function relationship of the free molecule in solution and in complexes with ribosomal proteins (L5, L18, L28) or transcription factor IIIA (*32–35*). All mammalian 5S rRNAs have a highly conserved nucleotide sequence in loop D. Part of this sequence, GGGAG (Fig. 1), very much resembles the binding site of leadzyme (*12*, *17*, *18*). The leadzyme is a unique RNA that cleaves RNA in the presence of lead ions (*11*, *36*, *37*). Together with its small size, this makes the leadzyme an ideal candidate as a molecular probe for various RNA structure-function studies. We took an advantage of that fact and designed the smallest known natural catalytic RNA. It has an asymmetric loop with three and two nucleotides on each side of the loop (totally 5 nt) and a proposed cleavage site before the first residue of a longer strand of the loop, identical to the classical leadzyme described by the Uhlenbeck laboratory (*10*, *11*, *38–40*). The size of the internal bubble (5 nt) could be longer (*e.g.* 6 nt), although a smaller catalytic center ensures better specificity (*41*). In addition to the previous studies, we proved by acid treatment (data not shown) that cyclic phosphate is formed (*10*). We were interested in binding out whether stem IV and loop D of 5S rRNA or entire 5S rRNA molecules are involved in a leadzyme activity in two ways: i) as a ribozyme that binds Pb^{2+} (Figs. 3 and 4), creates complex with the RNA substrate (L5) of nucleotide sequence (5′)CGGUC↓GAGCCAU(3′) and cleaves it *in trans* at the single predetermined site (arrow), or ii) as the substrate (83)AUGG↓GAGACCG(93) (Fig. 5) to be cleaved specifically *in trans* by leadzyme (5′)CGGUGACCAU(3′), (L4). The cleavage reaction pattern for the first case confirms that the native 5S rRNA leadzyme or its stem IV and loop D have established a complex with the bubble surrounded by the double stranded RNA regions. This means that loop D of native 5S rRNA has to be single stranded along 10 nucleotides. Leadzyme cleavage is the result of a very precise stereochemistry of the reaction of the smallest catalytic site ever found for natural RNA molecules. In the second case, 5S rRNA was used as a substrate to be cleaved *in trans* by lead already bound to an oligonucleotide. The cleavage reaction within the asymmetric loop did not take place, which proves that the active center is not formed. It also means that loop D has a rigid conformation and rather limited possibility of exposing the scissile bond in such a way that leadzyme can break it down. A mechanism for leadzyme hydrolysis suggests that it relies primarily upon the ability of the RNA substrate to adopt the requisite transition-state geometry through inherent RNA flexibility (*18*). This indicates that conformational dynamics appear to play a key role in the catalytic function of the leadzyme (*17*, *18*, *40–43*, *53*). Quantitative analysis shows that the k_{obs} values of both model and 5S rRNAzymes are similar for this type of Pbzymes. The observed differences, which are rather small, are due to the varying experimental conditions (*10*). If so, this strongly suggests that L3 (model leadzyme) and 5S rRNAzymes have similar or identical structures and work in the same manner.

We demonstrated mutations that eliminate the activity of the leadzyme enzyme strand and also calf liver 5S rRNA, thus providing an important control for the authenticity of the leadzyme activity of 5S rRNA (*10*). Leadzyme specificity is achieved by the tight binding of Pb^{2+} to a particular site on the RNA molecule (*42*, *43*, *53*).

In the case of yeast $tRNA^{Phe}$, three high-occupancy Pb^{2+} binding sites have been located by X-ray crystallography, in the ribothymidine (T) loop, extra arm, and the anticodon (*44–47*). Only one of them, that in the T loop, appears to cause specific cleavage of the loop D of the $tRNA^{Phe}$ molecule, because the Pb^{2+} ion is placed in the correct site with respect to a 2′ hydroxyl group. The region of the tRNA molecule where cleavage *in cis* occurs is flexible enough to aquire a conformation suitable for the cleavage reaction. If so, the specificity of leadzyme provides the basis for structural consideration and demonstrates how catalytic RNAs can be applied to the structural analysis of nucleic acids, *e.g.* 5S rRNA. The tetra loop structure has been proposed for various parts of large RNAs (*48*). Four nucleotides in the loop are structurally constrained and explain the unusual thermodynamic stability of the hairpin (*49*). In theory, the strong tetra loop structure of the GNRA type cannot form a double helix with a complementary oligoribonucleotide. However, this could be possible in the presence of unfolders (*50–52*). In this case, the longer loop D of 5S rRNA, comprising nucleotides (83)AUGGGAGACCG(93), meets easily the structural requirements for leadzymes formation. Our results are in agreement with the cleavage pattern of the plant 5S rRNA loop D induced by RNase H in the presence of complementary oligodeoxynucleotides (*7*).

Although it is known that lead cleaves different RNA species including 5S rRNA, one should add that under the conditions used in our experiments, the eukaryotic 5S rRNA molecule is not hydrolysed in loop D. This native molecule can be probed with lead at higher concentrations (*54*). Finally, one can ask what, if anything, is the biological role of natural leadzymes? Do they exist in native RNA sequences? Surprisingly, the answer is positive. Data bank search showed that there are ca. 2000 potential leadzyme RNA sequences, including two in human 28S rRNA (*19*). Currently, we are also searching for substrate sequences. In the data bank, we have found over 200 substrate sequences, among which are mRNAs encoding proteins, including those involved in pathogenesis, and also ribosomal RNA. This suggests that the 5S rRNA leadzyme and substrate sequences may help to explain the toxicity of lead. The leadzyme motif occurs frequently in mammals and bacteria and its presence may be significant, since there is evidence that the motif is being expressed in RNA and may therefore be involved is some cleavage activity (*53*). Leadzyme in coding sequences could be involved in self-cleavage (cleavage *in cis*) (*19*), but their presence in stable RNA (*e.g.* tRNA, 5S rRNA) could be used to break down neighboring sequences *in trans*. This finding can explain the molecular toxicity of lead. Lead poisoning results in pervasive neurological problems in both children and adults. Lead might target proteins involved in calcium mediated signal transduction and bind to zinc binding domains such as Zn fingers with a Kd of about 10^{-9}–10^{-14} M, causing a disruption of DNA-binding activity of protein and transcription factors (*54*). In addition, RNA not only can bind lead, but at the same time it acquires catalytic properties that can destroy protein biosynthesis machinery (ribosomes), messenger RNAs and other RNA.

This work was supported by a grant from the Polish Committee for Scientific Research (to M.B.) and the Deutsche Forschungsgemeinschaft, the Bundesministerium fur Forschung und Technologie, and the Fonds der Chemischen Industie E.V.

REFERENCES

1. Barciszewska, M., Erdmann, V.A., and Barciszewski, J. (1996) Ribosomal 5S RNA: tertiary structure and interactions with proteins. *Biol. Rev.* **71**, 1–25
2. Barciszewska, M.Z., Szymanski, M., Erdmann, V.A., and Barciszewski, J. (2000) 5S ribosomal RNA. *Biomacromolecules* **1**, 297–302
3. Szymanski, M., Barciszewska, M., Barciszewski, J., and Erdmann, V.A. (2000) 5S ribosomal RNA database Y2K. *Nucleic Acids Res.* **28**, 166–167
4. Brunel, C., Romby, P., Westhof, E., Ehresmann, C., and Ehresmann, B. (1991) Three-dimensional model of *Escherichia coli* ribosomal 5S RNA as deduced from structure probing in solution and computer modeling. *J. Mol. Biol.* **221**, 293–308
5. Correll, C.C., Freeborn, B., Moore, P.B., and Steitz, T.A. (1997) Metals, motifs and recognition in the crystal structure of a 5S rRNA domain. *Cell* **91**, 705–712
6. Dallas, A. and Moore, P.B. (1997) The loop E-loop D of *Escherichia coli* 5S rRNA: the solution structure reveals an unusual loop that maybe important for binding ribosomal proteins. *Structure* **5**, 1639–1653
7. Barciszewska, M.Z., Lorenz, S., Erdmann, V.A., and Barciszewski, J. (1990) Structural analysis of plant ribosomal 5S RNAs. Visualisation of novel tertiary interactions by cleavage of lupin and wheat 5S rRNAs with ribonuclease H. *Biochim. Biophys Acta* **1087**, 68–72
8. Barciszewska, M.Z., Lorenz, S., Joachimiak, A., Ulbrich, N., Erdmann, V.A., and Barciszewski, J. (1990) New model of tertiary structure of plant 5S rRNA is confirmed by digestions with alpha-sarcin. *FEBS Lett.* **269**, 83–85
9. Gluck, A. and Wool, I.G. (1996) Determination of the 28 S ribosomal RNA identity element (G4319) for alpha-sarcin and the relationship of recognition to the selection of the catalytic site. *J. Mol. Biol.* **256**, 838–848
10. Pan, T. and Uhlenbeck, O. (1992) A small metalloribozyme with a two-step mechanism *Nature* **358**, 560–563
11. Pan, T., Dichtl, B., and Uhlenbeck, O. (1994) Properties of an *in vitro* selected Pb2+ cleavage motif. *Biochemistry* **33**, 9561–9565
12. Doherty, E.A. and Doudna, J.A. (2000) Ribozyme structures and mechanisms. *Annu. Rev. Biochem.* **69**, 597–615
13. David, L., Lambert, D., Gendron, P., and Major, F. (2001) Leadzyme, *Methods Enzymol.* **341**, 518–540
14. Behlen, L.S., Sampson, J.R., DiRenzo, A.B., and Uhlenbeck, O. (1990) Lead-catalyzed cleavage of yeast tRNAPhe mutants. *Biochemistry* **29**, 2515–2523
15. Pan, T. and Uhlenbeck, O. (1992) *In vitro* selection of RNAs that undergo autolytic cleavage with Pb^{2+}. *Biochemistry* **31**, 3887–3895
16. Lemieux, S., Chartrand, P., Cedergren, R., and Major, F. (1998) Modeling active RNA structures using the intersection of conformational space: application to the lead-activated ribozyme. *RNA* **4**, 739–749
17. Wedekind, J.E. and McKay, D.B. (1999) Crystal structure of a lead-dependent ribozyme revealing metal binding sites relevant to catalysis. *Nat. Struct. Biol.* **6**, 261–267
18. Westhof, E. and Hermann, T. (1998) Leadzyme RNA catalysis. *Nat. Struct. Biol.* **6**, 208–209
19. Bordeau, V., Ferbeyre, G., Pageau, M., Paquin, B., and Cedergren, R. (1999) The distribution of RNA motifs in natural sequences. *Nucleic Acids Res.* **27**, 4457–4467
20. Barciszewska, M.Z., Erdmann, V.A., and Barciszewski. J. (1994) The dynamic conformation of plant cytoplasmic 5S rRNAs. *Phytochemistry* **37**, 113–117
21. Perbandt, M., Vallazza, M., Lipmann, C., Betzel, Ch., and Erdmann, V.A. (2001) Structure of an RNA duplex with an unu-

sual G-C pair in wobble-like conformation at 1.6 Å resolution. *Acta Cryst.* **D57**, 219–224
22. Barciszewska, M.Z., Huang, H.W., Marshall, A.G., Erdmann, V.A, and Barciszewski, J. (1992) Biochemical and NMR spectroscopy evidence for a new tertiary A-U base pair in lupin ribosomal 5 S RNA structure. *J. Biol. Chem.* **267**, 16691–16695
23. Barciszewska, M., Dirheimer, G., and Keith, G. (1983) The nucleotide sequence of methionine elongator tRNA from wheat germ. *Biochem. Biophys. Res. Commun.* **114**, 1161–1168
24. Ohmichi, T., Okumoto, Y., and Sugimoto, N. (1998) Effect of substrate RNA sequence on the cleavage reaction by a short ribozyme. *Nucleic Acids Res.* **26**, 5655–5661
25. Katahira, M., Kim, M.H., Sugiyama, T., Nishimura, Y., and Uesugi, S. (1998) Two metal binding sites in a lead ribozyme bound to competitively by Pb^{2+} and Mg^{2+}. *Eur. J. Biochem.* **255**, 727–733
26. Turner, B.G. and Summers, M.F. (1999) Structural Biology of HIV. *J. Mol. Biol.* **285**, 1–32.
27. Wyszko, E., Barciszewska, M.Z., Bald, R., Erdmann, V.A., and Barciszewski, J. (2001) The specific cleavage of HIV TAR RNA with anti-TAR hammerhead ribozyme. Structural and functional implications. *Int. J. Biol. Macromol.* **28**, 373–380
28. Moore, P.B. (1999) Structural motifs in RNA. *Annu. Rev. Biochem.* **68**, 287–300
29. Ban, N., Nissen, P., Hansen, J., Moore, P.B., and Steitz, T.A. (2000) The complete atomic structure of the large ribosomal subunit at 2.4 Å resolution. *Science* **289**, 905–920
30. Nissen, P., Hansen, J., Ban, N., Moore, P.B., and Steitz, T.A. (2000) The structural basis of ribosome activity in peptide bond synthesis. *Science* **289**, 920–930
31. Neely, L.S., Lee, B.M., Xu, J., Wright, P.E., and Gottesfeld, J.M. (1999) Identification of a minimal domain of 5 S ribosomal RNA sufficient for high affinity interactions with the RNA-specific zinc fingers of transcription factor. *J. Mol. Biol.* **291**, 549–560
32. Bogenhagen, D.F. (1993) Proteolytic footprinting of transcription factor TFIIIA reveals different tightly binding sites for 5S RNA and 5S DNA. *Mol. Cell Biol.* **13**, 5149–5158
33. Claussen, M., Rudt, F., and Pieler, T. (1999) Functional modules in ribosomal protein L5 for ribonucleoprotein complex formation and nucleocytoplasmic transport. *J. Biol. Chem.* **274**, 33951–33958
34. Zang, W.Q. and Romaniuk, P.J. (1995) Characterization of the 5S RNA binding activity of *Xenopus* zinc finger protein p43. *J. Mol. Biol.* **245**, 549–558
35. Westhof, E., Romby, P., Romaniuk, P.J., Ebel, J.P., Ehresmann, C., and Ehresmann. B. (1989) Computer modeling from solution data of spinach chloroplast and of *Xenopus laevis* somatic and oocyte 5 S rRNAs. *J. Mol. Biol.* **207**, 417–431
36. Kim, M.H., Katahira, M., Sugiyama, T., and Uesugi, S. (1997) Activation and repression of the activity of a lead ribozyme by a combination of Pb^{2+} and Mg^{2+}. *J. Biochem.* **122**, 1062–1067
37. Legault, P. and Pardi, A. (1997) Unusual dynamics and pKa shift at the active site of a lead dependent ribozyme. *J. Amer. Chem. Soc.* **119**, 6621–6628
38. Legault, P., Hoogstraten, C.G., Metlitzky, E., and Pardi, A. (1998) Order, dynamics and metal binding in the lead-dependent ribozyme. *J. Mol. Biol.* **284**, 325–335
39. Hoogstein, C.G., Legault, P., and Pardi, A. (1998) NMR solution structure of lead-dependent ribozyme: evidence for dynamics in RNA catalysis. *J. Mol. Biol.* **284**, 337–350
40. Hoogstraten, C.G., Wank, J.R., and Pardi, A. (2000) Active site dynamics in the lead-dependent ribozyme. *Biochemistry* **39**, 9951–9958
41. Wedekind, J.E. and McKay, D.B. (1998) Crystallographic structures of the hammerhead ribozyme: relationship to ribozyme folding and catalysis. *Ann. Rev. Biophys. Biomol. Struct.* **27**, 475–502
42. Werner, C., Krebs, B., Keith, G., and Dirheimer, G. (1976) Specific cleavages of pure tRNAs by plumbous ions. *Biochim. Biophys Acta* **432**, 161–175
43. Dirheimer, G., Ebel, J.P., Bonnet. J., Gangloff. J., Keith, G., Krebs, B., Kuntzel, B., Roy, A., Weissenbach, J., and Werner, C. (1972) Primary structure of transfer RNA. *Biochimie* **54**, 127–144
44. Brown, R.S., Hingerty, B.E., Devan, J.C., and Klug, A. (1983) Pb(II) – catalyzed cleavage of the sugar-phosphate backbone of yeast tRNAPhe – implications for lead toxicity and self splicing RNA. *Nature* **303**, 543–546
45. Brown, R.S., Devan, J.C., and Klug, A. (1985) Crystallographic and biochemical investigation of the lead(II)-catalyzed hydrolysis of yeast phenylalanine tRNA. *Biochemistry* **24**, 4785–4801
46. Jovine, L. Djordjevic, S., and Rhodes, D. (2000) The crystal structure of yeast phenylalanine tRNA at 2.0 Å resolution: cleavage by Mg(2+) in 15-year old crystals. *J. Mol. Biol.* **301**, 401–414
47. Shi, H. and Moore, P.B. (2000) The crystal structure of yeast phenylalanine tRNA at 1.93 Å resolution: a classic structure revisited. *RNA* **6**, 1091–1105
48. Costa, M. and Michel, F. (1995) Frequent use of the same tertiary motif by self-folding RNAs. *EMBO J.* **14**, 1276–1285
49. Varani, G. (1995) Exceptionally stable nucleic acid hairpins. *Ann. Rev. Biophys. Biomol. Struct.* **24**, 379–404
50. Buvoli, A., Buvoli, M., and Leinwand, L.A. (2000) Enhanced detection of tRNA isoacceptors by combinatorial oligonucleotide hybridization. *RNA* **6**, 912–918
51. Jankowsky, E. and Schwenzer, B. (1998) Oligonucleotide facilitators enable a hammerhead ribozyme to cleave long RNA substrates with multiple-turnover activity. *Eur. J. Biochem.* **254**, 129–134
52. Westhof, E. and Fritsch, V. (2000) RNA folding: beyond Watson-Crick pairs. *Structure* **8**, R55–R65
53. Godwin, H.A. (2001) The biological chemistry of lead. *Curr. Opin. Chem. Biol.* **5**, 223–227
54. Ciesiolka, J., Lorenz, S., and Erdmann, V.A. (1992) Different conformational forms of *Escherichia coli* and rat liver 5S rRNA revealed by Pb(II) induced hydrolysis. *Eur. J. Biochem.* **204**, 583–589.