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Characterization of the Lead(II)-Induced Cleavages in tRNAs in Solution and Effect of the Y-Base Removal in Yeast $tRNA^{Phe}$ †

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ABSTRACT: The specificity of lead(II)-induced hydrolysis of yeast $tRNA^{Phe}$ was studied as a function of concentration of Pb^{2+} ions. The major cut was localized in the D-loop and minor cleavages were detected in the anticodon and T-loops at high metal ion concentration. The effects of pH, temperature, and urea were also analyzed, revealing a basically unchanged specificity of hydrolysis. In the isolated 5′-half-molecule of yeast $tRNA^{Phe}$ no cut was found in the D-loop, indicating its stringent dependence on T–D-loop interaction. Comparison of hydrolysis patterns and efficiencies observed in yeast $tRNA^{Phe}$ with those found in other tRNAs suggests that the presence of a U59–C60 sequence in the T-loop is responsible for the highly efficient and specific hydrolysis in the spatially close region of the D-loop. The efficiencies of D-loop cleavage in intact yeast $tRNA^{Phe}$ and in $tRNA^{Phe}$ deprived of the Y base next to the anticodon were also compared at various Pb^{2+} ion concentrations. Kinetics of the D-loop hydrolysis analyzed at 0, 25, and 37 °C showed a 6 times higher susceptibility of $tRNA^{Phe}$ minus Y base ($tRNA^{Phe}$-Y) to lead(II)-induced hydrolysis than in $tRNA^{Phe}$. The observed effect is discussed in terms of a long-distance conformational transition in the region of the interacting D- and T-loops triggered by the Y-base excision.

A number of studies have shown that tRNAs can undergo conformational changes, although they tend to modulate their spatial configuration rather than undergoing dramatic conformational rearrangements [e.g., Gassen (1980) and Moras et al. (1985, 1986) and references cited therein]. For instance, removal of the Y base in the anticodon region in yeast $tRNA^{Phe}$ induces long-range conformational changes in this molecule [e.g., Cameron and Uhlenbeck (1973), Kearns et al. (1973), Wang et al. (1975), Davanloo et al. (1979), and Salemink et al. (1979)], a fact substantiated by an increased accessibility of its T-loop, leading to the modification of cytosine-60 by chloroacetaldehyde (Krzyzosiak & Ciesiolka, 1983). According to crystallographic studies of $tRNA^{Phe}$, C60 was found to be the closest ligand for lead ion binding (Brown et al., 1983, 1985; Rubin & Sundaralingam, 1983), which leads to specific cleavage of the tRNA in a spatially close region of the D-loop.

Considering these facts, and in agreement with the first observations by Werner et al. (1976), it seemed worthwhile to determine whether the excision of the Y base from yeast $tRNA^{Phe}$ would have any influence on the specificity of the Pb^{2+}-promoted cleavage reaction or, in other words, would be sensitive enough to monitor conformational changes in the altered $tRNA^{Phe}$. However, the relative lack of information concerning kinetic and biochemical characteristics of the cleavage reaction in $tRNA^{Phe}$ prompted us to examine the influence on the rate and specificity of this reaction of parameters such as the concentration of Pb^{2+} ions, pH, temperature, and tRNA structure. Once these variables had been optimized, the relative reactivities of yeast $tRNA^{Phe}$ and $tRNA^{Phe}$-Y[1] were studied. The results will be discussed in the light of long-distance conformational transitions between the anticodon and the T–D regions in tRNAs, in relation to mechanistic aspects proposed for Pb^{2+}-induced strand scission in yeast $tRNA^{Phe}$ as well as in the perspective of Pb^{2+} ions as a structural probe to test conformational features in tRNA and RNA molecules.

Materials and Methods

tRNAs. Yeast $tRNA^{Phe}$, $tRNA^{Asp}$, and $tRNA^{Val}$ were prepared from crude brewer's yeast tRNA (Boehringer) by countercurrent distribution (Dirheimer & Ebel, 1967) followed by column chromatographies (Keith et al., 1971). In the case of $tRNA^{Asp}$, purification was achieved by chromatography on a Sepharose 4B column (Giegé et al., 1986a). Pure *Escherichia coli* $tRNA^{Val}$ was from Boehringer. Yeast $tRNA^{Phe}$ was a gift from P. Remy. The 5′-half-molecule of $tRNA^{Phe}$ was prepared according to Phillipsen et al. (1968) by aniline/acetic acid (pH 4.5) treatment of 5′-end-labeled $tRNA^{Phe}$-Y.

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[1] Abbreviations: EDTA, ethylenediaminetetraacetic acid; Tris, tris(hydroxymethyl)aminomethane; $tRNA^{Phe}$-Y, phenylalanine-specific tRNA minus Y base.

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Table I: Quantitative Comparison of the Cleavage Induced in the Studied tRNAs at Various Concentrations of Pb^{2+} at 25 °C[a]

tRNAs	cuts	Pb^{2+} concn (mM)						
		0.03	0.1	0.3	1.0	3.0	10.0	30.0
Phe-Y (yeast)	D-loop	0.5	1.4	6.0	25.5	53.0	47.0	27.5
	AC-loop	–	–	–	–	–	4.5	7.0
	U33	–	–	–	–	–	0.6	1.0
	T-loop	–	–	–	–	–	–	4.0
Phe (yeast)	D-loop	0.4	1.5	6.5	24.0	43.0	38.0	25.0
	AC-loop	–	–	–	–	–	0.7	1.5
	U33	–	–	–	–	–	0.4	1.5
	T-loop	–	–	–	–	–	–	5.7
Val (yeast)	D-loop	1.7	10.5	23.5	47.0	71.5	66.0	18.0
	AC-loop	–	–	–	–	–	1.4	5.6
	T-loop	–	–	–	–	–	2.5	3.7
Val (*E. coli*)	D-loop	–	–	–	–	3.3	5.6	8.7
	AC-loop	–	–	–	–	1.6	3.1	8.5
	T-loop	–	–	–	–	1.7	2.6	2.6
Asp (yeast)	D-loop	–	–	–	–	1.1	3.9	12.8
	AC-loop	–	–	–	–	1.1	3.0	3.2 (35–36) 11.0 (AC-loop)
	T-loop	–	–	–	–	–	–	–

[a] The values are ratios between the amount of radioactivity (percent) of the corresponding electrophoretic bands of one lead cleavage and the total amount of radioactivity (percent) loaded on the gel. (–) means no Pb^{2+} cleavage.

End Labeling of tRNAs. The 5′-end labeling of tRNA was performed with [γ-^{32}P]ATP and T_4 polynucleotide kinase on tRNAs dephosphorylated with alkaline phosphatase (Silberklang et al., 1977). The 3′-end labeling of tRNAs was done on molecules deprived of their 3′-CCA end with snake venom phosphodiesterase and reconstituted with cold CTP, [γ-^{32}P]-ATP, and tRNA nucleotidyltransferase (Vlassov et al., 1981). Labeled tRNAs were purified by electrophoresis on 15% polyacrylamide gels in the presence of 8 M urea, eluted, and precipitated with ethanol.

Kinetic Studies. The tRNA concentrations used in these experiments were determined by the amount of carrier tRNA added to the reaction mixture. In all cases, carrier tRNA was of the same amino acid specificity as the labeled one. The procedure used to follow the pH dependence of Pb^{2+}-induced cleavages in yeast $tRNA^{Phe}$ is given below as one example. A mixture (120 μL) composed of 5′-end-labeled $tRNA^{Phe}$ ($\sim$2 $\times$ 10^6 cpm), carrier $tRNA^{Phe}$ (15 μM), and NaCl (73 mM) was split into 11-μL aliquots. Then, 2 μL of 100 mM buffer was added to each sample (sodium acetate for pHs 4.0–6.0; Tris-HCl for pHs 6.5–8.5). All tRNA solutions were incubated for 1 min at 55 °C. Then, 2 μL of $MgCl_2$ (100 mM) was added, and samples were cooled slowly (15 min) to reach room temperature. After preincubation at 25 °C (5 min), 5 μL of 2 mM $Pb(OAc)_2$ solution was added (controls were supplied with 5 μL of H_2O). Reactions were carried out in 20 μL at 25 °C for 1 h. Solutions of lead were prepared before use in order to avoid polymerization and precipitation of polynuclear species from aqueous solution (Burgers, 1978). Reactions were stopped by 20 μL of 7 M urea + dyes/20 mM EDTA. Before loading on the gels, the radioactivity level present in samples was controlled and brought to approximately the same value if variations were higher than 5%.

Electrophoresis, Autoradiography, and Quantitations. Electrophoresis was performed on 15% acrylamide/0.75% bis(acrylamide)/7 M urea slab gels (40 $\times$ 30 $\times$ 0.05 cm^3) at 1500 V for 2–5 h. Autoradiography was at either –80 or –20 °C without intensifying screens. Radioactivity present in the individual bands was determined by Cerenkov counting.

Results

Lead-Induced Cleavages in Yeast $tRNA^{Phe}$: Effect of Pb^{2+} Concentration. The first experiments were designed to establish the influence of Pb^{2+} ion concentratin on the cleavage specificity. The results obtained, using a constant tRNA concentration of 8 μM and a molar excess of Pb^{2+} ions ranging from 1.25 to more than 3000, are presented. At 20 μM lead the only detectable cleavage occurred at position 18 (not shown). The extent of strand scission at this position increases progressively with lead concentration to a value of $\sim$50%, the maximum yield being reached at 2.5 mM Pb^{2+} (Figure 1A, line 11, and Table I). Increasing the Pb^{2+} concentration to a 300-fold molar excess over tRNA still produces a highly specific cut at phosphate 18 accompanied only by two very weak cleavages at phosphates 16 and 17. These cuts are of secondary origin because they are only seen on 5′-end-labeled tRNA (compare Figure 1, parts A and C). The different susceptibility of phosphodiesters neighboring phosphate 18 to undergo secondary cleavages can be explained by the base composition of the corresponding nucleotides. The two guanine

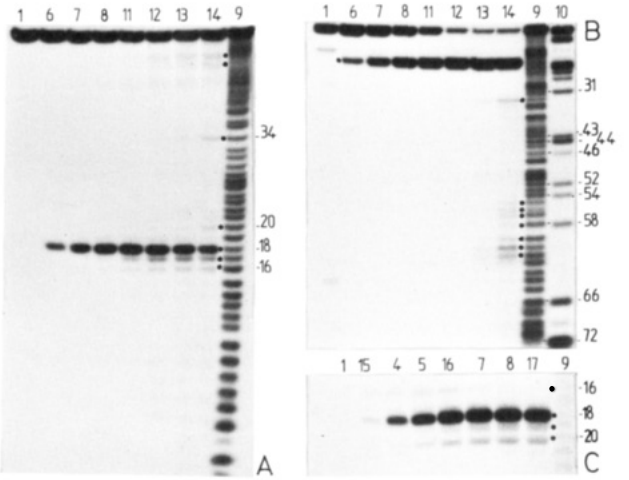

FIGURE 1: Effect of Pb^{2+} concentration on the specificity of cleavages in yeast $tRNA^{Phe}$. Constant reaction conditions: tRNA (8 μM), Tris-HCl (10 mM), pH 7.2, NaCl (40 mM), $MgCl_2$ (10 mM), and temperature 25 °C. Reactions were carried out with (A) 5′-labeled and (B and C) 3′-labeled tRNA. Reaction time was (A) 3 min, (B) 7 min, and (C) 15 min. The following concentrations (mM) of Pb(II) ions were used: (lane 4) 0.05, (lane 5) 0.1, (lane 6) 0.25, (lane 7) 0.5, (lane 8) 1.0, (lane 11) 2.5, (lane 12) 5.0, (lane 13) 10, (lane 14) 25, (lane 15) 0.02, (lane 16) 0.2, (lane 17) 2.0, (lane 1) control incubation without Pb(II) ions, (lane 9) formamide ladder, (lane 10) limited hydrolysis with ribonuclease T1. Experiments with the lowest Pb^{2+} concentrations, 0.01 and 0.025 mM (conditions 2 and 3), are not shown.

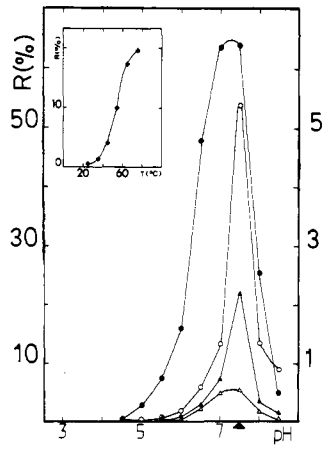

FIGURE 2: Influence of pH and of temperature on hydrolysis of yeast $tRNA^{Phe}$ in the D-loop. The quantitated cleavage efficiencies at phosphates 16 (▲), 17 (○), 18 (●), and 19 (△) are plotted against pH. The separate scale (right) applies for the cleavages at positions 16, 17, and 19. R is the ratio of the radioactivity corresponding to the analyzed RNA fragment with respect to the total radioactivity loaded per line. The final composition of the reaction mixture was 2×10^5 cpm of 5′-end-labeled $tRNA^{Phe}$, 8 μM carrier $tRNA^{Phe}$, 40 mM NaCl, 10 mM $MgCl_2$, 10 mM appropriate buffer, and 0.5 mM Pb^{2+}. Reactions were at 25 °C for 1 h. The influence of temperature was studied in the reaction mixtures as above except that Pb^{2+} was 0.1 mM. Reactions were for 10 min at 25, 35, 45, 55, 65, 75, and 85 °C. Only quantitated cleavages at phosphate 18 are given in the inset.

residues G18 and G19 will tend to form strong stacking interactions and, therefore, will be less flexible and less accessible to lead ions as compared to the two residues from the opposite side. Note that the interplay between primary and secondary cleavages in the D-loop applies basically to only two phosphodiesters at each side of phosphate 18.

The relationship between the concentration of Pb^{2+} and the efficiency of cleavage at phosphate 18 shown in Figure 1A indicates a saturation of the lead binding site at 2.5 mM Pb^{2+}, a fact also seen by Brown et al. (1983). In this respect, lead-induced cleavage in $tRNA^{Phe}$ shows one of the properties well-known for enzymatic reactions. Increasing the Pb^{2+}/tRNA ratio further reduces the amount of the pG1–D17p fragment generated. This is due to competing specific hydrolysis in other regions of the tRNA, i.e., in the anticodon loop (position 34) and in the entire T-loop comprising phosphates 55–61 (see Figure 1A,B). The predominant cut in the T-loop takes place at phosphate 60. The presence of cuts in the T-loop and anticodon region of tRNA labeled at either the 3′- or 5′-end indicates that these cleavages are at least in part of primary origin (i.e., they take place in the native tRNA).

Effect of pH. Except for the most recent studies of Brown et al. (1985), the effect of pH on the specificity and rate of Pb^{2+}-induced cleavage of yeast $tRNA^{Phe}$ had not been studied in detail. It was known, however, that the reaction rate was much lower at pH 5.0 than at pH 7.4 (Brown et al., 1983). The present studies were performed within the pH range of 4.5–8.5 with a constant Pb^{2+} ion concentration of 0.5 mM. Figure 2 shows that there is a qualitatively similar pH dependence for all the D-loop cleavages analyzed with a pH optimum of 7.5. This is close to the pK_a value of the Pb^{2+} aquo cation that was reported to be 7.2 (Burgers, 1978). The rate of the major cut at phosphate 18 decreases rather rapidly on both sides of the pH optimum.

A careful inspection of the results in Figure 2 shows that the pH optimum for the major cut and the primary cleavage at phosphate 18 is broader than that for hydrolysis at phosphates 16 and 17, which occurs mostly or entirely in a secondary fashion. It is also important to note that at pH 8.5 the major cut is accompanied by basically two faint satellite cleavages at phosphates 16 and 20 (data not shown). There is no tendency to increase the level of strand scission at phosphate 17 at this pH value as it was found at pH 9.0 by other authors (Brown et al., 1985). The decreased cleavage efficiency for all cuts in the D-loop at values above pH 7.5, despite the more favorable ionization state of the Pb^{2+} aquo cation, is most likely due to the known tendency of free $PbOH^+$ to undergo rapid polymerization at higher pH values (Burgers, 1978).

Effect of Temperature. In this experiment, a concentration of Pb^{2+} ions of 0.1 mM and a time of 5 min were kept constant while the temperature was varied from 25 to 85 °C. As can be seen in Figure 2 (inset), the rate of strand scission at phosphate 18 increases with temperature, reaching a maximum value between 65 and 75 °C. At 85 °C, the reaction loses its specificity and a lead-induced ladder of hydrolysis products is generated (not shown). It is striking, in light of the reaction mechanism proposed by Brown et al. (1983), that even at temperatures of 65–75°C there exists a configuration of ligands within yeast $tRNA^{Phe}$ that enables specific strand scission at phosphate 18 to occur. This apparent divergence can be yet explained by assuming that a reduction in the fraction of molecules with an intact configuration of ligands due to the progressive melting of the tRNA structure is offset by a greater rate of hydrolysis.

Effect of Urea. The results of the temperature-dependence study prompted us to examine the influence of another tertiary structure perturbing agent, urea, on the efficiency of cleavage. In 8 M urea in the absence of Na^+ and Mg^{2+} ions, the reaction occurs predominantly at phosphate 18, although the yield of the pG1–D17p fragment generated is reduced to 30% of the value observed in the absence of urea. In the absence of Mg^{2+} ions there is a reduced specificity in the cleavage pattern of tRNA; the presence of urea also reduces the cleavage specificity, and this effect is more pronounced in the absence of Mg^{2+} ions (not shown). It appears that the presence of counterions does not have a large stabilizing effect on the tRNA structure when it is exposed to a strong denaturing agent. The effects of both temperature and urea demonstrate that the forces stabilizing the interacting D- and T-loops of yeast $tRNA^{Phe}$ are not easily broken, even under very severe solution conditions.

Lead-Induced Cleavages in the 5′-Half-Fragment of Yeast $tRNA^{Phe}$. Since neither elevated temperature nor 8 M urea could induce the complete disappearance of the D-loop cleavage and unequivocally prove the rigorous requirements of T-loop proximity for cleavages in the D-loop, the following experiment was conducted with the 5′-half-fragment of the yeast $tRNA^{Phe}$ molecule. Identical molar quantities of yeast $tRNA^{Phe}$ and its 5′-half were treated separately with 0.2 mM Pb^{2+}, and the time dependence of the reaction was monitored. No cleavage was observed with the 5′-half-molecule over the time course, while the normal reaction occured with intact tRNA (Figure 3A). Since the structure of the 5′-half-fragment contains the complete D-stem and only tertiary interactions are lacking (Boyle et al., 1980, 1983), its resistance to hydrolysis is direct evidence that the cleavage in the D-loop is T-loop dependent, as was first suggested by Brown et al. (1983).

Specific Cleavages Induced by Lead in Other tRNAs. The pattern and efficiency of cleavages generated by Pb^{2+} in other tRNAs might shed some light on other features of tRNA

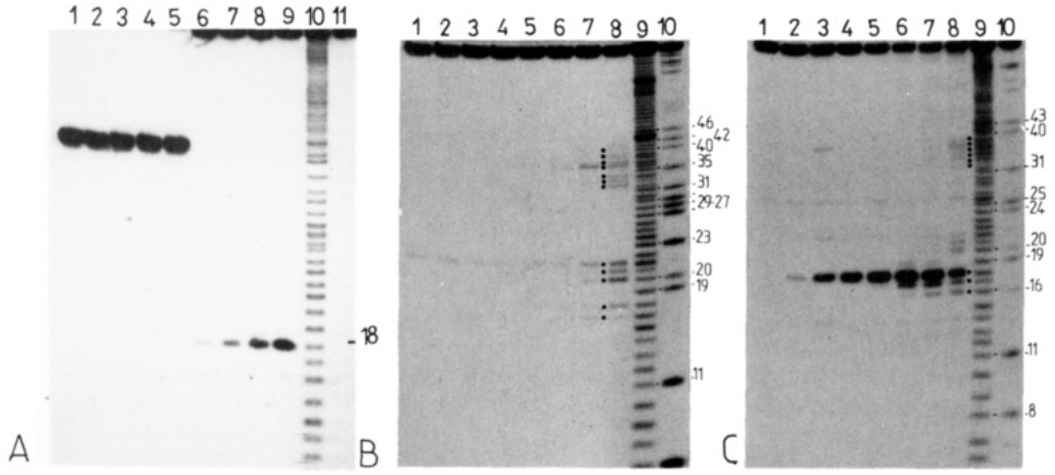

FIGURE 3: (A) Reactions of 5′-labeled $tRNA^{Phe}$ and its 5′-half-molecule with Pb^{2+} ions. The reaction mixtures were composed of 1.6 μM RNA, (3–5) × 10^5 cpm of labeled tRNA, 10 mM Tris-HCl, pH 7.2, 40 mM NaCl, 10 mM $MgCl_2$, and 0.2 mM Pb^{2+}. Incubations were at 25 °C for 2, 5, 15, and 30 min: (lanes 2–5) 5′-half-molecule, (lanes 6–9) $tRNA^{Phe}$. Lanes 1 and 11 represent controls without Pb^{2+} ions for the 5′-half-molecule and complete $tRNA^{Phe}$, respectively; lane 10 is the formamide ladder of $tRNA^{Phe}$. Hydrolysis patterns in (B) 5′-labeled yeast $tRNA^{Asp}$ and (C) yeast $tRNA^{Val}$ obtained at various Pb^{2+} concentrations. Reaction conditions were as described in the legend to Figure 1 (A and B). The following Pb^{2+} concentrations (mM) were used: (lane 2) 0.03, (lane 3) 0.1, (lane 4) 0.3, (lane 5) 1.0, (lane 6) 3.0, (lane 7) 10, (lane 8) 30, (lane 1) controls with no Pb^{2+} added, (lane 9) formamide ladder, (lane 10) limited hydrolysis with ribonuclease T1.

structure that are reactive toward lead. $tRNA^{Val}$ and $tRNA^{Asp}$ from yeast and $tRNA^{Val}$ from *E. coli* were the first molecules selected for these studies. Both yeast tRNAs had previously been reported to be cleaved by Pb^{2+} ions (Werner et al., 1976). The cleavage pattern obtained for yeast $tRNA^{Val}$ (Figures 3C and 4), which has the same T-loop sequence as yeast $tRNA^{Phe}$, closely resembles that of $tRNA^{Phe}$. The major strand scission occurs at phosphate 18 and is accompanied at high Pb^{2+} concentrations by weaker cuts in most of the D-loop. Some weaker cleavages in the anticodon loop also take place at the highest Pb^{2+}/tRNA ratio used. As is shown in Table I, yeast $tRNA^{Val}$ is an even better substrate for lead-induced hydrolysis than yeast $tRNA^{Phe}$. This agrees well with the results presented earlier (Werner et al., 1976). The results obtained with 3′-end-labeled yeast $tRNA^{Val}$ (gels not shown) also indicate the presence of weaker cleavages in the T-loop (Figure 4). Yeast $tRNA^{Asp}$, on the other hand, is much less reactive than $tRNA^{Phe}$ and $tRNA^{Val}$. In addition, the cleavage pattern is different (Figures 3B and 4). Almost the entire anticodon and D-loops undergo hydrolysis at the highest Pb^{2+} concentration (30 mM). It should be noted that the cuts in the anticodon stem were not observed in other tRNAs studied. The cleavage pattern of *E. coli* $tRNA^{Val}$ (Table I and Figure 4) qualitatively resembles that of yeast $tRNA^{Asp}$. It is interesting to note that some of the phosphodiester bonds in extensively cleaved regions of the studied tRNAs are strongly resistant to hydrolysis. These comprise phosphates 18 and 34 in $tRNA^{Asp}$ and phosphates 18, 19, and 34 in *E. coli* $tRNA^{Val}$. All these resistant phosphodiester bonds involve at least one purine residue on their side; thus, strong stacking interactions together with geometrical factors might be responsible for the observed effect.

Effect of the Y-Base Removal from Yeast $tRNA^{Phe}$. Comparative studies between $tRNA^{Phe}$ and $tRNA^{Phe}$-Y were undertaken by using the following approach. Both tRNAs were first subjected to treatment with various concentrations of Pb^{2+} ranging from 0.03 to 30 mM. Results summarized in Table I indicate that the major cut at phosphate 18 exhibits a similar efficiency in both tRNAs for Pb^{2+} concentrations of 1 mM and below. From 3 to 10 mM Pb^{2+} the reaction still retains its high specificity toward cleavage in the D-loop, but there is a distinct increase in the intensity of the major cleavage of $tRNA^{Phe}$-Y relative to $tRNA^{Phe}$. At 30 mM Pb^{2+} the difference between the two $tRNA^{Phe}$ species tends to disappear.

Hydrolysis of the anticodon loop at 10 and 30 mM Pb^{2+} is also clearly visible; again, it is more prominent in $tRNA^{Phe}$-Y. In contrast to $tRNA^{Phe}$, where the cut at phosphate 34 contributes more than 50% of the total anticodon loop hydrolysis, cuts of comparable or even greater intensity, comprising phosphates 37–39, are clearly apparent in $tRNA^{Phe}$-Y. Although the absolute values of the cleavages in the anticodon loop are much less than those in the D-loop, the severalfold difference of the intensity of these cuts between $tRNA^{Phe}$ and $tRNA^{Phe}$-Y reveals that there are significant conformational differences between the two molecules.

In another series of experiments, 3 mM Pb^{2+} was chosen to monitor the time course of the reactions; it is the concentration that gave the greatest difference in D-loop cleavage efficiency between the two tRNAs (Table I). The data show the time course of the reactions, which were carried out at 0, 25 (see Figure 5A for an example), and 37 °C. The cleavage efficiency at phosphate 18 is plotted versus time in Figure 5 B–D. Results indicate that the changed efficiency of cleavages in the high-reactivity region (D-loop) is coupled to differences in the low-reactivity region (anticodon loop). In fact, very weak cuts, similar to those observed at higher Pb^{2+} concentrations (except for the absence of the cut at phosphate 34), can be detected in the anticodon loop of $tRNA^{Phe}$-Y (Figure 5A, line 5). The large and easily measurable differences in the efficiencies of the major cleavage are greatest at low temperatures; they reach a factor of 6 at 0 °C.

Discussion

Importance of the T-Loop Conformation for D-Loop Cleavage by Lead. Crystallographic studies have shown that the characteristic feature of the Pb^{2+} binding site in the T-loop of yeast $tRNA^{Phe}$ is the existence of very short contacts with U59 and C60. There is a direct bond (2.2 Å) to O4 of U59 and another to N3 of C60 (2.8 Å). Contacts of less than 4 Å to these bases occur with N4 and O2 of C60 and with N3 of U59 (Brown et al., 1985). Our results support these crystallographic findings and bring more experimental data indicating the importance of the nature of bases 59 and 60 in the Pb^{2+} binding site in the T-loop. Indeed *E. coli* $tRNA^{Phe}$, having as does yeast $tRNA^{Asp}$ the U59–U60 sequence, gives much weaker cleavages in the D-loop. The weaker cleavage

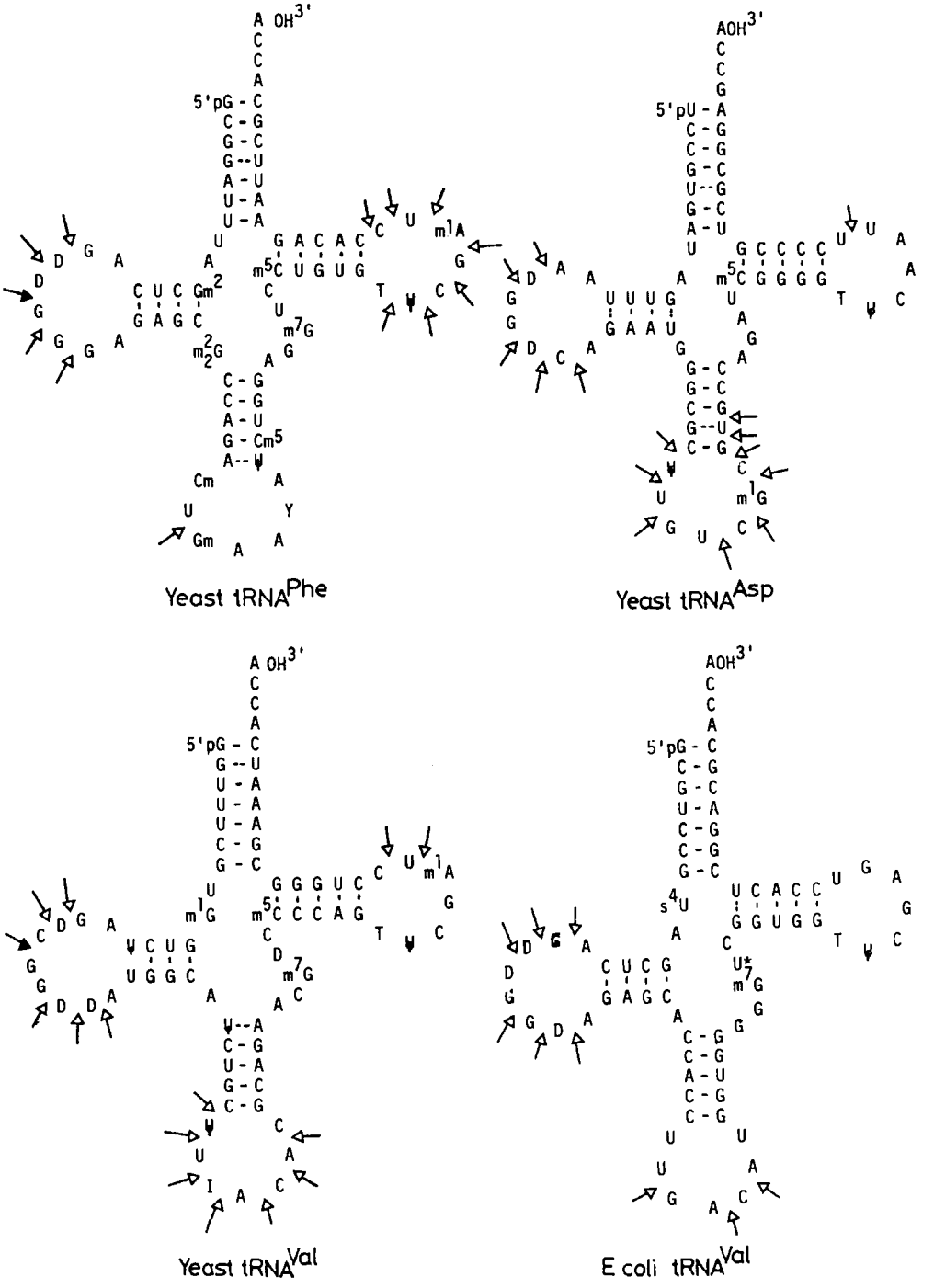

FIGURE 4: Cloverleaf structures of yeast $tRNA^{Phe}$, $tRNA^{Asp}$, and $tRNA^{Val}$ and *E. coli* $tRNA^{Val}$ with Pb^{2+}-induced cuts marked. Full arrows represent strong cleavage, and empty arrows represent moderate cleavage. Sequence data are from Sprinzl et al. (1987).

is also observed in *E. coli* $tRNA^{Val}$, which has the sequence G59–U60. One cannot totally exclude that the D-loop conformation also influences the lead cleavage pattern. However, *E. coli* $tRNA^{Val}$ and yeast $tRNA^{Val}$, possessing the same sequence in the D-loop, display different lead cleavage patterns (see Figure 4). Also, recent comparative studies in the phenylalanine tRNA system indicate that $tRNA^{Phe}$ molecules containing the U59–C60 sequence show a cleavage pattern similar to that of yeast $tRNA^{Phe}$ despite having some sequence differences in other regions of the molecule (Ciesiolka et al., 1987). This was also the case reported earlier for mammalian phenylalanine-specific tRNAs (Werner et al., 1976). Although more experimental data are required to make reasonable generalizations, it seems that the appearance and efficiency of cleavages in the D-loop of tRNA molecules are, at least to some extent, predictable.

Regions of Cleavages. It was suggested that the very poor stacking properties of dihydrouridine bases make them and their structural environment in the D-loop more flexible as compared with other regions of the molecule. This flexibility makes the D-loop elements easily accessible to the bound hydroxyl group of the lead aquo cation that affects the cleavage (Brown et al., 1985). Although strand scissions also occur in the anticodon loop of yeast $tRNA^{Phe}$, $tRNA^{Asp}$, $tRNA^{Val}$, and *E. coli* $tRNA^{Val}$ at high Pb^{2+} concentrations (see Figure 4), they show a much lower efficiency and, in most cases, lower specificity. It appears that the major factor responsible for this property may be a lower accessibility of the 2′-OH groups of the ribose residues to lead-bound hydroxyl groups because the anticodon loop components are more hindered in the

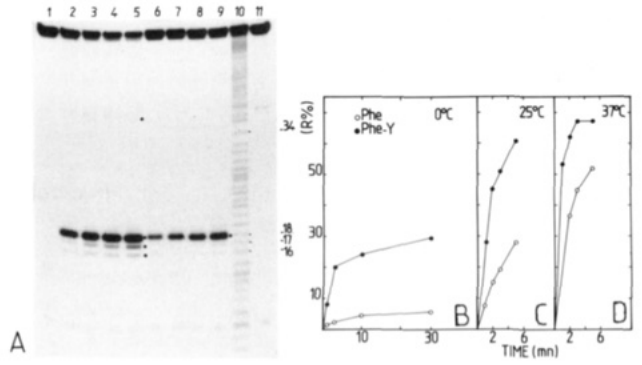

FIGURE 5: Comparative analysis of Pb^{2+}-induced cleavages in 5′-labeled yeast $tRNA^{Phe}$ and $tRNA^{Phe}$-Y at 0, 25, and 37 °C. For the conditions at 25 °C an autoradiogram is shown (A): (lanes 2–5) $tRNA^{Phe}$-Y, incubations for 1, 2, 3, and 5 min, respectively; (lanes 6–9) $tRNA^{Phe}$, incubations as above; (lanes 1, 11) incubation controls; (lane 10) formamide ladder. For the three temperature conditions, plots showing time dependence of the cleavages at phosphate 18 are presented in panels B–D. The Pb(II) concentration used was 3 mM.

stacked regions. In the anticodon loop of yeast $tRNA^{Phe}$, the most prominent of the minor cleavages occurs at phosphate 34. The Pb^{2+} distance to (O2′) U33 is 6.2 Å, which should make strand scission at this position very favorable for a mechanism involving proton abstraction from the 2′-hydroxyl (Brown et al., 1985). It is therefore likely that this and also other cleavages taking place at higher Pb^{2+} ion concentrations are effected by a mechanism similar to that proposed for the D-loop cleavage. Moreover, in the case of polynucleotide strand scissions in the T-loop of yeast $tRNA^{Phe}$, where the entire loop is hydrolyzed with low but comparable rates, the mechanism proposed by Brown et al. (1983) cannot explain the existing experimental data, because none of the ribose at the 2′-OH groups of the T-loop components lies less than 7 Å from the lead ion (Brown et al., 1985). According to Sundaralingam et al. (1984), the activation of the more remote 2′-OH groups could be effected by the hydroxyl groups bound to the lead ion via intervening water molecule(s). This could possibly be the case for the cleavages in the T-loop region generated most likely by the same lead ion that is responsible for the major cut in the D-loop. The discrepancies that exist about the cleavage at phosphate 60 (Sundaralingam et al., 1984) are more likely caused by different experimental conditions used by the various authors. From our studies, it is clear that not one but a whole spectrum of strand scissions occurs in the T-loop at Pb^{2+} concentrations above 10 mM (i.e., at a Pb^{2+}/tRNA ratio of greater than 10^3). In only one X-ray study (Rubin & Sundaralingam, 1983; Sundaralingam et al., 1984), tRNA crystals were soaked with a saturated $PbCl_2$ solution. This would assure a sufficient excess of Pb^{2+} ions for these cleavages to occur.

Long-Distance Conformational Transition in Yeast $tRNA^{Phe}$. The reason the differential reactivity of phosphate 18 in $tRNA^{Phe}$ and $tRNA^{Phe}$-Y is seen in only a certain range of high Pb^{2+} concentrations is unclear. One might speculate, however, that a relatively high concentration of lead ions (3–10 mM) is required to stabilize or induce a specific conformation of the anticodon loop in $tRNA^{Phe}$-Y that has the potential to trigger conformational changes elsewhere in the molecule affecting interactions in the region of the T- and D-loops. According to X-ray crystallographic studies, some structural elements of the Y base serve as strong ligands for a Pb^{2+} binding site (Brown et al., 1983, 1985; Rubin & Sundarlingam, 1983). Thus, the question arises of whether in the absence of the Y base an alternative set of ligands can be formed. The cleavages observed in the anticodon loop of $tRNA^{Phe}$-Y suggest that this may be the case, at least at Pb^{2+} concentrations of 3 mM and higher. The greater extent of anticodon loop hydrolysis and different cleavage patterns comprising phosphates 36–38 might be due to a greater flexibility and more favorable distance of the "relaxed" 3′-part of the anticodon loop of $tRNA^{Phe}$-Y to the bound lead ion. However, since Pb^{2+} ion may assume a variety of coordination numbers and geometries (Brown et al., 1985) and since the precise geometry of the anticodon loop in $tRNA^{Phe}$-Y is unknown, this hypothesis remains to be formally addressed. Nevertheless, whatever the nature of the conformational differences in the anticodon region of these two tRNAs at 3 mM Pb^{2+} concentration and above, it is clear that a structural perturbation is propagated in the $tRNA^{Phe}$-Y molecule and influences the rate of the T-loop-dependent D-loop hydrolysis. The fact that D-loop cleavage is more rapid in $tRNA^{Phe}$-Y could be due to one or a combination of the following effects: (i) a higher binding affinity or better positioning of lead aquo cation onto a perturbed geometry of the U59–C60 sequence in the T-loop of $tRNA^{Phe}$-Y; (ii) a conformational rearrangement within the D-loop of $tRNA^{Phe}$-Y that brings the 2′-OH group of ribose 17 in a closer proximity to the lead ion bound hydroxyl group. The temperature dependence of the cut at phosphate 18 observed in this study, which clearly shows the stabilization of the "better cleavable" conformation of $tRNA^{Phe}$-Y at lower temperatures, cannot eliminate any of the above possibilities. Such conformational changes in the T–D region of $tRNA^{Phe}$-Y have been observed by using chloroacetylaldehyde modification (Krzyzosiak & Ciesiolka, 1983) and complementary oligonucleotide binding (Cameron & Uhlenbeck, 1973). It was also shown that anticodon–anticodon interactions in yeast $tRNA^{Asp}$ induce a conformational change in the T–D-loop region (Moras et al., 1985, 1986). It was proposed that the mechanism is based on a fine tuning of both anticodons with a transfer of flexibility to the end of the arm in the D- and T-loop region. This most likely mechanism of transfer would be propagated through base stacking (Giegé et al., 1986b). According to recent X-ray crystallographic studies (Brown et al., 1985), the distance between the Pb^{2+} (site 1) and Mg^{2+} ions (site 3) in yeast $tRNA^{Phe}$ is only 2.1 Å. This means that both ions cannot be present in the same tRNA molecule at the same time. In addition, Mg^{2+} ions have been shown to retard the rate of Pb^{2+}-induced cleavage of yeast $tRNA^{Phe}$ in solution (Labuda et al., 1985). The above observation suggests a third possibility that could account for the observed difference in the rate of D-loop cleavage in the $tRNA^{Phe}$/$tRNA^{Phe}$-Y system. A magnesium ion release from its strong binding site in the D-loop upon Pb^{2+} ion binding in the anticodon might facilitate Pb^{2+} binding to the T-loop of $tRNA^{Phe}$-Y and the subsequent cleavage reaction. This brings to mind the general mechanism proposed for the transmission of signals in nucleic acids (Labuda et al., 1984). According to this model mechanism, any external signal that can affect Mg^{2+} binding at one site of the molecule may be transmitted along the polynucleotide chain by inducing changes in the distribution of bound Mg^{2+} ions. Both the anticodon loop (from which the Y base has been excised) and the interacting D- and T-loop region (where the structure becomes altered) have strong Mg^{2+} binding sites. Such a Mg^{2+} ion mediated mechanism was also considered earlier to explain the results of chemical modification studies (Krzyzosiak & Ciesiolka, 1983). In the present case, however, the combined action of Mg^{2+} and Pb^{2+} ions seems to trigger a conformational change that is detected by the activity of the latter. However, more experimental work is required to identify and characterize

other structural consequences of Y base removal from yeast $tRNA^{Phe}$. Studies are now in progress using a battery of chemical and enzymatic probes, including other metal ions. A better understanding of this simple model system will shed some light on the mechanism of conformational transitions taking place in more complex natural systems.

Summarizing, it appears that lead is useful for probing the folded structure in tRNAs. This has been recently applied to study the conformation of several yeast $tRNA^{Phe}$ mutants (Sampson et al., 1987). The application of the lead reaction as a probe, which was intuitively predicted 12 years ago (Werner et al., 1976) and is at present restricted to yeast $tRNA^{Phe}$ and some structurally related species, can also be extended to other folded RNA structures for which no X-ray data are yet available (e.g., ribosomal RNAs, messenger RNAs, snRNAs).

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