Site-Selective Hydrolysis of tRNA by Lanthanide Metal Complexes

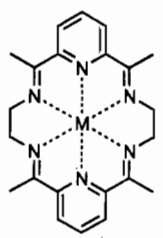
Nobuhiro Hayashi,[†] Naoya Takeda,[‡] Tetsuro Shiiba,[§] Morio Yashiro,[‡] Kimitsuna Watanabe,*[,‡] and Makoto Komiyama*[,‡]

Department of Life Chemistry, Tokyo Institute of Technology, 4259 Nagatsuta, Yokohama, Kanagawa 227, Japan, Department of Industrial Chemistry, Faculty of Engineering, University of Tokyo, Hongo, Tokyo 113, Japan, and Institute of Materials Science, University of Tsukuba, Tsukuba, Ibaraki 305, Japan

Received February 19, 1993

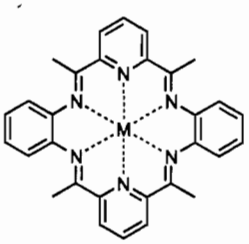
Site-selective scission of tRNAs has been attracting much interest, mainly because of potential applications to structural probing of tRNAs and their molecular manipulation. Elegant metal complexes, which recognize the specific tertiary structures in tRNA and cleave the ribose residue(s) there, were reported.[1-3] However, examples of site-selective hydrolysis of phosphodiester linkages in tRNA are less abundant.[4] These types of scissions are advantageous, at least for some purposes, since the RNA fragments thus obtained can be enzymatically ligated when necessary.

Recently lanthanide metal ions[5] and complexes[6] showed remarkable catalyses for the hydrolyses of RNAs. The findings have indicated that appropriately designed lanthanide complexes are highly potent for the site-selective hydrolytic scission of tRNAs. Here we report that lanthanide metal complexes of hexaimine macrocyclic ligands L_1 and L_2 hydrolyze $tRNA^{Phe}$ much more site-selectively than lanthanide metal ions without the ligands. Significant dependence of the site selectivity on the ligand structure is described.



M - L_1

M = La(III), Ce(III), Eu(III)



M - L_2

M = La(III), Ce(III)

$tRNA^{Phe}$ from brewers yeast (Boehringer Mannheim) was ^{32}P-labeled either at the 3′-end[7] or 5′-end and was purified by gel electrophoresis. The tRNA without Mg^{2+} ion was prepared by the elution of the tRNA from the gel using a buffer without Mg^{2+}.[8] Lanthanum(III), cerium(III), and europium(III) complexes with L_1 and L_2 (nitrate salts) were prepared by the reported procedures.[9,10] The hydrolysis of the tRNA was carried out at 30 °C and pH 7.5 (HEPES buffer) under the conditions that $[tRNA]_0 = 0.1$ mM (residual concentration), [lanthanide complex or ion]$_0$ = 0.5 mM, and $[MgCl_2]_0 = 1$ mM (except for the case of the tRNA without Mg^{2+}).[11] The cleavage products were analyzed by electrophoresis on a 20% polyacrylamide gel (7 M urea) and were quantified by using a Gilford Response II scanning microdensitometer. All the hydrolysis fragments were unambiguously characterized by use of both the 5′-end labeled and the 3′-end labeled tRNAs.[12] The sites for the primary hydrolysis were determined in the early stage of the reaction (usually within 20 min) and were differentiated from the secondary cleavage sites by following the time course of the hydrolysis.

All the L_1 and L_2 complexes promptly and selectively hydrolyzed the $tRNA^{Phe}$ (Figure 1). The cleavage profiles are schematically depicted in Figure 2.[13] Only four phosphodiester linkages (16, 20, and 21 in the D-loop and 36 in the anticodon loop) were cleaved by the Ce–L_1 complex (Figure 1a). The L_2 complex showed still larger selectivity: the hydrolysis site was the phosphate 16 alone, except for the marginal cleavages at the phosphates 20 and 36 (b).[11] The cleavage profiles for the La and Eu complexes of L_1 and L_2 were virtually identical with those for the corresponding Ce complexes.[14,15]

Significantly, the site selectivities by the L_1 and L_2 complexes are much higher than those of the metal ions without the ligands: Ce(III) and Eu(III) ions cleaved the phosphodiester linkages 16, 17, 18, 20, and 21 in the D-loop and 34 and 36 in the anticodon loop (Figure 1c and Figure 2b).[16] Thus the L_1 ligand in the lanthanide complexes suppresses the cleavages at the phosphates 17, 18, and 34, and the ligand L_2 additionally inhibits the cleavage at the phosphate 21. The cleavages by the complexes (as well as by the metal ions) are totally hydrolytic, since the resultant fragments comigrate in gel electrophoresis exactly with those obtained by alkaline hydrolysis and by RNase T_1 digestion. The cleavages of diribonucleotides ApA and UpU yielded only the hydrolytic products according to HPLC analysis.[5,17]

In contrast with the highly site-selective scissions by the L_1 and L_2 complexes, 1:1 complexes of Ce(III) and Eu(III) ions with iminodiacetate exhibited virtually the same selectivities as the metal ions. Apparently the macrocyclic ligands L_1 and L_2 dictate the scission sites to the complexes.

† Tokyo Institute of Technology.
‡ University of Tokyo.
§ University of Tsukuba.

(1) Pyle, A. M.; Barton, J. K. *Prog. Inorg. Chem.* **1990**, *38*, 413.
(2) Murakawa, G. J.; Chen, C. B.; Kuwabara, M. D.; Nierlich, D. P.; Sigman, D. S. *Nucleic Acids Res.* **1989**, *17*, 5361.
(3) (a) Chow, C. S.; Behlen, L. S.; Uhlenbeck, O. C.; Barton, J. K. *Biochemistry* **1992**, *31*, 972. (b) Chow, C. S.; Barton, J. K. *Biochemistry* **1992**, *31*, 5423. (c) Sitlani, A.; Long, E. C.; Pyle, A. M.; Barton, J. K. *J. Am. Chem. Soc.* **1992**, *117*, 2303.
(4) Ciesiolka, J.; Marciniec, T.; Krzyzosiak, W. J. *Eur. J. Biochem.* **1989**, *182*, 445 and references therein.
(5) Komiyama, M.; Matsumura, K.; Matsumoto, Y. *J. Chem. Soc., Chem. Commun.* **1992**, 640.
(6) Morrow, J. R.; Buttrey, L. A.; Shelton, V. M.; Berback, K. A. *J. Am. Chem. Soc.* **1992**, *114*, 1903.
(7) England, T. E.; Uhlenbeck, O. C. *Nature* **1978**, *275*, 560.
(8) A TBE buffer (90 mM Tris-HCl (pH 7.5), 90 mM boric acid, and 2 mM EDTA) was used as the elution buffer for the preparation of the tRNA without Mg^{2+}. The tertiary structure in the tRNA was greatly diminished as evidenced by circular dichroism spectroscopy: Hayashi, N.; Watanabe, K. Unpublished results.
(9) Arif, A. M.; Backer-Dirks, J. D. J.; Gray, C. J.; Hart, F. A.; Hursthouse, M. B. *J. Chem. Soc., Dalton Trans.* **1987**, 1665.
(10) Radecha-Parayzek, W. *Inorg. Chim. Acta* **1981**, *54*, L251; **1985**, *109*, L21.
(11) The solubility of Ln–L_2 is poor, so it is not completely dissolved at the conditions employed. The observed cleavage pattern using this compound is different from those using other catalysts or that of the control experiment, indicating that this compound can cleave tRNA with its characteristic selectivity.
(12) All fragments were completely assigned by the modified method of Donis-Keller using ribonucleases T1, PhyM, U2, and CL3 (Donis-Keller, H.; Maxam, A. H.; Gilbert, W. *Nucleic Acids Res.* **1977**, *48*, 2013).
(13) Throughout this paper, the phosphodiester linkage is numbered by the ribonucleoside residue located in the 3′-side.
(14) The Eu–L_1 complex preferentially cleaved the phosphate 36 to 34 (as the Ce–L_1 complex did), whereas the order was reversed for the La–L_1 complex.
(15) The Eu–L_1 complex is sufficiently stable under the reaction conditions employed.[6] The metal ions of reduced concentrations gave different cleavage patterns from those by the complexes, ruling out the possibility of the cleavage by the metal ions released from the complexes.
(16) A similar result was reported in ref 4, although the reaction conditions were different and thus the cleavage profile was not strictly identical with the present one.
(17) Takeda, N.; Yashiro, M.; Komiyama, M. Unpublished results.

0020-1669/93/1332-5899$04.00/0

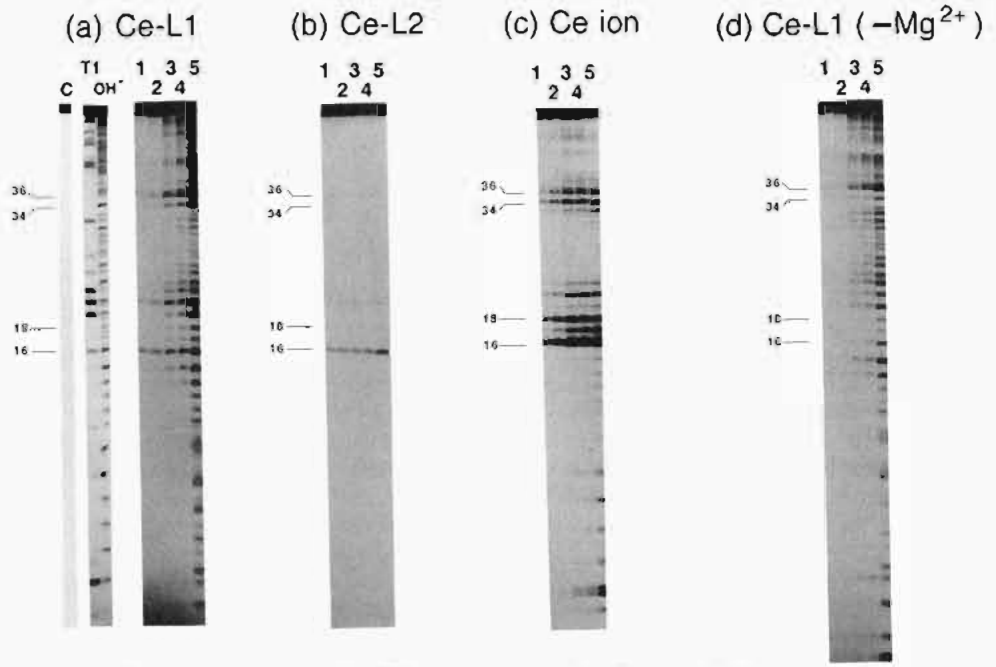


Figure 1. Autoradiograms of the 5′-end labeled yeast $tRNA^{Phe}$ cleaved by (a) the Ce–L_1 complex, (b) the Ce–L_2 complex, and (c) Ce(III) ion (lanes 1–5, incubation with the complex or the metal ion for 5 min, 20 min, 80 min, 3 h, and 21 h, respectively). (d) shows the pattern for the Ce–L_1 complex-catalyzed hydrolysis of the tRNA without Mg^{2+}, which does not have the specific tertiary structure (see text for detail). The patterns for the control (C) (80 min), RNase T_1 digestion (T_1: G-specific), and alkaline digestion (OH^-) are presented in (a).

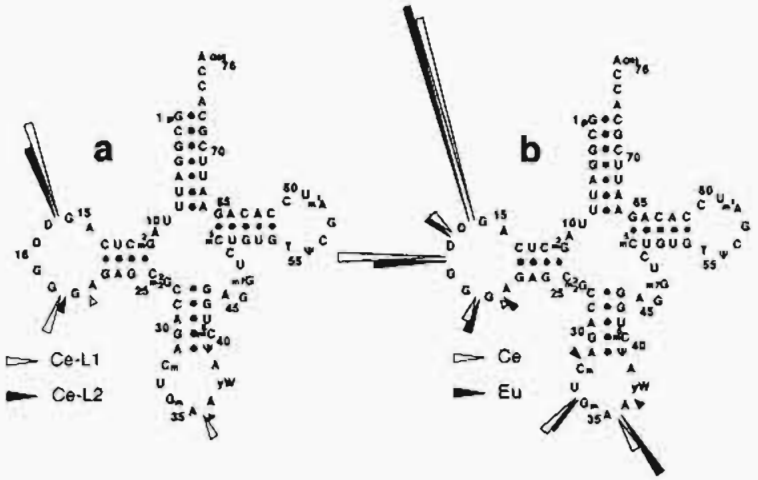


Figure 2. Summary of the hydrolysis sites of $tRNA^{Phe}$ for the cleavage by the Ce complexes (a) and by the metal ions (b). The lengths of the arrows correspond to the extent of hydrolysis after incubation for 5 min; the cleavage profiles for the Eu and La complexes are similar to those for the Ce complexes.

The specific tertiary structure of the tRNA is definitely required for both the efficiency and the site selectivity of the catalyses by the complexes. The $tRNA^{Phe}$ without Mg^{2+} ion, which cannot satisfactorily maintain the tertiary structure any more,[18] was hydrolyzed by the Ce–L_1 complex much less efficiently than the tRNA with Mg^{2+} (compare part d in Figure 1 with part a). The cleavage, which was detectable only after a long period of time, was almost nonselective (lanes 3–5 in part d). On the other hand, the Ce–L_2 complex did not hydrolyze the tRNA without Mg^{2+} to a measurable extent even for 21 h (data not presented): its catalysis was effective only for the hydrolysis of the tRNA having the specific tertiary structure. These results are consistent with those for the prolonged hydrolysis of the tRNA with Mg^{2+} by the complexes (Figure 1a,b). The site selectivity of the Ce–L_1 complex gradually disappeared due to the secondary hydrolysis (part a, lanes 3–5), whereas the secondary hydrolysis was not perceived at all for the Ce–L_2 complex (part b, lanes 3–5).

The present site-selective cleavages proceed via the binding of the complexes to the tRNA near the corresponding cleavage sites. The L_1 and L_2 complexes are more site selective than the metal ions in the cleavage, since the bulky and hydrophobic ligands render the bindings more selective. No promotion of the site selectivity by iminodiacetate, a less bulky and polar ligand, supports the argument.

In conclusion, lanthanide metal complexes of L_1 and L_2 hydrolyze $tRNA^{Phe}$ with high site selectivities. The specificity is strongly dependent on the ligand structure. Molecular design of the complexes showing still larger site selectivities is currently under way in our laboratory.

Acknowledgment. This work was partially supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture of Japan and by the Nissan Science Foundation.

(18) Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1984; Chapter 15.6.