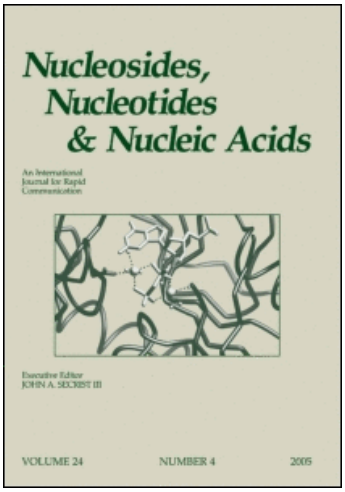

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Effect of Cu^{2} on Complex Formation Between a Deoxyribozyme and Its Substrates

Naoki Sugimoto[a]; Noboru Wakizaka[a]
[a] Department of Chemistry, Faculty of Science, Konan University, Kobe, Japan

To cite this Article Sugimoto, Naoki and Wakizaka, Noboru(1998) 'Effect of Cu^{2} on Complex Formation Between a Deoxyribozyme and Its Substrates', Nucleosides, Nucleotides and Nucleic Acids, 17: 1, 565 — 574
To link to this Article: DOI: 10.1080/07328319808005199
URL: http://dx.doi.org/10.1080/07328319808005199

NUCLEOSIDES & NUCLEOTIDES, 17(1-3), 565-574 (1998)

EFFECT OF Cu^{2+} ON COMPLEX FORMATION BETWEEN A DEOXYRIBOZYME AND ITS SUBSTRATES†

Naoki Sugimoto* and Noboru Wakizaka

Department of Chemistry, Faculty of Science, Konan University, 8-9-1 Okamoto, Higashinada-ku, Kobe 658, Japan

ABSTRACT: Effect of metal ions on secondary-structure formation of a deoxyribozyme-two substrates complex has been investigated by using surface plasmon resonance and secondary-structure predicting calculation. The result showed that Cu^{2+} not only acts on the ligation reaction but also plays the role of a promoter which makes an active conformation of the deoxyribozyme-substrate complex.

INTRODUCTION

Ribozymes derived from group I and II introns,[1,2] ribonuclease P,[3,4] satellite RNAs of tobacco ringspot virus,[5] and human hepatitis delta virus,[6] and so on are well known to cleave a phosphodiester backbone of RNAs with nucleophile attacking. Artificial ribozymes such as a leadzyme (lead-dependent ribozyme)[7] has been also drawing an attention, because the leadzyme has small size and high sequence selectivity,[8] and so it is hoped to have wide applications as well as hammerhead ribozymes.[9] Some folded single-strand DNA are also promising to exhibit catalytic activities similar to those of ribozymes and protein enzymes.[10,11] Very recently, Joyce *et al.* reported a DNA enzyme (deoxyribozyme) which has ability to recognize an RNA substrate through base pairing and cleave it.[12]

On the other hand, little is known about ribozymes with ligase activity. A few years ago, one deoxyribozyme with ligase activity was identified by using *in vitro* selection strategy.[13] Figure 1 shows the deoxyribozyme together with its substrates, S1

---

†This paper is dedicated to the late Professor Tsujiaki Hata.

*To whom correspondence should be addressed. Phone: +81-78-435-2497
Fax: +81-78-435-2539 E-mail: sugimoto@konan-u.ac.jp

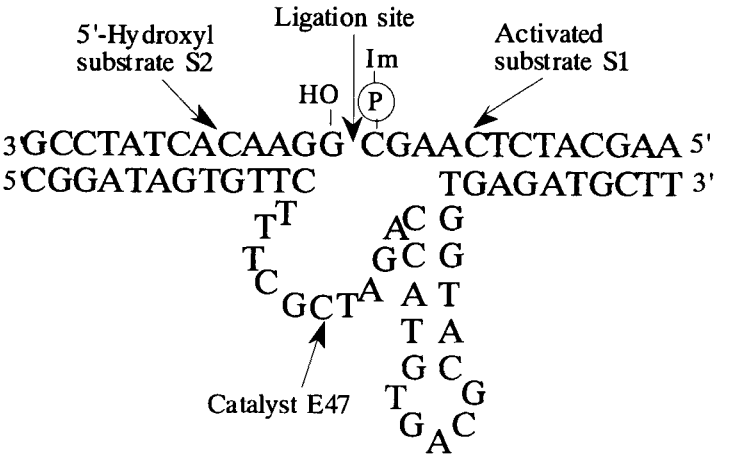

FIG. 1. The secondary structure of a deoxyribozyme, E47, with DNA ligase activity and its substrates, S1 and S2.

and S2. This deoxyribozyme has the following characteristics:[13,14] (1) First-substrate S1, of the deoxyribozyme bears a phosphorimidazolide in its 3' terminal as a ligation reagent. (2) The ligation activity of the deoxyribozyme depends on Mg^{2+} and Cu^{2+}. In ribozyme reactions, divalent cations are required for chemistry and structural stabilization of folded RNA.[15] It is showed that Mg^{2+} plays an important role in a ribozyme cleavage reaction as a catalytic activation.[16,17] On the other hand, Cu^{2+} may have a high affinity for bases of nucleic acids and could form base-base complex more easily than other divalent cations.[18] Therefore, Cu^{2+} may play a key role in the recognition step of the deoxyribozyme with its substrates as well as the ligation step.

In this study, we have investigate the complex formation of the deoxyribozyme (E47) with its substrates (S1 and S2) by using surface plasmon resonance (SPR) and secondary-structure predicting calculation in order to determine quantitatively effect of Cu^{2+} on the recognition step of the deoxyribozyme reaction.

EXPERIMENTAL

Materials

The deoxyribozyme (E47) and its second substrate (S2) were synthesized chemically on a solid support using phosphoramidite procedures and purified with HPLC after deblocking operations.[19] The first substrate (S1) which bears a 5'-biotin group was also synthesized chemically on a solid support using DNA and biotin phosphoramidite

procedures. These oligonucleotides were further purified and desalted with a C-18 Sep-Pak column. Final purity of the oligonucleotides was confirmed by HPLC to be greater than 98%.

Single strand concentrations of the oligonucleotides were determined by measuring the absorbance at 260 nm at high temperature as described previously.[20] Extinction coefficients of single strands were calculated from mononucleotide and dinucleotide data by using a nearest-neighbor approximation.[21]

Streptavidin, N-hydroxyscinimide (NHS), and N'-(3-dimethylaminopropyl)-carbodiimide hydrocloride (EDC) were purchased from Pharmacia Biosensor AB, Sweden (Amino Coupling Kit).

SPR measurements

Streptavidin was covalently coupled to the surface of carboxymethylated dextran coated sensor chip using Amine Coupling Kit. Carboxymethylated dextran was activated with 20 μL NHS and EDC, followed by 30 μL streptavidin in 10 mM acetate (pH 5.0) at 200 μg/mL. Excess reaction groups on the sensor chip were deactivated with ethanolamine hydrochloride.

After then, 5'-biotinylated substrate 1 (S1) probe was immobilized on the surface of carboxymethylated dextran coated sensor chip by using a streptavidin-biotin reaction (host-guest reaction). In kinetic measurements, each sample (80 μL) of 2.0, 1.5, 1.0, 0.5, and 0.25 μM E47 and S2 was injected over immobilized S1 and E47-S1 complex, respectively. Each reaction step is shown in Fig. 2. The running buffers contain 30 mM HEPES (pH 7.4) and 50 mM Mg^{2+} at which no ligation occurred,[13] or 50 mM Mg^{2+}, 10 μM Cu^{2+} at which the ligation occurred.[13] The SPR experiments were carried out at 20, 25, 30, and 37 °C.

SPR kinetics

The association and dissociation rate constants (k_a and k_d) in the steps of S1+E47 and S1-E47 complex+S2 can be determined with the following equations.[22]

$$dR/dt = k_a [A] R_{max} - (k_a [A] + k_d) R \quad (1)$$

$$k_{obs} = k_a [A] + k_d \quad (2)$$

where [A] is the molecular concentration of injected sample, R_{max} is the maximum binding capacity of the immobilized probe, and R is the response for sample binding to immobilized probe. The rate constants, k_a and k_d, are obtained with the slope and intercept of the plots in eq.2, respectively. When the intercept is too small to give a correct value of k_d, k_d can also be determined as an average value with dissociation phases at kinetic curves by the following equation:

$$\ln(R_n'-R_n) = - k_d t_n + \ln\{a (1 - \exp[- k_d (t_n' - t_n)]) \quad (3)$$

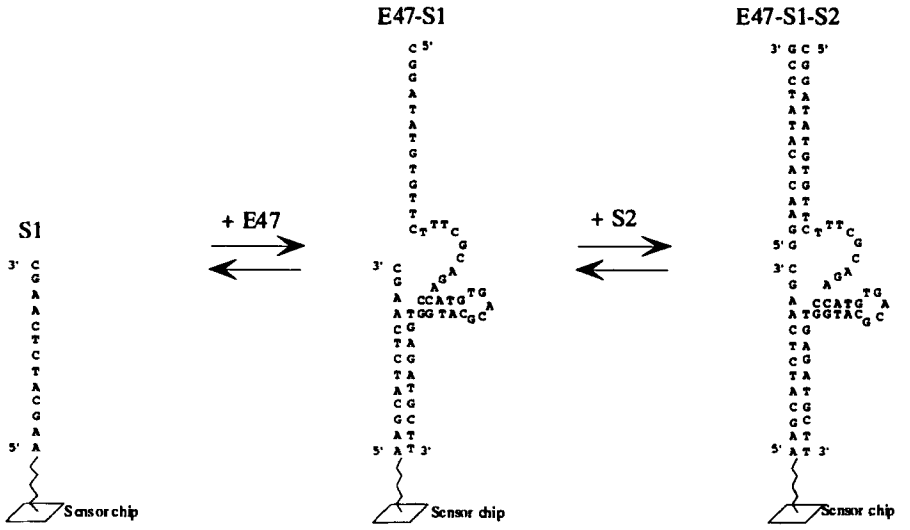

FIG. 2. The two recognition steps of E47, S1, and S2 in SPR measurement.

where R_n' and R_n are the relative values of response in RU (response unit) at time t_n' and t_n, respectively. The obtained values of k_a and k_d at various temperatures give activation energies, $E_{a,a}$ and $E_{a,d}$, of the association and dissociation, respectively.

Secondary structure prediction of DNA

Stable secondary structures of S1-E47-S2 complex were calculated by using nearest-neighbor parameters for DNAs as described previously.[23-25] Our calculation method is based on the program of Zucker and Stiegler[26] which was used to predict RNA secondary structures. We used our new thermodynamic parameters of DNA.[19,27] Energetically stable secondary structures of DNA were searched from the matrix of the energy values obtained by thermodynamic calculation for the possible base pairing. The program for calculation runs in MS-DOS environments. Structures of pseudoknots were not included in the program.

RESULTS AND DISCUSSION

SPR sensorgrams and rate constants

Figure 3 shows SPR sensorgrams of binding step of E47 to immobilized S1 in the presence and absence of Cu^{2+}. The sensorgram consists of two phases; (1) an

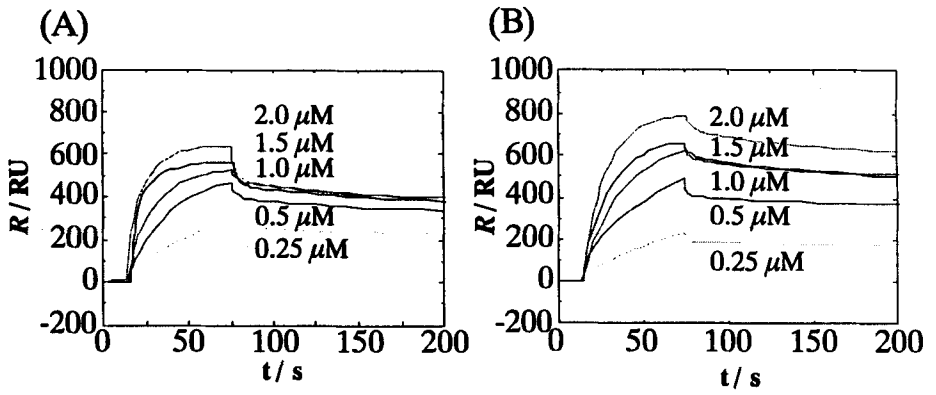

FIG. 3. Kinetic curves (sensorgrams) of E47 binding to immobilized S1 in (A) 50 mM Mg^{2+}, and (B) 50 mM Mg^{2+} and 10 μM Cu^{2+} at 20 °C. E47 concentrations are given at each curve.

association phase in which injected sample was supplied with immobilized target on the sensor chip and (2) a dissociation phase in which the bounded sample was washed by running buffer. The figure shows that amount of E47 bound to immobilized S1 increased by addition of Cu^{2+}, which was also found in the step of S2 binding to S1-E47 complex (data not shown).

Figures 4 and 5 show dR/dt vs. R and k_{obs} vs. [A] plots, respectively, for the association phase in the step of E47 binding to immobilized S1. However, since in Fig. 5 dissociation rate constants k_d were too small to calculate in eq. 2, the k_d values were obtained in the dissociation phase by using eq. 3.

Kinetic behaviors in the binding steps of E47 to S1 and S2 to S1-E47 complex.

Tables 1 and 2 list the association and dissociation rate constants in the binding steps of E47 to immobilized S1 and S2 to S1-E47 complex, respectively, together with the activation energies. As shown in Tables 1 and 2, k_a and k_d for the binding step of E47 to S1 in the presence of Cu^{2+} are not so different from those in the absence of Cu^{2+}. The difference of the k_a values for the binding process of S2 to S1-E47 complex is small in the presence and absence of Cu^{2+}.

On the other hand, surprisingly, the k_d values of S2 and S1-E47 complex formation decreased with adding Cu^{2+}; for example, at 30 °C only 10 μM Cu^{2+} addition led to decreased k_d from 101 s^{-1} to 14.2 s^{-1}. Addition of Cu^{2+} also changed dramatically temperature dependency of k_d for the S2 and S1-E47 complex formation. The activation

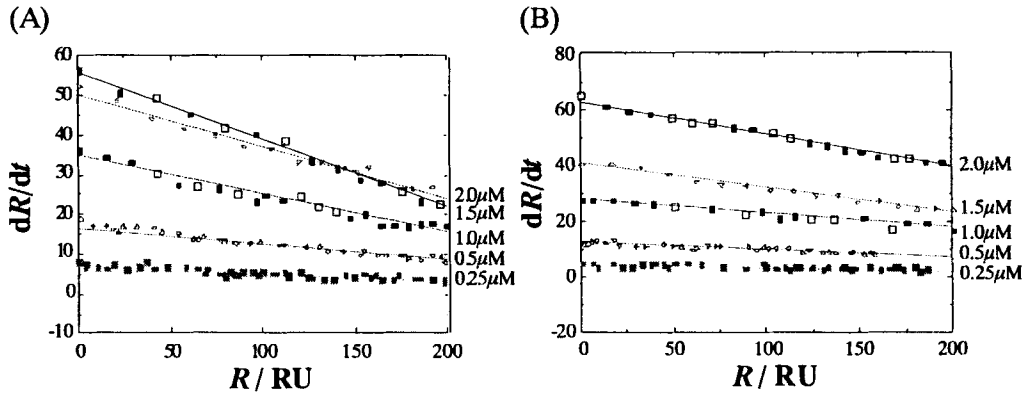

FIG. 4. dR/dt vs. R in (A) the absence and (B) presence of Cu^{2+} for the association phase of the curves in Fig. 3. E47 concentrations are given at each line.

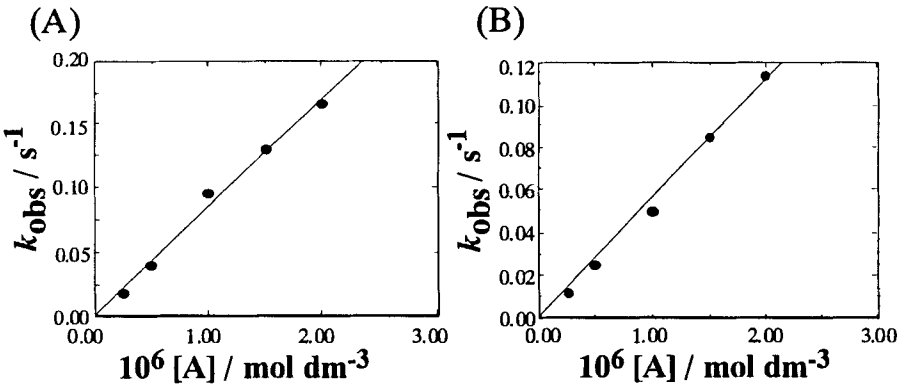

FIG. 5. k_{obs} vs. [A] in (A) the absence and (B) presence of Cu^{2+}.

energy in this step with Cu^{2+} was 17.3 kcal mol^{-1} smaller than that without Cu^{2+}. As shown in Fig. 6 in which the energy diagram of the binding steps with/without Cu^{2+} is illustrated, these results suggest that Cu^{2+} affect the stability of S1-E47-S2 complex larger than the stability of each transition state, that is, Cu^{2+} may play a role of destabilizer of the complex. In this point, this metal ion may increase a potential energy of enzyme-substrate complex and then promotes next ligation, although general enzymes only decrease activation energy of chemical transition state.

Stable secondary structure of S1-E47-S2 complex.

Figure 7 shows the most stable secondary structure of S1-E47-S2 complex calculated in the absence of Cu^{2+} together with that postulated in the presence of Cu^{2+}.[13]

TABLE 1. Kinetic parameters in the binding step of E47 to S1.[a]

	50 mM Mg^{2+} & 10μM Cu^{2+}		50 mM Mg^{2+}	
T (°C)	$10^{-4}k_a$ / ($M^{-1}s^{-1}$)	$10^{2}k_d$ / (s^{-1})	$10^{-4}k_a$ / ($M^{-1}s^{-1}$)	$10^{2}k_d$ / (s^{-1})
20	4.5	1.04	8.0	1.01
25	8.1	1.22	10.2	1.09
30	13.5	1.17	17.2	1.35
37	21.8	2.16		
E_a / kcal mol^{-1}	16.9	8.0	13.5	5.1

[a] All experiments were done in 30 mM HEPES (pH 7.4), 50 mM Mg^{2+} or 50 mM / 10 μM Cu^{2+}. The estimated errors of k_a, k_d, and E_a are ± 4 %, ± 4 %, and ± 8 %, respectively.

TABLE 2. Kinetic parameters in the binding step of S2 to S1-E47 complex.[a]

	50 mM Mg^{2+} & 10 μM Cu^{2+}		50 mM Mg^{2+}	
T (°C)	$10^{-4}k_a$ / ($M^{-1}s^{-1}$)	$10^{2}k_d$ / (s^{-1})	$10^{-4}k_a$ / ($M^{-1}s^{-1}$)	$10^{2}k_d$ / (s^{-1})
20	25.8	2.48	49.0	5.68
25	31.7		79.6	24.4
30	91.8	14.2	104	101
37	152	60.2		
E_a / kcal mol^{-1}	19.2	33.6	13.4	50.9

[a] All experiments were done in 30 mM HEPES (pH 7.4), 50 mM Mg^{2+} or 50 mM / 10 μM Cu^{2+}. The estimated errors of k_a, k_d, and E_a are ± 4 %, ± 4 %, and ± 8 %, respectively.

The postulated secondary structure consists of three helices, one hairpin loop, and a multibranched loop in the center of E47. The ligation site is between G and C in the multibranched loop. This deoxyribozyme depend on Mg^{2+} and Cu^{2+} for ligase activity. It is considered that the deoxyribozyme forms the most stable structure (left in Fig. 7) in the presence of only Mg^{2+} or Na^{+}, and each cation contributes to the complex formation. However, in this structure the ligation site in which imidazole group in S1 react with hydroxyl group in S2, is in stem region. So, Cu^{2+} may play the role of a breaker of double-stranded structure, because Cu^{2+} has a high affinity for base complextion and then may break Watson-Crick base pairs more easily than other divalent cations. In the binding step of S2 to S1-E47 complex, S1-E47-S2 complex may be induced to a

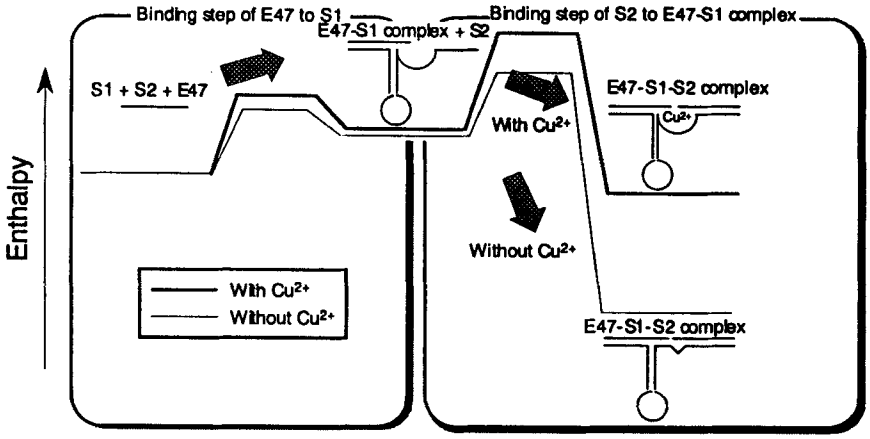

FIG. 6. The energy diagram of the binding steps of S1 and E47, and S2 and S1-E47 complex. The upper and lower lines indicate enthalpy changes with and without Cu^{2+}, respectively.

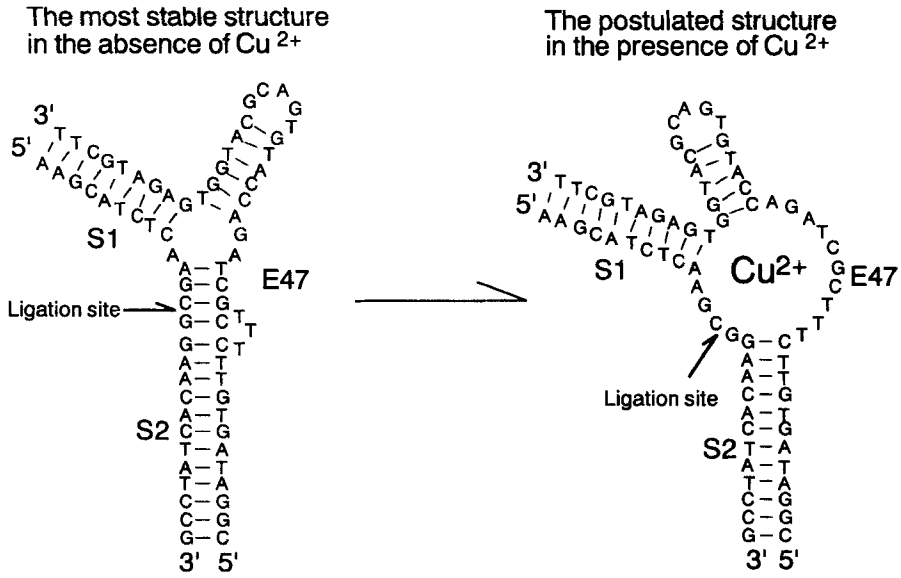

FIG. 7. S1-E47-S2 secondary structures calculated in the absence of Cu^{2+} (left) and postulated in the presence of Cu^{2+} (right).

conformational change by Cu^{2+}, and then the cation makes an internal loop near the ligation site in the complex.

Previous study of this deoxyribozyme showed pH-rate profile of the reaction at a single-turnover condition and the bell-shaped profile was displayed with Cu^{2+}.[13] The result indicated that Cu^{2+} may play the role of catalysis as a proton-transfer medium and decrease activation energy in the ligation. The combinated effect of metal ions was studied for ribozymes such as *Tetrahymena* ribozyme, RNase P, and leadzyme. The result of the leadzyme indicated that Nd^{3+} and Pb^{2+} influence directly the stability of a leadzyme-subatrate complex and also act as general acid and base.[8] In *Tetrahymena* ribozyme and RNase P, the two classes of the metal ion binding sites for making specific structure and an active site were identified.[28,29] The result of the structure prediction in this study suggested that Cu^{2+} not only acts on the ligation reaction but also plays the role of a promoter which makes an active conformation of the deoxyribozyme-substrate complex. This suggestion was also supported by the kinetic and thermodynamic results in the recognition steps described above.

ACKNOWLEDGMENT

This work was supported in part by grants from the Ministry of Education, Science Sports, and Culture, Japan to NS. The authors thank T. Ohmichi for helpful comments.

REFERENCES

1 Kruger, K.; Grabowski, P. J.; Zaug, A. J.; Sands, J.; Gottschling, D. E.; Cech, T. R. *Cell*, **1982** *31*, 147-157.

2 Zuag, A. J.; Cech, T. R. *Science*, **1986** *231*, 470-475.

3 Guerrier-Takada, C.; Altoman, S. *Science*, **1984** *223*, 285-286.

4 Guerrier-Takada, C.; Haydock, K.; Allen, L.; Altoman, S. *Biochemistry*, **1986** *25*, 1509-1515.

5 Prody, G. A.; Bakos, J. T.; Buzayan, J. M.; Schneider, I. R.; Bruening, G. *Science*, **1986** *241*, 1577-1580.

6 Wu, H.; Lai, M. M. C. *Science*, **1989** *243*, 652-654.

7 Sugimoto, N.; Ohmichi, T. *FEBS Letters*, **1996** *393*, 97-100.

8 Ohmichi, T.; Sugimoto, N. *Biochemistry*, **1997** *36*, 3514-3521.

9 Sullenger, B. A.; Cech, T. R. *Science*, **1993** *262*, 1566-1569.

10 Luebke, K. J.; Dervan, P. B. *J. Am. Chem. Soc.*, **1989** *111*, 8733-8735.

11 Li, T.; Nicolaou, K. C. , *Nature*, **1994** *369*, 218-224.

12 Santoro, S. W.; Joyce, G. F. *Proc. Natl. Acad. Sci. U.S.A.*, **1997** *94*, 4262-4266.

13 Cuenoud, B.; Szostak, J. W. *Nature*, **1995** *375*, 611-614.

14 Chu, B. C. F.; Kramer, F. R.; Orgel, L. E. *Nucleic Acids Research*, **1986** *14*, 5591-5603.

15 Sugimoto, N.; Kierzek, R.; Turner, D. H. *Biochemistry*, **1988** *27*, 6384-6392.

16 Pyle, A. M. *Science*, **1993** *261*, 709-714.

17 Bratty, J.; Chartrand, P.; Ferbeyre, G.; Cedergren, R. *Biochemica et Biophysica Acta*, **1993** *1216*, 345-359.

18 Jia, X.; Marzilli, L. G. *Biopolymers*, **1991** *31*, 23-44.

19 Sugimoto, N.; Nakano, S.; Yoneyama, M.; Honda, K. *Nucleic Acids Res.*, **1996** *22*, 4501-4505.

20 Sugimoto, N.; Kierzek, R.; Freier, S. M.; Turner, D. H. *Biochemistry*, **1986** *25*, 5755-5759.

21 Richards, E. G. *Handbooks of Biochemistry and Molecular Biology*; *Nucleic Acids* (Fasman, G. D., Ed) 3rd. ed., **1975** *1*, 597, CRC Press.

22 O'Shannessy, D. J.; Brigham-Burke, M.; Soneson, K. K.; Hensley, P.; Brooks, I. *Analytical Biochemistry*, **1993** *212*, 457-468.

23 Sugimoto, N.; Kawai, H.; Sasaki, M. *Prog. Poly. Phys. Jpn.*, **1993** *36*, 725-728.

24 Sugimoto, N.; Sasaki, M. *Chem.Lett.*, **1991**, 345-348.

25 Sugimoto, N.; Sasaki, M.; Tanaka, A. *Supramolec. Chem.*, **1993** *2*, 99-102.

26 Zuker, M.; Stiegler, P. *Nucleic Acids Res.*, **1981** *9*, 133-148.

27 Tuner, D. H.; Sugimoto, N.; Freier, S. M. (1990) in Saenger, W. R. (ed.) *Landolt-Börnstein Nucleic Acids* Vol.1c, chap. 3.6, Berlin, Germany.

28 Guerrier-Takada, C. A.; Gardiner, K.; Marsh, T.; Pace, N.; Altman, S. *Biochemistry*, **1986** *25*, 1509-1515.

29 Grosshans, C. A.; Cech, T. R. *Biochemistry.*, **1989** *28*, 6888-6894