Two Histidine Residues Are Essential for Ribonuclease T_1 Activity as Is the Case for Ribonuclease A†

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ABSTRACT: Ribonuclease T_1 (RNase T_1, EC 3.1.27.3) is a guanosine-specific ribonuclease that cleaves the 3′,5′-phosphodiester linkage of single-stranded RNA. It is assumed that the reaction is generated by concerted acid–base catalysis between residues Glu-58 and His-92 or His-40. From the results of chemical modification and NMR studies, it appeared that the residue Glu-58 was indispensable for nucleolytic activity. However, we have recently demonstrated that Glu-58 is an important but not an essential residue for catalytic activity, using the methods of genetic engineering to change Glu-58 to Gln-58 etc [Nishikawa, S., Morioka, H., Fuchimura, K., Tanaka, T., Uesugi, S., Ohtsuka, E., & Ikehara, M. (1986) *Biochem. Biophys. Res. Commun. 138*, 789–794]. In the present paper, we report that mutants of RNase T_1 with residue Ala-40 or Ala-92 have almost no activity, while mutants that contain Ala-58 retain considerable activity. These results show that the two histidine residues, His-40 and His-92, but not Glu-58, are indispensable for the catalytic activity of the enzyme. We propose a revised reaction mechanism in which two histidine residues play a major role, as they do in the case of RNase A.

A guanosine-specific ribonuclease, ribonuclease T_1 (RNase T_1, EC 3.1.27.3), was isolated from *Aspergillus oryzae* (Sato & Egami, 1957). RNase T_1 is a small (M_r 11 000) acidic and globular protein containing two disulfide bonds and a very stable enzyme. Moreover, it is relatively easy to purify large amounts of RNase T_1. For these reasons, RNase T_1 has been studied well, and its reaction mechanism is considered to be the following. In the first step of the reaction, that is, the transesterification step, the carboxyl group of Glu-58 abstracts a proton from the 2′-OH of the ribose moiety, and either His-92 or His-40 protonates the 5′-O of the adjacent ribose moiety to generate a cyclic 2′,3′-phosphate intermediate. In the second step, either His-92 or His-40 abstracts a proton from a water molecule, and Glu-58 adds a proton to the 2′-O of the ribose, thereby hydrolyzing the cyclic 2′,3′-phosphate intermediate.

This mechanism was proposed by Takahashi from results of studies involved mainly with chemical modification of RNase T_1 (Takahasi, 1970). Some discrepancies can be observed, however, between the results of chemical modification and those of NMR studies (Arata et al., 1979), and it is not known whether His-92 or His-40 acts as the general acid in the reaction. Furthermore, Osterman and Walz proposed a modified model, in which the residue Glu-58 is replaced by a Glu-58–His-92 pair (Osterman & Waltz, 1979). These catalytic residues are well conserved in guanine-specific ribonucleases from other microorganisms (Takahasi & Moore, 1982; Uchida & Egami, 1971), and furthermore, recent crystallographic studies on an RNase T_1–2′-GMP complex showed the catalytic residues to be located near the phosphate group (Heinemann & Saenger, 1982; Sugio et al., 1985). A recent approach to the study of the relationship between structure and function of RNase T_1 has exploited the techniques of protein engineering (Ikehara et al., 1986). In our previous paper it was reported that, because Glu-58 can be replaced by Gln-58 without complete loss of activity, Glu-58 is not essential, although it is still important, for enzymatic activity (Nishikawa et al., 1986).

In order to clarify the relationship between Glu-58, His-92, and His-40, we changed these amino acids to alanine by replacing appropriate regions of the gene with synthetic oligonucleotide fragments, using the method reported previously (Ikehara et al., 1986; Nishikawa et al., 1986).

Experimental Procedures

DNA ligase, polynucleotide kinase, and various restriction endonucleases were purchased from Takara Shuzo (Kyoto, Japan) or New England Biolabs (Beverly, MA). RNase T_1 was a gift from Sankyo (Tokyo, Japan).

All materials were of reagent grade and were obtained from commercial sources as described previously (Ikehara et al., 1986).

Site-Directed Mutagenesis. Construction of mutant genes and plasmids was essentially as reported previously (Ikehara et al., 1986; Nishikawa et al., 1986). The codon for Glu-58, His-40, of His-92 in RNase T_1 was changed to the codon for Ala by use of the following deoxyoligomers as the sense and antisense strands, in that order (italics indicate the changed codons): for Glu-58 → Ala, d(ACTAC*GCT*TGGCCGATC) and d(CCA*AGC*GTAGTACGGAG); for His-40 → Ala, d(*GCA*AAATACAACAAC) and d(GTATTT*TGC*CGGGTAAG); for His-92 → Ala, d(GTTATCACC*GCT*ACC) and d(AGAAGCGCCGGT*AGC*). The genes were inserted at the *Bgl*II–*Sal*I site of pGH-L9 (Ikehara et al., 1984). Plasmids containing the inserted mutant genes were obtained by standard procedures in *Escherichia coli* HB101, and the nucleotide sequences of the mutant genes were verified by the dideoxy method (Sanger et al., 1977).

Expression of Genes and Purification of Mutant Enzymes. The RNase T_1 mutants were prepared as a fusion protein to a part of human growth hormone in *E. coli* under the control

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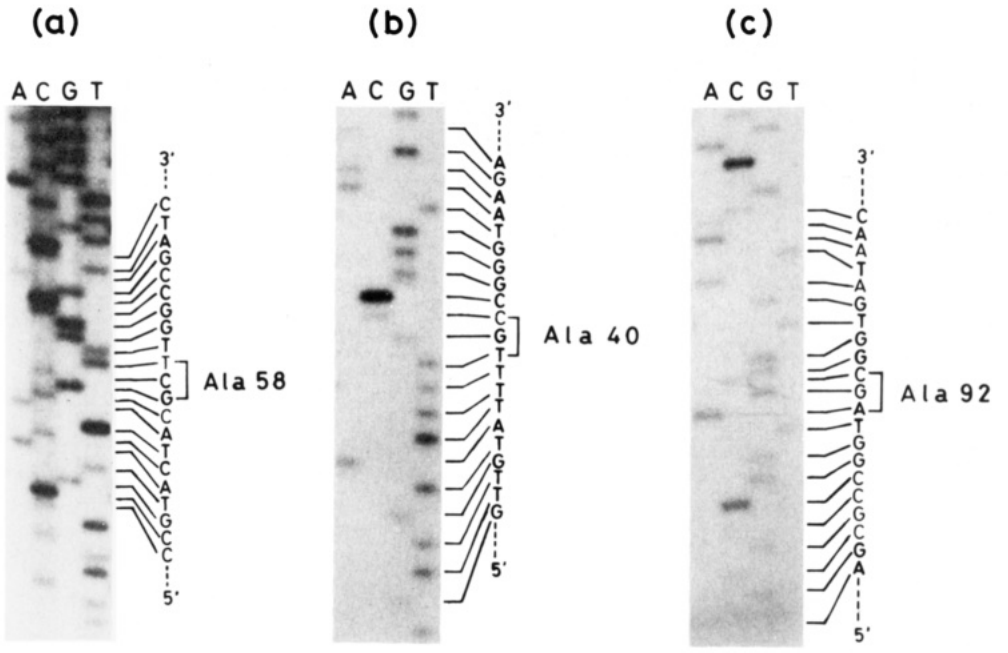


FIGURE 1: Autoradiogram of the DNA sequencing gel showing the nucleotide sequence around the mutation site of the mutant gene. The DNA sequences of the coding strand in Ala-58 mutant (a) and complementary strands in Ala-40 mutant (b) and Ala-92 mutant (c).

of the trp promoter (Ikehara et al., 1986; Nishikawa et al., 1986). Cells were grown and induced with 3-indoleacrylic acid as described (Hallewell & Emtage, 1980; Ikehara et al., 1986). Mutant enzymes were purified by the procedures described by Nishikawa et al. (1986) with some modifications. The cells were suspended in 50 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) (pH 8.0), 30 mM NaCl, and 10 mM ethylenediaminetetraacetic acid (EDTA) and treated with lysozyme (1 mg/mL) at 0 °C for 30 min. They were disrupted by freezing and thawing for five cycles instead of by sonication, and the crude extract was incubated with DNase I (1 μg/mL) at 0 °C for 30 min. After centrifugation, precipitates were dissolved in standard buffer which contained 7 M urea and were loaded onto a DE-52 column as described (Nishikawa et al., 1986). The treatment with cyanogen bromide was performed in 70% formic acid but without 2-mercaptoethanol. Mutant enzymes were purified without any special refolding procedures, such as oxidation and renaturation, by anion-exchange HPLC (TSK-gel, DEAE-2SW, Toyo Soda Co Ltd.) as the final step, with a linear gradient of NaCl (0–0.5 M) in 20 mM Tris-HCl (pH 7.5). The purity of mutant enzymes was checked by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). Protein concentrations were determined by Lowry's method or by use of an extinction coefficient of RNase T_1 of $A^{1\%}_{278} = 19$ (Takahashi et al., 1970) in the case of purified mutant enzymes.

Analysis of Nucleolytic Activity and Enzyme Kinetics. Enzymatic activity was measured by analysis of the cleavage of [5′-^{32}P]pGpC. The standard reaction mixture consisted of 150 μM [5′-^{32}P]pGpC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, and the indicated amount of enzyme in a volume of 10 μL at 37 °C. The enzymatic activities were linear within at least 10 min with the amount of protein used as follows: RNase T_1, 0.45 nM; Asp-58, Gln-58, and Ala-58 mutants, 5.4–9 nM; Ala-40 mutant, 198 nM. The product of the reaction, [5′-^{32}P]pGp, was separated from [5′-^{32}P]pGpC by DEAE-cellulose thin-layer (CEL 300 DEAE/HR-2/15, Macherey-Nagel) chromatography by with partially hydrolyzed RNA solution (homomixture VI; Jay et al., 1974) as solvent system and detected by autoradiogram (homochromatography, Brownlee & Sanger, 1969). pGp could contain pG>p since pGp and pG>p are not separated in this system. Radioactivity of each spot was measured in a liquid scintillation counter. Kinetic constants were obtained from analysis of Lineweaver–Burk plots (4–6 times repeated). The amounts of substrates and enzymes are given in the legends to Figures 3 and 4 and Table I.

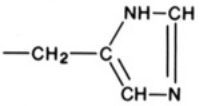
Computer Graphics. Molecular models were generated from the atomic coordinates of the X-ray structure of RNase T_1–2′-GMP or –3′-GMP complex obtained by X-ray crystallography (Sugio et al., 1985a,b; Hakoshima et al., unpublished results) and were displayed on a graphic terminal, Tectronics 4017, with the program NPLOT and on a color graphic terminal, NEC N6970, with the program MOLDISP in the Crystallographic Research Center, Institute for Protein Research, Osaka, University. Both of the terminals are hosted by an NEC ACOS-850 computer.

Results and Discussion

Nucleolytic Activities of Mutant Enzymes. The genes for several mutants of RNAse T_1 were constructed by the same way as reported previously (Ikehara et al., 1986; Nishikawa et al., 1986). The mutations were confirmed by DNA sequence determination (Figure 1) with the dideoxy method (Sanger et al., 1977). The mutant genes were expressed in *E. coli* HB101, and mutant enzymes were purified to homogeneity as assessed by SDS–PAGE (Figure 2). By substitution of a charged group with a methyl group, it may be possible to elucidate the function of specific amino acid residues in the catalytic reaction. Since it is assumed, from crystallographic studies, that the residues of interest do not interact with other regions of the enzyme, the substitutions that we made should not change the overall structure.

The enzymatic activity was measured by analysis of the hydrolysis of [5′-^{32}P]pGpC with a homochromatography system. Figure 3 shows one typical example of this homochromatogram for checking activities of mutant enzymes. Kinetic constants for the wild-type and mutant enzymes and their relative activities are given in Figure 4 and Table I. Some parts of this table have been already published in the previous paper (Nishikawa et al., 1986). These relative activities were mainly obtained from the comparison of initial

Table I: Kinetic Constants and Relative Activity of Wild-Type and Mutant Enzymes[a]

mutation	side chain	K_m (μM)	k_{cat} (min^{-1})	k_{cat}/K_m (min^{-1} μM^{-1})	relative activity (%)
wild type	$-CH_2CH_2COOH$	536[b]	75 700[b]	142.5[b]	100
Glu-58 → Asp	$-CH_2COOH$	435[b]	5 940[b]	13.7[b]	10
Glu-58 → Gln	$-CH_2CH_2CONH_2$	453[b]	827[b]	1.82[b]	1
Glu-58 → Ala	$-CH_3$	165	1 033	7.10	5
wild type					
His-40 → Ala	$-CH_3$				0.01
His-92 → Ala	$-CH_3$				0

[a] Enzyme kinetics were measured under standard conditions in mixtures containing 133.3–1000 μM [5′-^{32}P]pGpC and 5–10 nM mutant enzyme. After 5 min the reaction was stopped by the addition of dilute HCl (final concentration 0.2 N) and analyzed by homochromatography. [b] These data are taken from published results (Nishikawa et al., 1986).

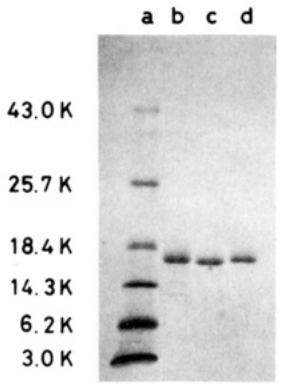


FIGURE 2: The 15% SDS–PAGE of purified mutant enzymes. The protein bands were stained by Coomassie Brilliant Blue R. (Lane a) Molecular weight standards; (lane b) Ala-40 mutant RNase T_1; (lane c) Ala-92 mutant RNase T_1; (lane d) wild-type RNase T_1.

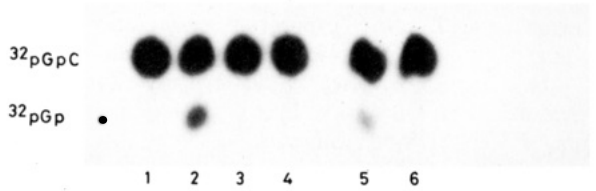


FIGURE 3: Homochromatogram of the products of hydrolysis of [5′-^{32}P]pGpC by mutant enzymes. Enzymatic actiivty was measured under the conditions described under Experimental Procedures. [5′-^{32}P]pGpC (150 μM) and mutant enzyme were incubated together in a total volume of 10 μL. After 5 min the reaction was stopped by the addition of diluted HCl (final concentration 0.2 N). In this reaction condition the amount of product was proportional to incubation time at least until 10 min. After incubation for 5 min, the reaction was analyzed by homochromatography. (Lane 1) Marker pGpC; (lane 2) mutant enzyme having Ala-58, 4.5 nM; (lanes 3 and 5) mutant enzyme having Ala-40, 4.5 and 198 nM, respectively; (lane 4 and 6) mutant enzyme having Ala-92, 4.5 and 297 nM, respectively.

velocities of enzymes. The 90% decrease in activity observed for the Asp-58 mutant enzyme may be due to the fact that the shorter carboxyl side chain is less favorable for abstraction of the 2′-OH proton. However, with the mutant enzymes that have Gln-58 or Ala-58, in which the carboxy group is replaced by an amide group and the carboxyethyl group is replaced by a methyl group, respectively, 1–5% activity is retained. By contrast, substitution of either of the histidine residues by an alanine residue caused a complete or an almost complete loss of activity (Figure 3, lanes 3–6). Very low activity was observed in the case of the Ala-40 mutant, where a 44-fold higher concentration of the mutant enzyme was used with respect to that of Ala-58 mutant (Figure 3, lane 5). On the other hand,

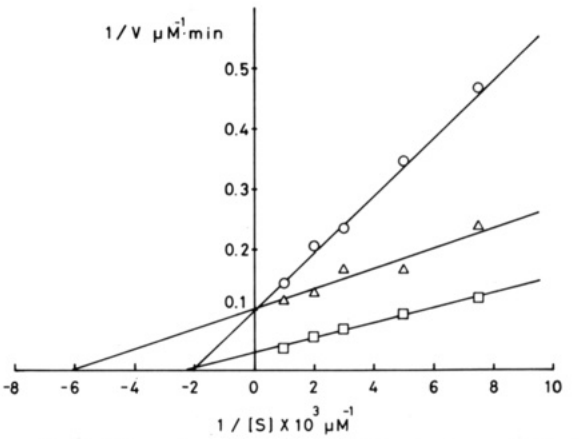


FIGURE 4: Double-reciprocal plot of initial velocity vs substrate, [5′-^{32}P]pGpC, concentration. The conditions of kinetic experiments are described under Experimental Procedures and in the footnote of Table I. Enzyme concentrations are as follows: Asp-58 mutant, 5.4 nM (□); Gln-58 mutant, 9 nM (○); Ala-58 mutant, 9 nM (△).

no activity was detected in the case of the Ala-92 mutant even when a 66-fold higher concentration of the mutant enzyme was used (Figure 3, lane 6). Relative activities of these mutants at histidine residues were 0.01 and 0% of that of wild type. This result indicates that both histidine residues are indispensable for catalytic activity.

For the mutant enzymes that contain Asp-58 and Gln-58, the K_m values are almost identical with that of the wild-type enzyme, but for the mutant enzyme that contains Ala-58, the K_m is significantly different (approximately one-third that of the wild-type enzyme). This result indicates that the substrate binds more strongly to the mutant enzyme that contains Ala-58 than to the wild-type and the other two mutant enzymes. This tighter binding could be caused by the enlargement of the binding pocket as a result of the shorter side chain. Because the activities of the mutant enzymes that contained Ala-40 and Ala-92 were very low and not detectable, respectively (Figure 3), we were not able to obtain the kinetic constants under the same conditions as employed for the enzymes with mutations at residue 58.

New Reaction Mechanism for RNase T_1. We propose a new mechanism for the action of RNase T_1 (Figure 5), in the first step of which His-40 acts as a general base catalyst and His-92 as a general acid catalyst; Glu-58 would then ensure an acidic environment around His-40 and enhance its basicity. Substitution of Glu-58 with Asp, Ala, or Gln may reduce the basicity of the His-40 residue, making abstraction of the proton from the 2′-OH of the ribose moiety more difficult. In con-

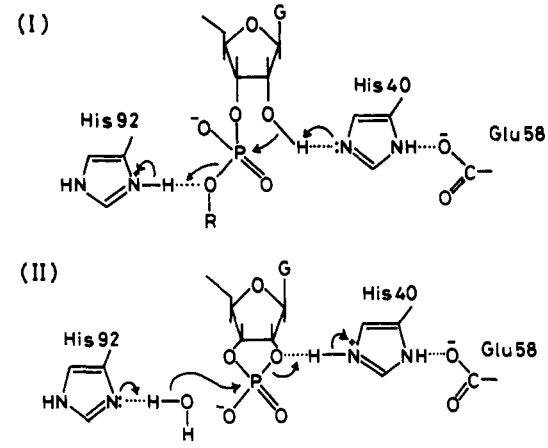


FIGURE 5: Newly proposed reaction mechanism for RNase T_1. (I) The first step, i.e., the transesterification, generates a cyclic 2′,3′-phosphate intermediate. (II) The second step, i.e., hydrolysis, generates the 3′-phosphate.

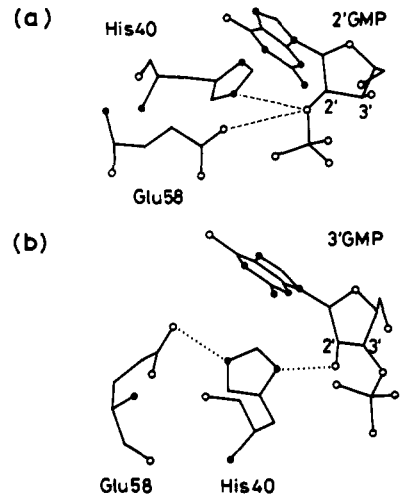


FIGURE 6: Arrangement of guanylic acid, His-40, and Glu-58 residues in crystals of an RNase T_1–2′-GMP complex (a) and an RNase T_1–3′-GMP complex (b). Open circles indicate oxygen atoms, and closed circle indicate nitrogen atoms. (a) Broken lines indicate the distance between 2′-O and Glu-58 $O^{\epsilon 2}$ and between 2′-O and His-40 $N^{\epsilon 2}$. (b) Dotted lines indicate hydrogen bonds between 2′-O and His-40 $N^{\delta 1}$ and between His-40 $N^{\epsilon 2}$ and Glu-58 $O^{\epsilon 2}$.

trast, substitution of His-40 or His-92 with alanine would remove what is now thought to be the actual catalytic residue, the imidazole group, such that enzymatic activity would be completely lost.

Three pieces of evidence support this newly proposed mechanism:

(i) The distances between 2′-O and His-40 $N^{\epsilon 2}$ and between 2′-O and Glu-58 $O^{\epsilon 2}$ in the RNase T_1–2′-GMP complex are, according to the crystal structure, 3.26 and 3.73 Å, respectively (Sugio et al., 1985a) (Figure 6a); i.e., Glu-58 is located 0.5 Å further from the 2′-O than His-40.

(ii) Crystallographic studies on an RNase T_1–3′-GMP complex revealed two hydrogen-bonds, 3′-GMP 2′-O–His-40 $N^{\delta 1}$ and His-40 $N^{\epsilon 2}$–Glu-58 $O^{\epsilon 2}$ (Sugio et al., 1985b; Hakoshima et al., unpublished results) between these residues (Figure 6b). Although the 3′-GMP is a product and not a substrate for the reaction, this alignment is well suited to the new model in which His-40 acts as a trigger and Glu-58 as an assistant to the reaction.

(iii) RNase A is another example of a ribonuclease that generates a cyclic 2′,3′-phosphate intermediate. The first step in the cleavage reaction catalyzed by RNase A is carried out by two histidine residues, His-12 and His-119, with His-12 acting as a general base and His-119 as a general acid (Findlay et al., 1961). Moreover, it has been demonstrated, in an experiment involving a synthetic oligopeptide, that the interaction between His-119 and Asp-121 is important for full activity (Okada et al., 1984).

The newly proposed mechanism for RNase T_1 is very similar to the reaction mechanism proposed for RNase A, in spite of no homology between their amino acid sequences and also no similarities in their tertiary structures. It should be noted, however, that there is a difference in the location of Glu-58 in RNase T_1 and Asp-121 in RNase A. Glu-58 is located near His-40, which is the acceptor of a proton in the first step (I in Figure 5), while Asp-121 is located near His-119 (Umeyama et al., 1979; Borkakoti, 1983), which is the donor of a proton in the first step and corresponds to His-92 in RNase T_1. These differences in structural configuration may explain the phenomenon in which the cyclic 2′,3′-phosphate intermediate tends to accumulate more often during the reaction of RNase T_1 than during that of RNase A (Irie et al., 1970; Richards & Wyckoff, 1971).

What could cause the apparent discrepancy between results from studies of chemical modification and those involving protein engineering? It is possible that the inactivation of RNase T_1 by carboxymethylation (Takahashi et al., 1967) of Glu-58 could result from steric hindrance caused by the extended side chain and/or loss of the carboxyl group from a critical position.

It would be most interesting to find out if this mechanism is also involved in the catalytic activity of other RNases. Similar guanosine-specific RNases have been found from microorganisms in both eukaryotes and prokaryotes (Uchida & Egami, 1971; Takahashi & Moore, 1982). The amino acid residues corresponding to Glu-58 and His-92 of RNase T_1 are all well conserved in these RNases. On the other hand, His-40 is conserved in RNases (RNase Ms, RNase F_1, RNase U_2, etc.) derived from lower eukaryotes but not in bacterial RNases (RNase St, RNase Ba, and RNase Bi). They have threonine or asparatic acid in analogous positions.

A similar catalytic triad to 2′-OH–His-40–Glu-58 (Figure 5) is already found in serine proteases as the form OH-(Ser)-His-Asp and has been termed the "charge relay system" (Blow et al., 1969). There is no homology in terms of amino acid sequences and tertiary structures among serine proteases such as trypsin (mammalian) and subtilisin (bacteria), but the catalytic triad, Ser-His-Asp, and substrate binding site among these enzymes have a similar configuration. This example is thought to be a typical case of "divergent evolution" in enzymes. We continue studying further to clarify whether this divergent evolution exists between RNase T_1 (lower eukaryote) and RNase A (mammalian) or not.

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REFERENCES

Arata, Y., Kimura, S., Matsuo, H., & Narita, K. (1979) *Biochemistry 18*, 18–24.

Blow, D. M., Birktoft, J., & Hartley, B. S. (1969) *Nature (London) 221*, 337–340.

Borkakoti, N. (1983) *Eur. J. Biochem. 132*, 89–93.

Brownlee, G. G., & Sanger, F. (1969) *Eur. J. Biochem. 11*, 395–399.

Findlay, D., Herries, D. G., Mathias, A. P., Rabin, B. R., & Ross, C. A. (1961) *Nature (London) 190*, 781-784.
Hallewell, R. A., & Emtage, S. (1980) *Gene 9*, 27-47.
Heinemann, U., & Saenger, W. (1982) *Nature (London) 299*, 27-31.
Ikehara, M., Ohtsuka, E., Tokunaga, T., Taniyama, Y., Iwai, S., Kitano, K., Ikari, T., Kobayashi, M., Miyake, T., Shibahara, S., Ono, A., Ueda, T., Tanaka, T., Baba, H., Miki, T., Sakurai, A., Oishi, T.,Chisaka, O., & Matsubara, K. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 5956-5960.
Ikehara, M., Ohtsuka, E., Tokunaga, T., Nishikawa, S., Uesugi, S., Tanaka, T., Aoyama, Y., Kikyodani, S., Fujimoto, K., Yanase, K., Fuchimura, K., & Morioka, H. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 4695-4699.
Irie, S., Itoh, T., Ueda, T., & Egami, F. (1970) *J. Biochem. (Tokyo) 68*, 163-170.
Jay, E., Bambara, R., & Wu, R. (1974) *Nucleic Acids Res. 1*, 331-353.
Nishikawa, S., Morioka, H., Fuchimura, K., Tanaka, T., Uesugi, S., Ohtsuka, E., & Ikehara, M. (1986) *Biochem. Biophys. Res. Commun. 138*, 789-794.
Okada, Y., Teno, N., Miyao, J., Mori, Y., & Irie, M. (1984) *Chem. Pharm. Bull. 32*, 4585-4592.
Osterman, H. L., & Walz, F. J., Jr. (1979) *Biochemistry 18*, 1984-1988.
Richards, F. M., & Wyckoff, H. W. (1971) *Enzymes (3rd Ed.) 4*, 647-806.
Sanger, F., Nicklen, S., & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 5463-5467.
Sato, K., & Egami, F. (1957) *J. Biochem. (Tokyo) 44*, 753-768.
Sugio, S., Amisaki, T., Ohishi, H., Tomita, K., Heinemann, U., & Saenger, W. (1985a) *FEBS Lett. 181*, 129-132.
Sugio, S., Oka, K., Ohishi, T., Tomita, K., Heinemann, U., & Saenger, W. (1985b) *FEBS Lett. 183*, 115-118.
Takahashi, K. (1970) *J. Biochem. (Tokyo) 67*, 833-839.
Takahashi, K., & Moore, S. (1982) *Enzymes (3rd Ed.) 15-B*, 435-468.
Takahashi, K., Stein, W. H., & Moore, S. (1967) *J. Biol. Chem. 242*, 4682-4690.
Takahashi, K., Uchida, T., & Egami, F. (1970) *Adv. Biophys. 1*, 53-98.
Uchida, T., & Egami, F. (1971) *Enzymes (3rd Ed.) 4*, 205-250.
Umeyama, H., Nakagawa, S., & Fujii, T. (1979) *Chem. Pharm. Bull. 27*, 974-980.

Raman Spectroscopic Study of Left-Handed Z-RNA†

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ABSTRACT: The solvent conditions that induce the formation of a left-handed Z form of poly[r(G-C)] have been extended to include 6.5 M NaBr at 35 °C and 3.8 M $MgCl_2$ at room temperature. The analysis of the A → Z transition in RNA by circular dichroism (CD), ^1H and ^{31}P NMR, and Raman spectroscopy shows that two distinct forms of left-handed RNA exist. The Z_R-RNA structure forms in high concentrations of NaBr and $NaClO_4$ and exhibits a unique CD signature. Z_D-RNA is found in concentrated $MgCl_2$ and has a CD signature similar to the Z form of poly[d(G-C)]. The loss of Raman intensity of the 813-cm^{-1} A-form marker band in both the A → Z_R-RNA and A → Z_D-RNA transitions parallels the loss of intensity at 835 cm^{-1} in the B → Z transition of DNA. A guanine vibration that is sensitive to the glycosyl torsion angle shifts from 671 cm^{-1} in A-RNA to 641 cm^{-1} in both Z_D- and Z_R-RNA, similar to the B → Z transition in DNA in which this band shifts from 682 to 625 cm^{-1}. Significant differences in the glycosyl angle and sugar pucker between Z-DNA and Z-RNA are suggested by the 16-cm^{-1} difference in the position of this band. The Raman evidence for structural difference between Z_D- and Z_R-RNA comes from two groups of bands: First, Raman intensities between 1180 and 1600 cm^{-1} of Z_D-RNA differ from those for Z_R-RNA, corroborating the CD evidence for differences in base-stacking geometry. Second, the phosphodiester stretching bands near 815 cm^{-1} provide evidence of differences in backbone geometry between Z_D- and Z_R-RNA.

Although RNA adopts a wide variety of tertiary structures in vivo, only the A-form secondary structure has customarily been observed for double-stranded regions. DNA, by contrast, has been shown to exist in vitro in a variety of double-helical conformations, e.g., A, B, C, D, and Z forms, although most natural DNA is thought to exist in the right-handed B-conformational family under physiological conditions (Wang, 1979). However, under conditions such as high salt concentration (Pohl & Jovin, 1972), low concentrations of certain metal complexes (Behe & Felsenfeld, 1981; Woisard et al., 1985), and superhelical torsional stress (Peck et al., 1982), DNA of certain base sequences, most notably alternating purines and pyrimidines, can adopt the left-handed Z conformation. It has recently been shown that double-stranded RNA consisting of alternating cytidines and guanosines, poly[r(G-C)], can also adopt a conformation similar to the left-handed Z form of DNA; this form has been called Z-RNA (Hall et al., 1984).

The formation of Z-RNA was originally documented by

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