RNA Hydrolysis by the Cooperation of Carboxylate Ion and Ammonium Ion

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Nonenzymatic hydrolysis of RNA has been widely attempted because of potential application to molecular biology, biotechnology, and therapy.[1−4] Mimics of the active sites of ribonuclease were prepared by using the cooperation of imidazolyl and/or aliphatic amino residues.[3a,5,6] Catalytically active metal ions and metal complexes were also reported.[7−9] These catalysts were attached to RNA binding molecules for site-specific or sequence-specific scission of RNA.[2,4,10] However, the catalysis by a negatively charged residue has never been documented. We report here a direct participation of carboxylate ion in RNA hydrolysis. The conjugates of glycine or iminodiacetate with anthraquinone (**1** and **2** in Scheme 1) hydrolyze tRNA under physiological conditions, due to intramolecular cooperation of carboxylate ion(s) and ammonium ion.

Efficient scission of yeast $tRNA^{Phe}$ by **1** at 37 °C and pH 7 has been clearly shown by polyacrylamide gel electrophoresis (Figure 1, lane 6). All the fragments (both the 3′-end and the 5′-end ^{32}P-labeled ones) comigrated with the corresponding alkaline hydrolysis products (lane 1), confirming the hydrolytic character of the scission. The hydrolysis is ascribed to the cooperation of the carboxylate ion and the ammonium ion in terms of the following results: (i) Neither the ethyl ester of **1** (**3**) nor the *N*-acetyl derivative (**4**) is active for RNA hydrolysis. (ii) The conjugate **5**, which has an amino residue but no carboxylate ion,[11] shows no catalysis. The hydrolysis rate is virtually independent of pH in the pH range 6−8 (lanes 5−7: the pK_a values of glycine are 2.35 and 9.78).[12]

The proposed cooperation is furthermore substantiated by the efficient hydrolysis of tRNA by the iminodiacetate−anthraquinone conjugate **2** (lanes 8−10). As expected, the diethyl ester of **2** (**8**) is not active. Interestingly, the scissions by **1** and **2** are site selective. 5′-Cytidine-adenosine-3′ sites are hydrolyzed by **1** (the closed wedges in Figure 2),[13] whereas the 3′-sides of guanosine are the preferential scission sites by **2** (the open wedges).

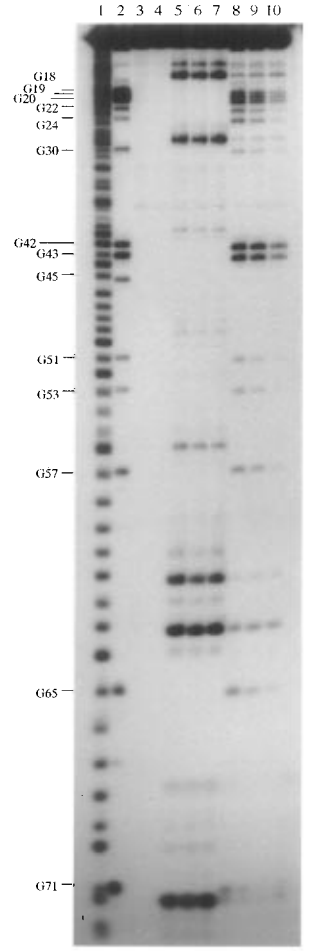
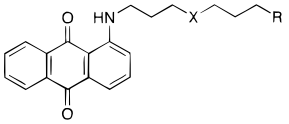


Figure 1. Autoradiographs of a 15% denaturing polyacrylamide gel electrophoresis for the scission of the $tRNA^{Phe}$, ^{32}P-labeled at the 3′-end, by **1** and **2** (1 mM) at 37 °C for 4 h. Lane 1, alkaline hydrolysis; lane 2, ribonuclease T1 (G-specific); lane 3, no treatment; lane 4, control reaction at pH 7; lane 5, with **1** at pH 6; lane 6, **1** at pH 7; lane 7, **1** at pH 8; lane 8, with **2** at pH 6; lane 9, **2** at pH 7; lane 10, **2** at pH 8. Hydrolyses were carried out in the solutions containing 10 mM HEPES (for pH 7 and 8; cacodylate was used for pH 6), 1 mM EDTA, and 10^{-6}−10^{-7} M tRNA.

Scheme 1



1 : X = -N(CH$_3$)-, R = -NHCH$_2$CO$_2$H
2 : X = -N(CH$_3$)-, R = -N(CH$_2$CO$_2$H)$_2$
3 : X = -N(CH$_3$)-, R = -NHCH$_2$CO$_2$C$_2$H$_5$
4 : X = -N(CH$_3$)-, R = -N(COCH$_3$)(CH$_2$CO$_2$H)
5 : X = -N(CH$_3$)-, R = -NH$_2$
6 : X = -O-, R = -NHCH$_2$CO$_2$H
7 : X = -N$^+$(CH$_3$)$_2$-, R = -NHCH$_2$CO$_2$H
8 : X = -N(CH$_3$)-, R = -N(CH$_2$CO$_2$C$_2$H$_5$)$_2$
9 : X = -O-, R = -N(CH$_2$CO$_2$H)$_2$
10 : X = -N$^+$(CH$_3$)$_2$-, R = -N(CH$_2$CO$_2$H)$_2$

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(13) An intercalator attached with two imidazolyl residues also cleaved the 5′-pyrimidine-adenosine-3′ sites in tRNA: ref 10.

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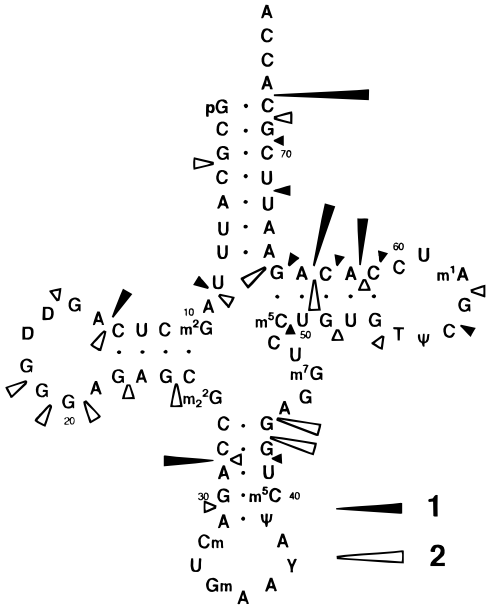

Figure 2. Scission profiles for the hydrolysis of $tRNA^{Phe}$ by **1** (the closed wedges) and by **2** (the open wedges); the length of the arrow corresponds to the magnitude of scission. Lanes 6 and 9 in Figure 1 were analyzed by densitometry. The scission sites were assigned by using both the 3′-end and the 5′-end ^{32}P-labeled tRNAs.

The anthraquinone moieties in **1** and **2** are definite requisites, since free glycine and iminodiacetate without them showed no measurable catalysis even at 0.5 M. These aromatic moieties bind to the tRNA, increasing the local concentration of the catalytic residue in its vicinity. Thus, the plot of the hydrolysis rate vs $[\mathbf{1}]_0$ showed a gradual saturation, attaining a plateau above $[\mathbf{1}]_0 = 0.5$ mM. An "intercalation-like" binding of **1** with calf thymus DNA has been indicated by the hypochromic change in UV/visible absorption spectra: the binding constant at pH 7 and 25 °C is 4.79×10^4 M^{-1}.[11]

Quite significantly, the G-specificity of **2** disappeared when its central amine was quaternarized (**10** cleaved the CA sites only faintly). It is strongly indicated that a hydrogen bonding of this ammonium ion with a G residue is responsible for the G-specific and efficient RNA hydrolysis.[14] Consistently, **9**, in which the amino residue in **2** is replaced by an ethereal oxygen,

(14) The hydrogen accepting site of G is tentatively assigned to its 6-O atom, which is positioned favorably for the purpose.

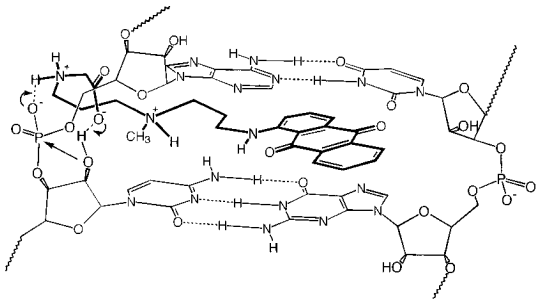

Figure 3. Proposed mechanism for the hydrolysis of $tRNA^{Phe}$ by **1**.

is inactive for the catalysis. However, the CA specificity and the activity of **1** were virtually unchanged on the quaternarization (**7**), since the central ammonium ion enhances the binding of **1** to the tRNA simply by the electrostatic interaction.[15] The activity of **6** is about $^1/_7$ of that of **1**.

The proposed mechanism for the catalysis by **1** is schematically depicted in Figure 3. The anthraquinone is incorporated between two nucleic acid bases in the tRNA, placing the cleaver at the scission site. The carboxylate ion as a general base catalyst activates a 2′-OH residue for the intramolecular attack toward the phosphorus atom, which is cooperatively promoted by the general acid catalysis by the ammonium ion. Intrinsically reactive CA linkages are overwhelmingly hydrolyzed. The catalysis by **2** prevails at the G-site, since the repulsion between the negatively charged iminodiacetate and the negative charges of the tRNA can be compensated by the hydrogen bonding of the central ammonium ion with G (*vide ante*).[16] Direct nucleophilic attack by the carboxylate ion toward the phosphorus atom is ruled out, since DNA is not hydrolyzed to a measurable extent.

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(15) The possibility of acid−base cooperation of the central ammonium ion with the carboxylate is ruled out by this result.

(16) The mechanism is supported by the decrease of the hydrolysis rate by **2** on increasing pH from 6 to 8 (Figure 1). The central ammonium ion (pK_a 7.2), which is essential for the catalysis, is gradually deprotonated here. In the case of **1**, the effect is less explicit, mainly because the cleaver is neutral in the net charge and thus is not repelled much by the negative charges of the tRNA. Consistently, **6** is somewhat active.