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Ribose Recognition by Ribonuclease T_1: Difference Spectral Binding Studies with Guanosine and Deoxyguanosine†

Frederick G. Walz, Jr.

ABSTRACT: The binding of ribonuclease T_1 with guanosine (Guo) and deoxyguanosine (dGuo) was studied in experiments employing ultraviolet difference spectroscopy in the pH range 3–9 at 0.2 M ionic strength and 25 °C. Similar experiments were also conducted with γ-carboxymethyl-glutamate-58 ribonuclease T_1 at pH 5.0. At most pH values the characteristic difference spectrum and association constant were obtained. The binding constant for dGuo was ~550 M^{-1} and did not significantly vary in the pH range 3.5–9.0. The binding constant for Guo increased from pH 3.5 to 5.0, was constant between pH 5.0 and 7.0 (~3200 M^{-1}), and decreased at higher pH values. The binding of Guo and dGuo with ribonuclease T_1 could also be distinguished in terms of the wavelength for maximal difference absorbance, λ_{max}, between pH 5.0 and 7.0. At higher and lower pH values, λ_{max} for Guo approached that found fr dGuo. On the other hand, the value of the binding constant (~6500 M^{-1}) and the nature of the difference spectra for Guo and dGuo binding with γ-carboxymethyl-glutamate-58-ribonuclease T_1 at pH 5.0 were identical. These results suggest that the discrete interaction of the Guo 2′-hydroxyl group with ribonuclease T_1 involves the γ-carboxylate of glutamate-58 and an imidazolium group at the active site.

It is recognized that RNase[1] T_1 (EC 3.1.4.8) catalyzes RNA depolymerization via a two-step process including: (1) *Transesterification* in which the phosphoester bond between a guanosine 3′-P residue and the 5′ oxygen of the adjacent nucleoside group is cleaved with concomitant formation of a guanosine 2′,3′-P residue, and (2) *hydrolysis* in which the guanosine 2′,3′-P residue is specifically hydrolyzed to its 3′-monophosphate product (Takahashi et al., 1970). The simple fact that RNase T_1 polynucleotide substrates require a guanosine 2′-hydroxyl group for endonucleolytic cleavage immediately suggests the primary importance of the enzyme's interaction with this group in substrate recognition and catalysis. In this regard, a mechanism for the RNase T_1 catalyzed transesterification has been proposed (Takahashi, 1970a) which suggests, in part, that the substrate Guo 2′-hydroxyl

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[1] Abbreviations used are: RNase, ribonuclease; γ-CM-Glu-58-RNase T_1, RNase T_1 having a γ-carboxymethyl group on Glutamate 58; nucleoside designations follow the recommendation of the IUPAC-IUB commission as reported in *Biochemistry 9,* 4025 (1970); Tris, 2-amino-2-hydroxymethyl-1,3-propanediol; uv, ultraviolet; NMR, nuclear magnetic resonance.

group at least transiently interacts with the γ-carboxylate group of Glu-58. An approach towards a further elucidation of this interaction is to study the nature of RNase T_1 binding with ribose and deoxyribose substrate analogues.

Equilibrium binding studies in our laboratory (Walz and Hooverman, 1973; Walz and Terenna, 1976) and elsewhere (Oshima and Imahori, 1972) have demonstrated that the affinities of RNase T_1 for a variety of monomeric and dimeric ligands having a Guo 2′-hydroxyl group are generally much greater than those of corresponding 2′-deoxyribose compounds. The present report concerns an investigation into the nature of this affinity difference by studying the pH dependence of RNase T_1 binding with Guo and dGuo using uv difference spectroscopy. Experiments were conducted in the pH range 3–9 and information regarding the nature of the binding process was obtained from difference spectra per se and binding constants derived from ligand titration studies at each pH. Similar experiments were also conducted at pH 5.0 with catalytically inactive γ-CM-Glu-58-RNase T_1. Evidence is summarized that supports the conclusion that the discrete binding of the Guo 2′-hydroxyl group with the enzyme involves Glu-58 and an active site His residue.

Materials and Methods

Ribonuclease T_1 was prepared as described previously (Walz and Hooverman, 1973). γ-CM-Glu-58-RNase T_1 was prepared according to the method of Takahashi et al. (1967). This enzyme derivative was 99.97% inactivated as determined by initial velocity measurements using GpC as a substrate (Zabinski and Walz, submitted for publication). The concentrations of RNase T_1 and γ-CM-Glu-58-RNase T_1 were both determined using 2.1×10^4 M^{-1} cm^{-1} as a molar extinction coefficient at 278 nm. Guanosine and deoxyguanosine were obtained from P-L labs, Inc. and their concentrations were determined using a molar extinction coefficient of 1.37×10^4 M^{-1} cm^{-1} at 253 nm and pH 7.0.

The ultraviolet difference spectra resulting from RNase T_1 nucleoside binding was obtained in the same manner as described previously (Walz and Hooverman, 1973) except that a Cary 118C scanning spectrophotometer was employed in the present study. Difference spectra were determined by scanning in the range of 240–310 nm. All experiments were conducted at 25 °C in a standard buffer solution containing 0.1 M KCl, 0.05 M Tris, 0.05 M sodium acetate adjusted to the desired pH with acetic acid. At pH 3.0 and 3.5 the same buffer with sodium lactate–lactic acid replacing sodium acetate–acetic acid was used. Experiments were conducted at pH 4.0 comparing the lactate and acetate buffers and no difference was observed. The concentrations of RNase T_1 or γ-CM-Glu-58-RNase T_1 used were $\sim 4.3 \times 10^{-5}$ M and the concentration of the nucleosides ranged from $\sim 4 \times 10^{-5}$ M to $\sim 6 \times 10^{-4}$ M. For the determination of the pH-induced difference spectra for Guo and dGuo, a standard buffer at the desired pH and a solution of 0.1 M HCl and 0.1 M KCl (pH 1.10) were prepared. Identical small volumes of Guo or dGuo in distilled water were added to both solutions, and the difference spectra were determined using the pH 1.1 solution as the sample and the standard buffer system at the desired pH as the blank.

All pH measurements were conducted at 25 °C using a Radiometer pH M-26 pH meter.

Results and Treatment of the Data

At a given pH, difference spectra were determined using a constant concentration of protein (RNase T_1 or γ-CM-Glu-58-RNase T_1) and at least ten different concentrations of Guo or dGuo. These experimental difference spectra were treated as follows: (1) the average and standard deviation of the wavelength at which the difference absorbance (ΔA) reached a maximum, λ_{max}, was computed; (2) in each experiment, ΔA values at 5-nm intervals from 240 to 310 nm were normalized to that at λ_{max} and these were used to calculate an average, normalized difference spectrum. Under a given set of experimental conditions, the deviations of the normalized difference spectra were apparently random and not correlated to the concentrations of the nucleoside. The pH-induced difference spectra for Guo and dGuo were treated in the same manner. However, in these experiments, the results were extremely reproducible and only two or three difference spectra were obtained for each experiment. Some normalized difference spectra for Guo and dGuo are presented in Figure 1 and λ_{max} values are listed in Table I. The normalized differece spectra for Guo and dGuo binding with λ-CM-Glu-58-RNase T_1 (not shown) were identical to each other and had essentially the same shape as those shown in Figure 1a for nucleoside binding with the native enzyme, except for different λ_{max} values (Table I).

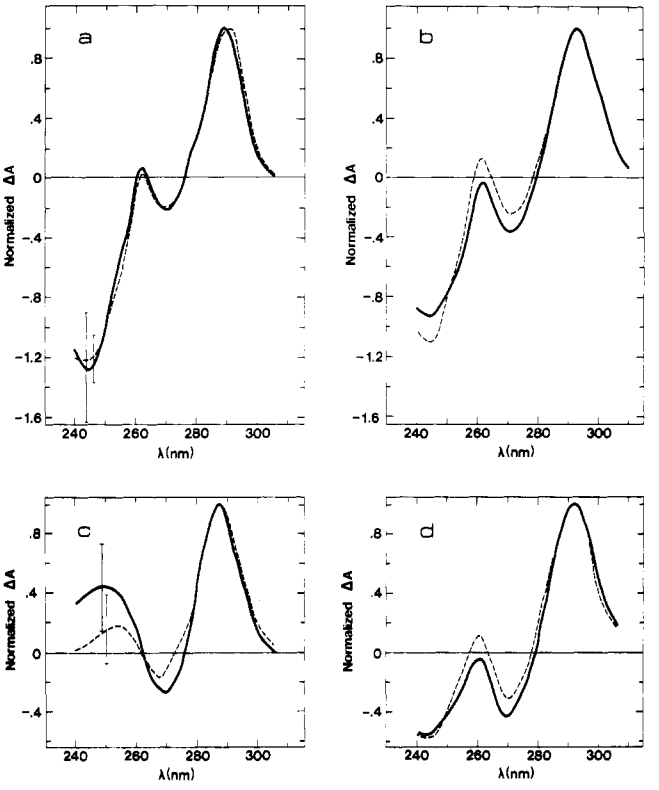


FIGURE 1: Normalized difference spectra for guanosine and deoxyguanosine induced by binding with RNase T_1 and pH difference. Experiments were conducted at 0.2 M ionic strength and 25 °C, other conditions were as described under Materials and Methods. Difference spectra were normalized to ΔA at λ_{max}. (a) RNase T_1 binding difference spectra at pH 7.0. Error bars represent standard deviation for ten experiments; (- - -) Guo; (—) dGuo. (b) pH-induced difference spectra. The pH of blank and sample cells was 7.0 and 1.1, respectively; the concentration of the nucleosides was 3.4×10^{-5} M. (- - -) Guo; (—) dGuo. (c) RNase T_1 binding difference spectra at pH 9.0. Error bars represent standard deviations of ten experiments; (- - -) Guo; (—) dGuo. (d) pH-induced difference spectra. The pH of blank and sample cells was 9.0 and 1.1, respectively; the concentration of nucleosides was 3.4×10^{-5} M. (- - -) Guo; (—) dGuo.

In order to determine the association constant, K, and the difference molar extinction coefficient, $\Delta\epsilon_{290}$, for enzyme binding with Guo and dGuo, ΔA was measured at 290 nm for a given total enzyme concentration, $[E]_0$, and total nucleoside

TABLE I: Values of the Wavelength of the Maximal Difference Absorbance from Difference Spectra of Guanosine and Deoxyguanosine Induced by Ribonuclease T_1 and γ-CM-Glu-58-ribonuclease T_1 Binding or pH Change.[a]

Difference Spectra	pH[b]	Guo λ_{max} (nm)	dGuo λ_{max} (nm)
RNase T_1 binding	3.0	288.7 ± 1.1	287.9 ± 0.5
	3.5	289.6 ± 0.5	288.9 ± 0.8
	4.0	290.6 ± 0.4	
	5.0	290.5 ± 0.3	289.0 ± 0.4
	7.0	290.7 ± 0.2	288.9 ± 0.3
	8.0	289.7 ± 0.5	
	8.5	289.3 ± 0.4	288.3 ± 0.7
	9.0	287.6 ± 0.2	287.3 ± 0.5
γ-CM-Glu-58-RNase T_1 binding	5.0	289.8 ± 0.3	289.7 ± 0.2
pH change (sample at pH 1.1)	3.5	291.8	291.8
	7.0	292.6 ± 0.2	292.5 ± 0.2
	8.5	292.0	291.8
	9.0	292.0	291.8

[a] Experiments were conducted as described under Materials and Methods. [b] pH refers to the value used in binding experiments and to the blank buffer solution in pH change experiments.

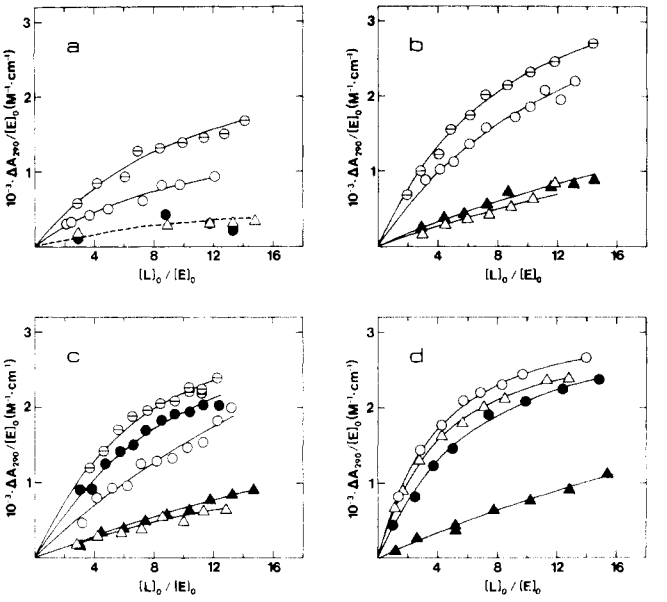


FIGURE 2: Experimental and theoretical plots of the difference absorbance at 290 nm divided by the total RNase T_1 concentration vs. the ratio of the total nucleoside and total enzyme concentrations at different pH values. Experiments were conducted at 0.2 M ionic strength and 25 °C; other conditions were as described under Materials and Methods. The solid theoretical curves were calculated as described in the text. (a) (△) dGuo, pH 3.0, [RNase T_1] = 4.22×10^{-5} M; (●) Guo, pH 3.0, [RNase T_1] = 4.22×10^{-5} M; (○) Guo, pH 3.5, [RNase T_1] = 4.54×10^{-5} M; (⊖) Guo, pH 4.0, [RNase T_1] = 4.52×10^{-5} M. (b) (△) dGuo, pH 3.5, [RNase T_1] = 4.44×10^{-5} M; (▲) dGuo, pH 7.0, [RNase T_1] = 4.25×10^{-5} M; (○) Guo, pH 8.5, [RNase T_1] = 4.13×10^{-5} M; (⊖) Guo, pH 6.0, [RNase T_1] = 4.10×10^{-5} M. (c) (△) dGuo, pH 8.5, [RNase T_1] = 4.31×10^{-5} M; (▲) dGuo, pH 9.0, [RNase T_1] = 4.20×10^{-5} M; (○) Guo, pH 9.0, [RNase T_1] = 3.94×10^{-5} M; (●) Guo, pH 8.0, [RNase T_1] = 4.63×10^{-5} M; (⊖) Guo, pH 7.0, [RNase T_1] = 4.52×10^{-5} M. (d) (▲) dGuo, pH 5.0, [RNase T_1] = 4.95×10^{-5} M; (●) Guo, pH 5.0, [RNase T_1] = 4.72×10^{-5} M; (△) dGuo, pH 5.0, [γ-CM-Glu-58-RNase T_1] = 4.52×10^{-5} M; (○) Guo, pH 5.0, [γ-CM-Glu-58-RNase T_1] = 4.56×10^{-5} M.

concentration, $[L]_0$. The binding isotherms obtained by varying $[L]_0$ are shown in Figure 2. These could be described by the equilibrium expression (Walz and Hooverman, 1973)

$$K = \frac{[EL]}{[E][L]} = \frac{\Delta A_\lambda/\Delta\epsilon_\lambda}{([E]_0 - \Delta A_\lambda/\Delta\epsilon_\lambda)([L]_0 - \Delta A_\lambda/\Delta\epsilon_\lambda)} \quad (1)$$

where λ = 290 nm, and [EL], [E], and [L] are the concentrations of the enzyme–nucleoside complex, the free enzyme, and the free ligand, respectively. The best-fit values for K and $\Delta\epsilon_{290}$ were determined by finding the value of $\Delta\epsilon_{290}$ that minimized the coefficient of variation for K (Walz and Hooverman, 1973). Best-fit values of K and $\Delta\epsilon_{290}$ were obtained for all experiments except those conducted at pH 3.0. The average value of the coefficient of variation was 8.4% for all data sets and no value exceeded 12%. The values of K and $\Delta\epsilon_{290}$ for enzyme binding Guo and dGuo are listed in Table II. Theoretical binding curves were calculated using these values of K and $\Delta\epsilon_{290}$ with eq 1 and are shown with the experimental data in Figure 2.

Discussion

The results of this investigation strongly suggest that two RNase T_1 acid–base groups are involved in interacting with the 2′-hydroxyl group of a bound Guo residue. The supporting evidence for this proposal can be grouped under the following categories: (1) the nature of the difference spectra for RNase T_1 binding with Guo and dGuo as a function of pH, (2) the affinity difference of Guo and dGuo with RNase T_1 as a function of pH, and (3) the effect of specific chemical modification of the RNase T_1 active-site residue, Glu-58, on the binding of Guo and dGuo.

It has been proposed that the difference spectra resulting from RNase T_1 binding with various guanine-containing ligands result primarily from protonation of the N^7 position of this base group in the enzyme–ligand complex (Oshima and Imahori, 1972; Epinatjeff and Pongs, 1972; Walz and Hooverman, 1973). This proposal was based on the substantial qualitative similarity of RNase T_1–guanine ligand binding difference spectra with that obtained by comparing the absorption spectra of guanine nucleosides or nucleotides at neutral and low pH (where the N^7 position of Guo is unprotonated and protonated, respectively). These similarities between RNase T_1 binding and pH-induced difference spectra for Guo and dGuo are illustrated with some typical results in Figure 1. The pH-induced difference spectrum conducted at pH 9.0 (Figure 1d) is different from that at pH 7.0 (Figure 1b) due to the partial ionization of the guanine N^1-C^6-OH system at the former pH. As anticipated, the RNase T_1-binding difference spectra showed a similar change for these pH values (compare Figure 1a and 1c). A closer scrutiny of the two kinds of difference spectra observed with Guo and dGuo revealed some subtle differences in the *binding* of these nucleosides with RNase T_1. As shown in Table I, the values of λ_{max} for the pH-induced difference spectra were the same for Guo and dGuo at each pH tested, whereas, for the RNase T_1-binding difference spectra, λ_{max} values were significantly different for these nucleosides in the pH range 4–7. (This characteristic difference was also observed for RNase T_1 binding of 5 Guo- and 13 dGuo-containing monomeric and dimeric ligands, i.e., all dGuo ligands had λ_{max} values of 289 ± 0.5 nm and all Guo-containing ligands had λ_{max} values of 291 ± 0.5 at pH 5.0, 0.2 M ionic strength and 25 °C (Walz and Terenna, 1976).) Beyond the pH range 4–7, the values of λ_{max} for RNase T_1–Guo binding were significantly lower than those found within this range; furthermore, at pH 3.0, 3.5, 8.5, and 9.0 the values

TABLE II: Values of the Binding Parameters for Ribonuclease T_1 and γ-CM-Glu-58-ribonuclease T_1 with Guanosine and Deoxyguanosine.

Enzyme	pH	Guo		dGuo	
		$\frac{10^{-3}\,\Delta\epsilon_{290}}{(M \times cm)^{-1}}$	$\frac{10^{-3}\,K}{(M^{-1})}$	$\frac{10^{-3}\,\Delta\epsilon_{290}}{(M \times cm)^{-1}}$	$\frac{10^{-3}\,K}{(M^{-1})}$
RNase T_1	3.5	1.95	1.69 ± 0.19	3.40	0.50 ± 0.07
	4.0	3.05	2.03 ± 0.20		
	5.0	3.40	3.42 ± 0.25	3.90	0.55 ± 0.04
	6.0	4.20	3.13 ± 0.30		
	7.0	3.85	3.06 ± 0.18	3.50	0.62 ± 0.06
	8.0	4.10	2.00 ± 0.19		
	8.5	4.20	1.90 ± 0.14	3.15	0.50 ± 0.04
	9.0	5.90	0.89 ± 0.10	3.60	0.57 ± 0.03
γ-CM-Glu-58-RNase T_1	5.0	3.30	6.90 ± 0.62	3.10	6.51 ± 0.28

of λ_{max} (Table I) and the normalized difference spectra were the same within experimental error for the binding of both nucleosides. The changes in λ_{max} for RNase T_1–dGuo binding had only borderline significance over the pH range investigated (Table I). These results indicate that the RNase T_1 *complexes* with Guo and dGuo are different and this difference is dependent on the ionized state of two acid–base groups of the RNase T_1–Guo complex.

The binding of RNase T_1 with Guo and dGuo could not be distinguished at pH 3.0, either qualitatively by their normalized difference spectra and λ_{max} values (Table I), or quantitatively by their binding curves (Figure 2a). Binding of the enzyme with *both* nucleosides at this pH was apparently at a lower level than that obtained with dGuo at higher pH's, and best-fit values of K and $\Delta\epsilon_{290}$ could not be obtained from the data. The best-fit values of K that were obtained at other pH values are plotted in Figure 3. In the case of RNase T_1–Guo binding, K significantly increased from pH 3.0 to 5.0, was constant from pH 5.0 to 7.0, and significantly decreased from pH 7.0 to 9.0, whereas, for RNase T_1–dGuo binding, K was essentially pH independent over this pH range. The pH dependence of K for RNase T_1–Guo binding, shown in Figure 3, is consistent with the results of previous RNase T_1 binding studies, conducted at single ligand concentrations, using difference spectroscopy (Epinatjeff and Pongs, 1972) and gel-filtration (Takahashi, 1970b) techniques. Since Guo is not significantly ionized in the pH range investigated, it is likely that the pH dependence of K for binding of this ligand reflects ionizations of enzyme acid–base groups. Furthermore, the curve in Figure 3 describing the pH dependence of K for RNase T_1–Guo binding indicates that optimal enzyme affinity for this nucleoside requires one enzyme acid–base group in its unprotonated form having a pK_a value in the range 3.0–5.0 and another required in its protonated form having a pK_a value in the range 7.0–9.0.

The results at this point can be summarized as follows: (1) the affinity of RNase T_1 with Guo, as indicated by K, is significantly greater than that of dGuo from pH 4.0 to 7.0; (2) the nature of the RNase T_1 complex with Guo, as indicated by λ_{max}, is significantly different from that with dGuo from pH 4.0 to 7.0; (3) the pH dependence of K and λ_{max} for RNase T_1–Guo binding is bell shaped from pH 3.0 to 9.0, whereas, these parameters for dGuo are essentially independent of pH over this range; and (4) the values of K and λ_{max} for RNase T_1–Guo binding approach those for RNase T_1–dGuo binding at the extreme pH values tested. These facts are consistent with the proposal that the enzyme acid–base groups described above

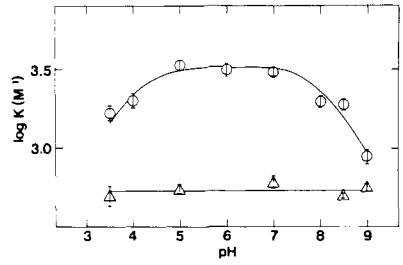


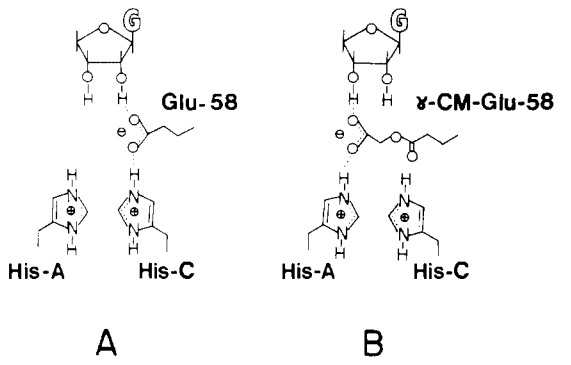
FIGURE 3: Plot of the logarithm of the association constant for RNase T_1 binding with Guo and dGuo vs. pH. Experiments were conducted at 0.2 M ionic strength and 25 °C, other conditions were as described under Materials and Methods. The binding constants were calculated as described in the text; error bars represent standard deviations. (○) Guo; (△) dGuo.

for RNase T_1–Guo binding are involved in interacting with the 2′-hydroxyl group.

The results of chemical modification studies have indicated the presence of Glu-58 (Takahashi et al., 1967), two His (Takahashi, 1970a; Irie, 1970), and possibly Arg-77 (Takahashi et al., 1970) at the active site of RNase T_1. More specific evidence from NMR chemical shift studies of the three imidazole C-2 protons of the enzyme (Rüterjans et al., 1969; Rüterjans and Pongs, 1971) has supported the proposal of two active site His residues (designated His-A and His-C). In addition, these studies indicated that the γ-carboxyl group of Glu-58 is adjacent to the imidazole group of His-C in the native enzyme, whereas, the newly introduced carboxyl group of catalytically inactive γ-CM-Glu-58-RNase T_1 interacts with the imidazole group of His-A (Rüterjans and Pongs, 1971). It was also concluded that the His-A residue interacts with the phosphate group in the Guo-3′-P–RNase T_1 complex. Finally, kinetic studies of the RNase T_1 catalyzed transesterification of GpC and GpU (Zabinski and Walz, submitted) suggested that one unprotonated carboxyl and two protonated imidazole groups of the enzyme are required for substrate binding and/or catalysis. In view of this information, it is tempting to speculate that the enzyme acid–base groups, proposed above, to constitute a binding locus for a Guo 2′-hydroxyl group, represent Glu-58 and one of the active site His residues.

In the present study, the involvement of the γ-carboxyl group of Glu-58 in interacting with the Guo 2′-hydroxyl group was indicated from binding experiments using Guo and dGuo with γ-CM-Glu-58-RNase T_1 (see Figure 2). The basis for this conclusion is that the significant differences in λ_{max} (Table I) and K (Table II) determined for RNase T_1 binding with Guo

Chart I



and dGuo at pH 5.0 are eliminated by the introduction of a carboxymethyl group on Glu-58. In fact, the binding of Guo and dGuo with the modified enzyme was identical in all respects tested except for a minor difference in $\Delta\epsilon_{290}$ values. Interestingly, the values of K at pH 5.0 in Table II indicate that the affinity of Guo is significantly increased with γ-CM-Glu-58-RNase T_1 when compared with the native enzyme. The same phenomenon was previously reported by Takahashi (1970b), although its significance was not discussed.

Considering the present results and those from previous studies, a reasonable working hypothesis can be proposed regarding the binding of Guo and dGuo with RNase T_1 and γ-CM-Glu-58-RNase T_1 which can be summarized as follows: (1) the unprotonated γ-carboxyl group of Glu-58 is paired with the protonated imidazole group of His-C and binds with the 2′-hydroxyl group of a Guo residue complexed with the native enzyme; and (2) the newly introduced carboxyl group of γ-CM-Glu-58-RNase T_1 forms a similar pair with His-A and binds with the 3′-hydroxyl group of either Guo or dGuo complexed with the modified enzyme. This hypothesis is roughly visualized in Chart I. The designations and carboxyl group interactions of the His residues are taken from NMR studies (Rüterjans and Pongs, 1971). The array of His-A and Glu-58 residues with the Guo 2′-hydroxyl group in Chart IA is the same as that presented in a proposed mechanism for the RNase T_1 catalyzed transesterification (Takahashi, 1970a). The proposed carboxylate–hydroxyl group interactions would probably involve hydrogen bonding, which presumably is stabilized by the associated imidazolium group. In any event, the suggestion of active site carboxyl–imidazole interactions has precedents in x-ray crystallographic models of chymotrypsin (Blow et al., 1969) and RNase S (Richards and Wyckoff, 1971). The interactions proposed in Chart IA intuitively explain the pH dependence of RNase T_1–Guo binding and provide a simple basis for understanding the difference observed in the binding of the enzyme with Guo and dGuo. Likewise, the carboxylate–3′-hydroxyl interaction proposed in Chart IB accounts for the observed *identical* binding of Guo and dGuo with γ-CM-Glu-58-RNase T_1. The disposition of the carboxylate group of γ-CM-Glu-58-RNase T_1 adjacent to the 3′ position of the nucleoside is also consistent with the observation that the affinity of the enzyme with Guo-3′-P is considerably reduced upon γ-carboxymethylation of Glu-58 (Takahashi, 1970b; Oshima and Imahori, 1972; Walz, unpublished experiments).

A different role was previously proposed for the His-C and Glu-58 residues of RNase T_1 on the basis of NMR studies (Rüterjans and Pongs, 1971). The observation of an analogous effect on the His-C reonance in Guo and Guo-3′-P complexes with RNase T_1 led these authors to suggest that His-C and consequently Glu-58 were involved in interacting with the guanine moiety of these ligands. However, other interpretations can be considered, since these ligands share several groups in common besides a guanine moiety, such as 2′ and 5′ hydroxyls. Furthermore, if these enzyme groups are involved in interacting with the base group, it would be anticipated that γ-carboxymethylation of Glu-58 would have a common effect on the binding of Guo and Guo-3′-P: as discussed above, this chemical modification *increases* the enzyme's affinity with Guo and *decreases* its affinity with Guo-3′-P. In addition, the characteristics of the difference spectra for guanine nucleoside binding with the enzyme are essentially unperturbed by γ-carboxylmethylation of Glu-58, which also suggests that this residue is not in close proximity with the base group of the ligand. Finally, it is difficult to reconcile the proposed involvement of Glu-58 at the guanine recognition site (Rüterjans and Pongs, 1971) with the reasonable evidence that led to its proposed role as a catalytic group (Takahashi, 1970a).

In summary, the evidence presented in this study supports the view that Glu-58 and a His residue at the active site of RNase T_1 are the principal components of a binding locus for the Guo 2′-hydroxyl group. The proposed nature of the binding interaction at this site involves a carboxylate–imidazolium pair with the carboxylate group forming a hydrogen bond with the Guo 2′-hydroxyl. The significance of these results in terms of RNase T_1 catalysis will be the subject of a future report.

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