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Interaction of Guanine Nucleotides with Ribonuclease F_1' as Studied by Absorption and Circular Dichroism Spectroscopies

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Difference spectra have been observed in both absorption and circular dichroism upon binding of various guanine nucleotides to ribonuclease F_1'. The dissociation constants of the enzyme-nucleotide complexes have been determined based on the difference absorption spectra. The results have been compared with those obtained with ribonuclease T_1.

RNase[1] F_1 isolated from the culture medium of *Fusarium moniliforme* belongs to the RNase T_1 family which is composed of mostly guanine specific endoribonucleases of fungal origin. The members of this family show considerable structural and functional varieties so that comparisons among them would provide useful informations on their structure-function relationships. RNase F_1 is guanine specific[2] like RNase T_1, the representative of the family, but homology between them is only about 60%.[3] Moreover, disulfide bridges, which tend to be well conserved among homologous proteins, are located differently in these RNases.[4] They are, therefore, considered to be structurally distant homologs. Thus, it will be of interest to compare them from functional points of view. In this report, we have investigated interaction of RNase F_1 with some guanine nucleotides by means of absorption and CD spectroscopy.

It is known that binding of a guanine nucleotide to RNase T_1 induces the change of absorption[5] and CD[6,7] spectra in the region 240-310 nm. Namely, the spectrum of the mixture of RNase T_1 and a guanine nucleotide differs from the sum of the spectra of the enzyme and the nucleotide measured separately. Various explanations, some controversial, have been proposed for these phenomena. Among them, stacking interaction between the nucleotide and the aromatic (Trp and/or Tyr) residue(s) in the active site of the enzyme has been pointed out by us as the most plausible explanation.[8] This interaction has first been proposed by Sander and Ts'o as an explanation for the difference CD spectrum,[6] but it can be considered valid also for the difference absorption spectrum. However, it is not yet known which aromatic residues contribute to the difference spectra. In this respect, RNase F_1 is a natural mutant of choice to assess the role of Trp 59 in RNase T_1, because RNase F_1 has a Phe residue in place of Trp 59.[3] The Trp residue is the strongest chromophore in RNase T_1 and is adjacent to the active site residue Glu 58, thus it is considered to be one of the most probable candidates for the aromatic residues responsible for the change detected in difference spectra.

RNase F_1' (formerly F_2) was used in this study for the reason of supply. It is an isoform of RNase F_1 having an Asp residue at position 106, the C-terminus, in place of the Asn residue in RNase F_1 (unpublised results). It was prepared by the previously reported method.[9] The preparation, however, was unsuitable for precise spectroscopic measurements, because it contained a trace of brown contaminants. One more run of the affinity chromatography and the DEAE-cellulose column chromatography yielded a completely colorless sample, which was used in this study. 2'- and 3'-GMP were prepared from a commercially available mixture by separation on a Dowex 1 x 8 (Cl^- form) column. 2'- and 3'-BrGMP were obtained through bromination of 2'(3')-GMP according to a modified version of the previously reported procedure.[10] The detail of the new method will be described elsewhere.

All measurements were carried out in 10 mM (1 M = 1 mol dm^{-3}) sodium acetate-0.1 M NaCl (pH 5.6) with a Hitachi 220A spectrophotometer thermostatted at 25 °C and a JASCO J-40A spectropolarimeter equipped with a data processor J-DPZ. The molar absorbance of RNase F_1' at 280 nm was determined to be 1.39×10^4 M^{-1} cm^{-1} in the above solvent, based on quantitation of the protein with a JEOL JLC-200A amino acid analyzer after hydrolysis with 6 M HCl at 110 °C for 20 h. The methods for obtaining difference absorption spectra and determining the dissociation constants of RNase F_1'-ligand complex were described previously.[8]

Figure 1 shows the difference absorption spectra with 2'-GMP and 2'-BrGMP as ligands. The spectra were taken at about 16 μM concentration of the protein with 10% excess of the ligand. The difference absorbances actually observed were approximately 0.12 in a 2 cm light path at peak wavelengths. The results are shown in molar difference absorbance ($\Delta\varepsilon$) scale. Scale conversion was performed so that $\Delta\varepsilon$ at the peak wavelength ($\Delta\varepsilon_{max}$) gave the determined value shown in Table 1. 2'-GMP and 2'-BrGMP induced difference spectra of different shapes, the peaks being at 291 nm and 294 nm, respectively. The difference spectra with 3'- and 5'-GMP as ligands were the same as that with 2'-GMP, whereas the difference spectrum with 3'-BrGMP was slightly different from that with 2'-BrGMP: the crossover point was at 275 nm instead of 267 nm (data not shown). This minor difference between 2'-BrGMP and 3'-BrGMP has also been observed with RNase T_1.[8] Table 1 summarizes the determined dissociation constants (K_d) of RNase F_1'-guanine nucleotide complex together with the related parameters: λ_{max} and λ_o are the wavelengths at which the difference spectrum reaches a maximum and zero,

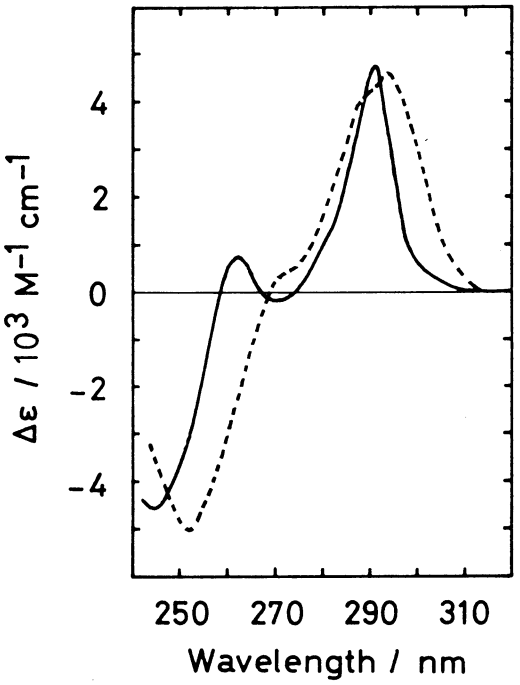

Fig. 1. Difference absorption spectra observed for RNase F_1' and 2'-GMP (——) or 2'-BrGMP (---).

Table 1. Dissociation constants of RNase F_1'-nucleotide complexes and related parameters

Nucleotide	λ_{max}/nm	λ_o/nm	$\Delta\varepsilon_{max}/10^3$ m^{-1} cm^{-1}	K_d/μM
2'-GMP	291	267	4.78	5.5
3'-GMP	291	267	4.50	13
5'-GMP	291	267	4.95	85
2'-BrGMP	294	267	4.66	1.2
3'-BrGMP	294	275	4.92	9.3

respectively. The dissociation constant changed depending on the position of phosphate in the nucleotides, the affinity decreasing in the following order: 2' > 3' ≫ 5'. The brominated nucleotide interacted with the enzyme more strongly than the corresponding parent nucleotides. Especially noteworthy is the dissociation constant for 2'-BrGMP which is 4.6 times lower than that for 2'-GMP.

Figure 2 shows the difference CD spectra with 2'-GMP and 2'-BrGMP as ligands. The spectra were taken at the following concentrations of protein and ligands: 40.3 μM RNase F_1' and 191 μM 2'-GMP; 34.0 μM RNase F_1' and 87.1 μM 2'-BrGMP. Under these conditions, more than 97% of the protein was expected to form the complex from the dissociation constants and the nucleotides exhibited very weak CD by themselves. Therefore, the difference spectrum was obtained as follows. The CD spectra of the protein in the presence and absence of a nucleotide were recorded and the difference between the two spectra was calculated with the data processor. The results are shown in a molecular ellipticity ([θ]) scale. With 2'-GMP as a ligand, a peak at 253 nm and a trough at 285 nm were observed. With 2'-BrGMP, the peak and the trough red-shifted to 260 nm and 294 nm, respectively, and dcreased in magnitude. The 3'-isomers gave similar difference spectra to those obtained with the corresponding 2'-isomers, although minor quantitative differences were observed (data not shown).

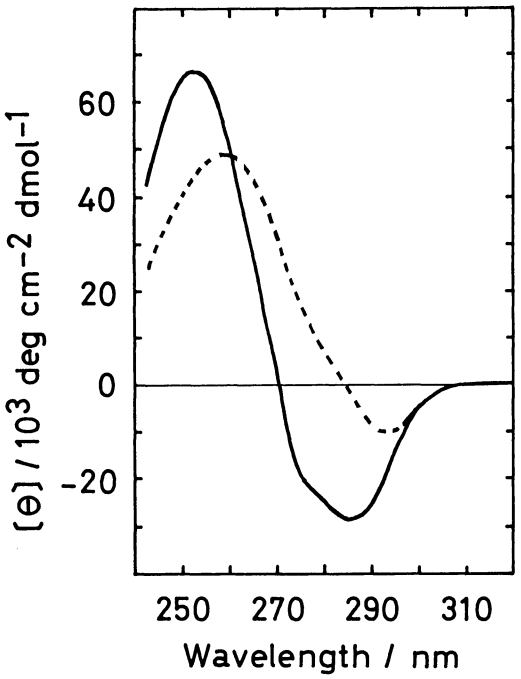

Fig. 2. Difference CD spectra observed for RNase F_1' and 2'-GMP (——) or 2'-BrGMP (- - -).
1 deg = 1 degree = (π/180) rad.

These results obtained by both absorption and CD spectroscopies are, as a whole, very similar to those observed with RNase T_1 in the following aspects: the shape and magnitude of the difference absorption[11] and CD[6] spectra, dependence of the affinity of nucleotides on the position of phosphate (2' > 3' ≫ 5'),[11] and

change of the spectra due to the introduction of a Br atom at C8 of the guanine base.[8] This extensive similarity is surprising, because the absorption and CD spectra of RNases T_1 and F_1' themselves differ considerably in reflection of the Trp → Phe replacement. A distinction, however, does exist: 2'-BrGMP binds 4.6 times more strongly to RNase F_1' than 2'-GMP, whereas both nucleotides bind to RNase T_1 with almost the same affinity.[8] Free 2'-BrGMP may have a conformation close to that of the guanine nucleotide bound to the active site of RNase F_1', thereby gaining some of the free energy necessary for the fixation of the nucleotide conformation that accompanies the binding.

In conclusion, the present study shows a high similarity but a subtle difference between the active sites of RNases F_1' and T_1 despite their structurally distant relation. This study also implicates that Trp 59 of RNase T_1 is not the origin of the difference absorption and CD spectra. Probably, the responsible residues are Tyr 42 and Tyr 45, which have been shown to be close to the bound guanine nucleotide by an X-ray crystallographic study.[12] These residues are both conserved in RNase F_1'. In addition, the following remarks should be made, though the aspects have been fully discussed with RNase T_1 by us.[8] BrGMP has a reduced electron density on N7 and a *syn* fixed conformation because of the bulky, electron-withdrawing Br substituent at C8. The strong interaction of 2'-BrGMP with RNase F_1' indicates that the electron density on N7 is not important and that the guanine nucleotide binds to the enzyme in a *syn* conformation.

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References

1) Abbreviations used are: RNase, ribonuclease; GMP, guanosine monophosphate; BrGMP, 8-bromoguanosine monophosphate. The prefix 2'-, 3'-, or 5'- shows the position of a phosphate group in a nucleotide. The three-letter symbols are used for amino acid residues.
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