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Kinetic Constants of Ribonuclease F_1 Acetylated at Lysine 62 toward High Molecular Weight RNA

(ribonuclease F_1/acetylation/enzyme kinetics)

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Kinetic constants toward high molecular weight RNA have been determined with native ribonuclease F_1 and the enzyme acetylated at lysine 62. The Michaelis constant and the maximum velocity are 0.75 mg/ml and 0.32 U/nM, respectively, with ribonuclease F_1 and 0.93 mg/ml and 0.13 U/nM, respectively, with the acetylated enzyme. These results suggest that lysine 62 does not constitute a phosphate binding subsite, but somehow participates in the catalytic breakdown of high molecular weight substrates.

Introduction

Ribonuclease (RNase) F_1 is a guanine specific endoribonuclease isolated from the culture filtrate of a phytopathogenic fungus, *Fusarium moniliforme* (1). It is a homolog of well known RNase T_1 and its structure-function relationship is now under investigation. Comparison of the primary structures of RNases belonging to the RNase T_1 family reveals a unique feature of RNase F_1 (Fig.1). At position 62, which is near the active site residue Glu 58, RNase F_1 has a lysine residue, whereas most other RNases except those of *Fusarium* genus and RNase U_1 have a hydrophobic residue such as Leu, Met or Val.

Hanazawa acetylated this Lys residue and examined enzymatic properties of the acetylated RNase F_1 (AcRNase F_1)(2). He found that AcRNase F_1 has the same binding affinity as RNase F_1 for 2'- or 3'-guanosine monophosphate (GMP) and that it shows the same enzymatic activity toward the low molecular weight substrates, 2',3'-cyclic GMP and guanylyl

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	55					60					65
RNase T_1	-Pro	Tyr	-Tyr	Glu	Trp	Pro	Ile	Leu	-Ser	-Ser	-Gly-
RNase N_1	-Pro	Trp	-Tyr	Glu	Phe	Pro	Ile	Leu	-Ser	-Ser	-Gly-
RNase C_2	-Asn	Tyr	-Tyr	Glu	Trp	Pro	Ile	Leu	-Ser	-Ser	-Gly-
RNase Ms	-Ser	Tyr	-Tyr	Glu	Tyr	Pro	Ile	Met	-Ser	-Asp	-Tyr-
RNase Pb	-Thr	Tyr	-Tyr	Glu	Phe	Pro	Ile	Leu	-Lys	-Ser	-Gly-
RNase Pch_1	-Thr	Tyr	-Tyr	Glu	Phe	Pro	Ile	Leu	-Arg	-Ser	-Gly-
RNase F_1	-Pro	Tyr	-Gln	Glu	Phe	Pro	Ile	(Lys)	-Ser	-Gly	-Gly-
RNase Fl_1	-Pro	Tyr	-Gln	Glu	Phe	Pro	Ile	Arg	-Thr	-Gly	-Gly-
RNase Fl_2	-Pro	Tyr	-Gln	Glu	Phe	Pro	Ile	Arg	-Thr	-Ser	-Gly-
RNase U_1	-Pro	Tyr	-Lys	Glu	Tyr	Pro	Leu	Lys	-Thr	-Ser	-Ser-
RNase U_2	-Pro	Trp	-Ser	Glu	Phe	Pro	Leu	Val	-Tyr	-Asn	-Gly-

Fig.1. Comparison of the primary stuctures from residue 55 to 65 of RNases belonging to the RNase T_1 family. The sequence number shown above is that for RNase T_1. Active site Glu 58 is underlined. Invariant and conservative residues are boxed with continuous and broken lines, respectively. Lys 62 of RNase F_1 is encricled. The origins of RNases are as follows: T_1, *Aspergillus oryzae*; N_1, *Neurospora crassa*; C_2, *A. clavatus*; Ms, *A. saitoi*; Pb, *Penicillium brevicompactum*; Pch_1, *P. chrysogenum*; F_1, *Fusarium moniliforme*; Fl_1 and Fl_2, *F. lateritium*; U_1 and U_2, *Ustilaga sphaerogena*.

-(3'-5')-cytidine. These results show that the acetylation of Lys 62 does not affect the basic recognition and catalysis of the enzyme. Interestingly, however, the activity toward high molecular wight RNA was lowered to some extent by the acetylation.

RNase F_1 has a blocked N-terminus and very few basic residues, Arg 17 and Lys 62, except for His 40, Arg 76 and His 91 which are implicated in the active site by homology with RNase T_1. When RNase F_1 acts on polyanionic RNA substrate, it is probable that some of the positively charged basic residues would play a role as a phosphate binding subsite. Therefore, in this study, we have determined the kinetic constants of AcRNase F_1 toward high molecular weight RNA to test the above hypothesis.

Materials and Methods

RNase F_1 was prepared from Toyocelase A as described previously (1). AcRNase F_1 was prepared by Hanazawa (2) according to the method of Riordan and Vallee (3). High molecular weight RNA of *Torula* yeast was purchased from Calbiochem. According to the supplier, it consists mainly of ribosomal RNA and contains minimal amount of transfer RNA and degradation products of ribosomal RNA.

RNA concentration was determined on the basis of absorbance at 260 nm (A_{260}). A solution with $A_{260}=1$ was assumed to have a concentration of 50 μg/ml. RNase F_1 and AcRNase F_1 were determined on the basis of A_{280}, using the molar absorbance of 1.39×10^4 M^{-1} cm^{-1} (4). Enzyme assay was carried out as described previously (4), except that the substrate was added at various concentrations (0.5 – 3 mg/ml). The enzyme concentrations used in the assay were 1.11 nM and 3.41 nM for RNase F_1 and AcRNase F_1, respectively.

Results and Discussion

Enzyme activities of RNase F_1 and AcRNase F_1 toward high molecular weght RNA was measured at various substrate concentrations and the results are shown in Fig.2 in the form of double reciprocal plots.

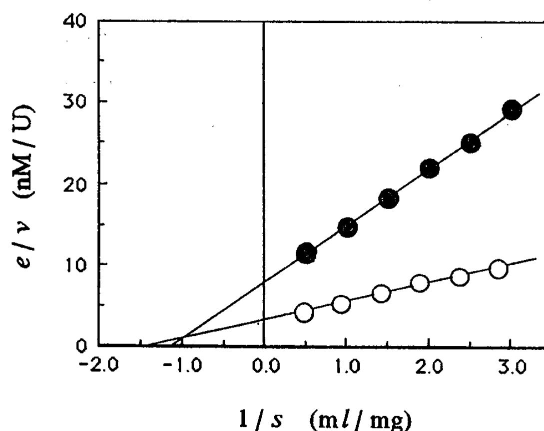

Fig.2. The double reciplocal plots for RNase F1 (○) and AcRNase F1 (●). *s* is substrate concentration, *v* is intial velocity, and *e* is enzyme concentration.

From these results, the Michaelis constant Km and the maximum velocity V toward the RNA were determined as follows:

RNase F_1; Km=0.75mg/ml, V=0.32U/nM,

AcRNase F_1; Km=0.93mg/ml, V=0.13U/nM.

On acetylation of Lys 62 of RNase F_1, the Km value remained virtually unchanged, whereas the V value was lowered to 40% of the original level. This suggests that Lys 62 is not a phoshate binding subsite. If it were the subsite, the Km value would have been increased.

Hanazawa reported that the acetylation did not affect the activity toward low molecular weight substrates (2). We cannot explain at present why the acetylation lowers the enzyme activity only toward the high molecular weight substrate. However, our observation suggests that there may be some factor(s) other than those in the active site which controls the enzyme activity toward polymer substrates. We are planning investigations to elucidate the mechanism of the contorol.

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