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Revision of the Amino Acid Sequence of Ribonuclease F1

(ribonuclease/amino acid sequence/peptide separation)

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The amino acid sequence of ribonuclease F1 reported earlier (Hirabayashi and Yoshida (1983) Biochem. Int. 7, 255-262) was revised. The revision was an interchange between residue 32 (Ser) and residue 36 (Thr). Thus, the correct sequence from residue 32 to 36 is -Thr-Ala-Gly-Ser-Ser-. The error in the original sequence had been pointed out by X-ray crystallography (Vassylyev et al. (1993) J. Mol. Biol. 230, 979-996) and confirmed chemically in the present study.

Ribonuclease (RNase) F1 is a guanine specific endoribonuclease isolated from the culture filtrate of a phytopathogenic fungus, *Fusarium moniliforme*. Hirabayashi and Yoshida (1) determined its amino acid sequence and showed that it was a homolog of well-known RNase T1 of *Aspergillus oryzae*. Aiming at determination of the positions of disulfide bonds, Yoshida and Naijo (2) digested native RNase F1 simultaneously by trypsin and chymotrypsin and separated the resulting peptides (TC-peptides). Then, they examined the amino acid compositions of cystine-containing peptides and established the disulfide pairs, Cys6-Cys102 and Cys24-Cys83. In addition, they isolated many other TC-peptides and showed that their amino acid compositions could be interpreted without contradiction with the reported sequence.

Later, however, the sequence turned out to contain some errors. In the course of investigating the position of deamidated asparagine residue, Hanazawa et al. (3) found that the C-terminal sequence should be -*Gly*-Thr-*Asn* rather than -*Asn*-Thr-*Gly* as initially reported. This correction is an interchange between a Gly residue and an Asn resisue, thus the error cannot be detected by amino acid analysis. It is, therefore, understandable that the error escaped detection by Yoshida and Naijo. Another error was found in the study of the tertiary structure of RNase F1 by X-ray crystallograpy (4). Usually, electron density maps derived from X-ray analyses are interpreted on the basis of the amino acid sequence determined chemically or genetically. However, in the case of RNase F1, some parts of the electron density map were so clear that the side chains in those parts were identified unambiguously without knowledge of the sequence. As a result, the sequence from residue 32 to 36 was revised as -*Thr*-Ala-Gly-Ser-*Ser*- from -*Ser*-Ala-Gly-Ser-*Thr*-. Again, the correction was an interchange between a Thr residue and a Ser residue. Here we present chemical evidence that the correction based on X-ray crystallography is indeed correct.

Yoshida and Naijo had isolated a peptide preparation named TCV-11 which might include the sequence in question. The amino acid composition of TCV-11 is shown in the first column of Table I. The composition was tentatively interpreted as that of a peptide from Gln28 to Tyr42 which should have the compsition shown in the middle column of Table I. RNase F1 has a Tyr residue at position 27, so this peptide seemed a reasonable product of chymotryptic cleavage. However, this peptide was not included in the paper, because some ambiguity remained in the interpretation. In fact, direct application of the Edman degradation to peptide preparation TCV-11 revealed that this preparation was a mixture of two peptides with the following sequences:

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Tyr-Gln-Asn-Asp-Asp-*Thr*-Ala-Gly-Ser-*Ser*-Thr-Tyr-?- (A)

Gln-Asn-Asp-Asp-?-Ala-Gly-Ser-*Ser*-Thr-Tyr-Pro-His-Thr-Tyr (B)

peptide A being the major component. At this stage, it was already apparent that the revision made on the basis of X-ray crystallography was correct. However, we tried to separate and sequence these two peptides. Separation of peptide preparation TCV-11 on an Asahipak GS-320 column (7.6 x 500 mm, Asahi Chemical Industry) using 0.1 M acetic acid as a solvent at a flow rate of 1.0 ml/min afforded two peptides, TCV-11-1 and TCV-11-2 (Fig. 1).

These peptides were subjected to further purification by reversed phase high performance liquid chromatography using an ODS column (Cosmosil 4.6 x 250 mm, Nacalai Tesque). After an isocratic elution for 5 min with 0.1% trifluoroacetic acid, a linear gradient elution was performed from 0% to 42% acetonitrile in 0.1% trifluoroacetic acid in 40 min. The flow rate was always 1.0 ml/min. Peptide TCV-11-1 yielded a major peptide TCV-11-1a at a retention time of 30.0 min followed by two small minor peptides. On the other hand, TCV-11-2 was homogeneous on the chromatography, eluting at 26.7 min (data not shown). The final homogeneous peptides were sequenced using an Applied Biosystems 477A protein sequencer and the results shown below were obtained:

TCV-11-1a, Gln-Asn-Asp-Asp-*Thr*-Ala-Gly-Ser-*Ser*-Thr-Tyr-Pro-His-Thr-Tyr

TCV-11-2, Tyr-Gln-Asn-Asp-Asp-*Thr*-Ala-Gly-Ser-*Ser*-Thr-Tyr.

The results clearly show that the revision made by X-ray crystallography is correct. The amino acid composition of peptide preparation TCV-11 can be explained as that of a mixture of TCV-11-1a and TCV-11-2 at a molar ratio of 0.6 : 1 (the last column of Table I). The final version of the amino acid sequence of RNase F1 is shown in Fig. 2.

Production of TCV-11-1a by chymotryptic digestion of RNase F1 is understandable in view of the known specificity of chymotrypsin. Cleavage at the C-terminal side of Tyr27 and Tyr42 would yield the peptide. On the contrary, TCV-11-2 must have arisen from cleavage at the C-terminal side of Tyr28 and Tyr38 that is followed by Pro39. As a textbook knowledge, chymotrypsin does not cleave a peptide bond at the N-terminal side of a Pro residue. However, this cleavage may occur albeit slowly, since TCV-11-2 was actually obtained. Recently, we have isolated and sequenced a cDNA encoding RNase F1 (to be published). The result confirmed the sequence shown in Fig. 2.

The Edman degradation on preparation TCV-11 was carried out by Dr. J. Hirabayashi at the

Table 1. Amino acid composition of peptide preparation TCV-11.

Amino acid	Observed	Interpreted as	
		Peptide Gln28-Tyr42	Mixture[a]
Histidine	0.55	1.0	0.6
Aspartic acid	3.16	3.0	3.0
Threonine	2.49	3.0	2.4
Serine	1.72	2.0	2.0
Glutamic acid	1.00	1.0	1.0
Proline	+	1.0	0.6
Glycine	1.51	1.0	1.0
Alanine	0.57	1.0	1.0
Tyrosine	1.60	2.0	2.0

The results are expressed in molar ratio.
[a]Calculated as a mixture of TCV-11-1a and TCV-11-2 at a ratio of 0.6:1

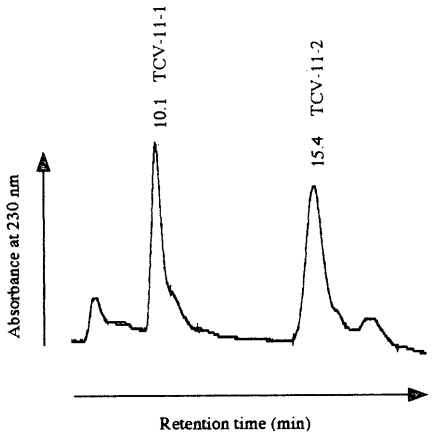

Fig. 1. Separation of peptide preparation TCV-11 on an Asahipak column. The retention time (min) of each peptide is shown on top of the peak. Experimental details are described in the text.

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  1  Pyr-Ser-Ala-Thr-Thr-Cys-Gly-Ser-Thr-Asn-
 11  Tyr-Ser-Ala-Ser-Gln-Val-Arg-Ala-Ala-Ala-
 21  Asn-Ala-Ala-Cys-Gln-Tyr-Tyr-Gln-Asn-Asp-
 31  Asp-Thr-Ala-Gly-Ser-Ser-Thr-Tyr-Pro-His-
 41  Thr-Tyr-Asn-Asn-Tyr-Glu-Gly-Phe-Asp-Phe-
 51  Pro-Val-Asp-Gly-Pro-Tyr-Gln-Glu-Phe-Pro-
 61  Ile-Lys-Ser-Gly-Gly-Val-Tyr-Thr-Gly-Gly-
 71  Ser-Pro-Gly-Ala-Asp-Arg-Val-Val-Ile-Asn-
 81  Thr-Asn-Cys-Glu-Tyr-Ala-Gly-Ala-Ile-Thr-
 91  His-Thr-Gly-Ala-Ser-Gly-Asn-Asn-Phe-Val-
101  Gly-Cys-Ser-Gly-Thr-Asn
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Fig. 2. The revised amino acid sequence of RNase F1. Pyr stands for a pyroglutamic acid residue.

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