Note

Identification of Cd-binding Peptides of Fission Yeast *Schizosaccharomyces pombe* by FRIT-FAB LC/MSYutaka KON-YA, Etsuro YOSHIMURA,
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Upon exposing fission yeast *Schizosaccharomyces pombe* to the Cd ion, Cd-peptide complexes are induced. These complexes are composed of Cd ions, acid-labile sulfides, and of peptides named cadystin, whose structures have been elucidated as $(\gamma\text{-Glu-Cys})_n\text{-Gly}$ ($n=2$ and 3).¹⁾ A family of these peptides with $n \geq 2$ has also been shown to occur in plants,²⁻⁵⁾ and the name phytochelatin has been proposed for this kind of peptide. The peptides are considered to be involved in detoxification and homeostasis of heavy metal ions.

There are a few reports which have shown the occurrence of desglycyl peptides in Cd-peptide complexes. One of the Cd-binding peptides purified from *Zea mays* has been proved to be lacking in Gly, being composed of Glu and Cys, by an amino acid analysis.⁶⁾ In addition, based on a comparison of the amino acid composition between an acid hydrolysate and enzymatic digest of the Cd-binding peptides from *S. pombe* and *Candida glabrata*, the presence of desglycyl peptides has been suggested.^{7,8)}

In this study, the peptides in a Cd-peptide complex of *S. pombe* were characterized by the recent coupling technique of high-performance liquid chromatography (HPLC) with fast atom bombardment mass spectrometry (Frit-FAB LC/MS^{9,10)}.

S. pombe (IAM 4779) was cultivated in a medium containing 2% glucose, 2% tryptone, 1% yeast extract and 1 mM CdCl₂ for 6 days. The cells were harvested and homogenized with quartz sand in buffer A (25 mM Tris-HCl, pH 7.5, containing 50 mM KCl and 1 μ M CdCl₂). The homogenate was centrifuged at $15,000 \times g$ for 30 min at 4°C, and the supernatant was applied to a Sephadex G-50 column (16 $\times$ 650 mm, Pharmacia) with buffer A. Cadmium and sulfhydryl groups in the fractions were measured by atomic absorption spectrometry and by the method of Ellman,¹¹⁾ respectively. The Cd and sulfhydryl-containing fractions (elution volume from 70 to 100 ml) were collected, loaded into a QAE-Sephadex A-25 column

(16 $\times$ 140 mm) that had been equilibrated with 25 mM Tris-HCl at pH 7.5, and chromatographed by a linear gradient of 0–1 M NaCl in the same buffer as that used for the equilibration. The fractions rich in Cd and sulfhydryl groups, which appeared at around 0.6 M NaCl, were collected, desalted by a Sephadex G-15 column and lyophilized. The dried material was dissolved in 0.1% trifluoroacetic acid (TFA) and subjected to FRIT-FAB LC/MS (JEOL model JMS-AX505W) with a 4.6 mm $\times$ 15 cm ODS column (Nomura Kagaku Co.). A combination of isocratic elution (0.1% TFA-acetonitrile = 93:7 for 5 min) and successive linear gradient elution (0.1% TFA-acetonitrile = 93:7 to 80:20 in 15 min) was employed, in which the flow rate of the eluent was 1.0 ml/min. The eluate was monitored by absorption at 210 nm, and a 10% glycerol solution was added to it just prior to its introduction to the MS spectrometer.

As shown in Fig. 1(A), the HPLC chromatogram gave four apparent peaks designated as P-1 to P-4, apart from that appearing at the void volume. Mass spectra of the peaks are shown in Fig. 1(B) to (E), respectively. In Fig. 1(B), a base peak appeared at m/z 540, which corresponds to the mass number of $[(\gamma\text{-Glu-Cys})_2\text{-Gly} + \text{H}]^+$. The mass numbers expected to have arisen from the fragmentation of $(\gamma\text{-Glu-Cys})_2\text{-Gly}$ are shown in Fig. 2, in which the ions observed in the spectrum are marked by asterisks. The nomenclature in Ref. 12 is used for of the fragment ions in the spectrum of $(\gamma\text{-Glu-Cys})_2\text{-Gly}$. More than a half of the N-terminal ions and only the Y"-series of the C-terminal ions can be seen in the spectrum. P-1 was, therefore, assigned as $(\gamma\text{-Glu-Cys})_2\text{-Gly}$, which many workers have isolated from this fungus.

The mass spectrum of P-2 showed a base peak at m/z 483, which corresponds to a substance lacking in Gly from $(\gamma\text{-Glu-Cys})_2\text{-Gly}$. In this mass spectrum, the N-terminal fragment ions which appeared in the P-1 spectrum were also observed. However, the peaks at m/z 179 and 308, the C-terminal fragment ions from P-1, did not occur, and peaks at m/z 122 and 251 emerged with minor intensity, which differ by a 57 mass unit from the C-terminal ions of P-1. These data supported P-2 being assigned to the peptide of $(\gamma\text{-Gly-Cys})_2$. In a similar manner, P-3 and P-4 could be assigned to $(\gamma\text{-Glu-Cys})_3\text{-Gly}$ and $(\gamma\text{-Glu-Cys})_3$, respectively.

The levels of the desglycyl peptides depended on the culturing period. They were not detected in the earlier stage of culture, while they increased with culturing period (data not shown). The synthetic pathway for the desglycyl peptides has not been clarified. According to the report¹³⁾ on the synthesis of the peptides, $(\gamma\text{-Glu-Cys})_2\text{-Gly}$ was formed by transpeptidation of the $\gamma\text{-Glu-Cys}$ moiety of glutathione to another glutathione, and $(\gamma\text{-Glu-Cys})_n\text{-Gly}$ with $n \geq 3$ was also successively formed by a similar transpeptidation. For the synthesis of the desglycyl peptides, there are two possible mechanisms. One is that the $\gamma\text{-Glu-Cys}$ moiety of glutathione is transferred to the $\gamma\text{-Glu-Cys}$ molecule that is the precursor of glutathione.

Fig. 1. FRIT-FAB LC/MS Analysis of the Cd-Binding Complex in *S. pombe*.

(A): Chromatogram monitored by the absorption at 210 nm. (B), (C), (D) and (E): Mass spectra of P-1, P-2, P-3 and P-4, respectively.

X	437	334	205	102*
Y''	411	308*	179*	76*
Z	394	291	162	59
$\gamma\text{Glu} - \text{Cys} - \gamma\text{Glu} - \text{Cys} - \text{Gly}$				
A	102*	205	334	437
B	130*	233*	362	465
C''	147*	250*	379*	482

Fig. 2. Possible Fragment Ions from the Peptide ($\gamma\text{-Glu-Cys}$)₂-Gly.

The ions observed in the mass spectrum shown in Fig. 1(B) are marked by an asterisk. In peptide $R_N\text{CONHR}_C$, where R_N and R_C are the N- and C-terminal moieties, respectively, the fragment ions are represented as follows: A, R_N^+ ; B, $R_N\text{CO}^+$; C'', $[R_N\text{CONH}_2 + \text{H}]^+$; X, CONHR_C^+ ; Y'', $[\text{H}_2\text{NR}_C + \text{H}]^+$; Z, R_C^+ . The number of prime indicates that of the hydrogen atom transferred.

The other is that Gly is released from the formed peptides by hydrolytic cleavage by carboxypeptidase. At present, it is unclear which process acts. These desglycyl peptides could form heavy metal complexes,⁷⁾ so that they would be considered to function as a detoxification substance for the metals together with the peptides, ($\gamma\text{-Glu-Cys}$)_n-Gly ($n \geq 2$). A further study is, therefore, needed to elucidate the synthetic mechanisms.

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