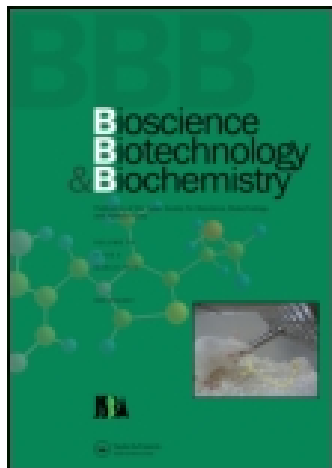


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Conversion of Glutathione into Cadystins and Their Analogs Catalyzed by Carboxy-peptidase Y

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Published online: 12 Jun 2014.

To cite this article: Imai Kunio, Obata Hitoshi, Shimizu Keisuke & Komiya Takashi (1996) Conversion of Glutathione into Cadystins and Their Analogs Catalyzed by Carboxy-peptidase Y, *Bioscience, Biotechnology, and Biochemistry*, 60:7, 1193-1194, DOI: [10.1271/bbb.60.1193](https://doi.org/10.1271/bbb.60.1193)

To link to this article: <http://dx.doi.org/10.1271/bbb.60.1193>

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Note

Conversion of Glutathione into Cadystins and Their Analogs Catalyzed by Carboxypeptidase Y

Kunio IMAI,[†] Hitoshi OBATA, Keisuke SHIMIZU, and Takashi KOMIYA

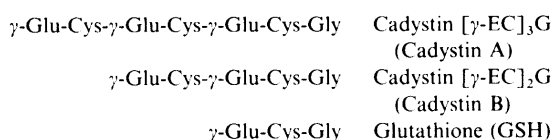
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Received January 19, 1996

Cadystins induced in a fission yeast treated with Cd²⁺ are the higher homologs of glutathione. In the present work, glutathione was incubated with Carboxypeptidase Y at a high substrate concentration. The reaction afforded not only the degraded product, but also cadystins and their analogs. A possible transformation pathway for glutathione by this enzyme is proposed.

Key words: cadystin; [γ -EC]_nG; glutathione; enzymic conversion; phytochelatin

A fission yeast, *Schizosaccharomyces pombe*, produced metallothioneine-like complexes Cd-BP 1 and 2, which were composed of unit peptides and cadmium ions, when the yeast was treated with the cadmium ion.¹⁾ The structures of the unit peptides, named cadystin A and B, were clarified as shown below, by using enzymic and chemical reactions, in 1984^{2a)}:



These peptides have a repeating unit of γ -Glu-Cys ending up with Gly, and they are the higher homologs of glutathione (GSH). In 1985, Grill *et al.* found cadystins and their higher homologs in plants, and called the peptides "phytochelatins."³⁾ In the present paper, we use the names "cadystin [γ -EC]₂G" and "cadystin [γ -EC]₃G" for cadystin B and A, respectively, to avoid unnecessary confusion.^{2c)} During the structural analysis of cadystins, interesting specificity of carboxypeptidase P (Takara Shuzo Co.) was found.²⁾ The enzyme hydrolyzed the Cys-Glu and Cys-Gly linkages of cadystins, but it did not cleave the γ -Glu-Cys bonds, the reaction thus affording Gly and a dipeptide, γ -Glu-Cys, in a high yield after reduction with 1,2-ethanedithiol. The specificity of the enzyme and the structural resemblance between cadystins and GSH suggested to us the possibility that carboxypeptidases would catalyze the conversion of GSH into cadystins.

In the present study, carboxypeptidase Y (CPase Y, Peptide Institute Co.) was employed instead of carboxypeptidase P, because the application of CPase Y for amide exchange has been well established. To facilitate the amide exchange, the reaction was carried out at a higher substrate concentration in a more basic reaction medium than the usual conditions used for digestion of peptides; otherwise, hydrolysis would predominate. The substrate, GSH (18.6 mg), was treated with 20 μ l of a CPase Y solution (2.5 mg in 500 μ l of water) in 110 μ l of a 34 mM phosphate buffer (pH 9.7) at 37°C. The reaction was carried out under a nitrogen atmosphere to prevent air oxidation of the substrate and the products. After appropriate intervals of the reaction time, one μ l of the reaction mixture was taken out and diluted with 100 μ l of 0.1% TFA. Each acidified sample of the reaction mixture was

analyzed by reversed-phase HPLC (RP-HPLC). A typical chromatogram of the mixture after 90 min of the reaction is shown in Fig. 1, and the time-course plots of the enzymic products are summarized in Fig. 2. The chromatogram shows that the reaction afforded 6 major products. These products must have been produced by the action of carboxypeptidase Y, as no reaction took place without the enzyme. Products 1, 3, 4, and 6 (P-1, P-3, P-4, and P-6) increased gradually until 240 min, and products 2 and 5 (P-2 and P-5) increased until 90 min and then decreased slowly (Fig. 2).

To characterize the products, the enzyme reaction was carried

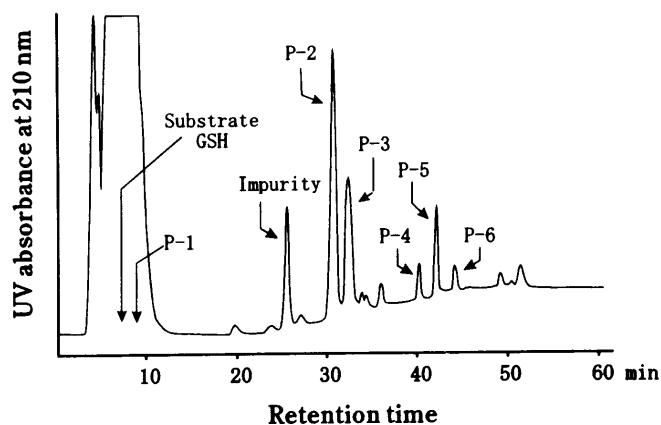


Fig. 1. High-performance Liquid Chromatogram of the Enzymic Products after 90 min of Incubation.

Chromatographic conditions: Column, Develosil ODS-5 (4.6 mm \times 150 mm); solvent, 0.10% CH₃CN in 0.1% TFA (60 min); flow rate, 0.5 ml/min; detection, UV absorbance at 210 nm.

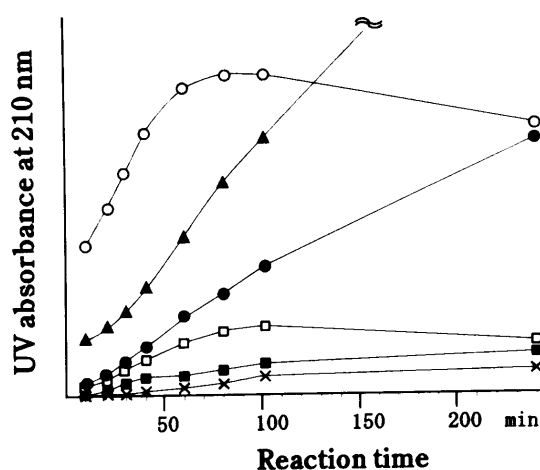


Fig. 2. Time-Course Plots of the Enzyme Reaction Products.

▲, P-1 ([γ -EC]); ○, P-2 (Cadystin [γ -EC]₂G); ●, P-3 ([γ -EC]₂); ×, P-4 (Dehydrated product of [γ -EC]₂); □, P-5 (Cadystin [γ -EC]₃G); ■, P-6 ([γ -EC]₃).

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out once again, and the reaction was stopped by the addition of TFA after 90 min. The major products were isolated by semi-preparative RP-HPLC under the same elution conditions as those used for analytical chromatography. Re-chromatography of the isolated products indicated that P-2 and P-5 had the same retention times as those of authentic cadystins $[\gamma\text{-EC}]_2\text{G}$ and $[\gamma\text{-EC}]_3\text{G}$.^{2b)} To compare the chromatographic behavior of P-2 and P-5 with that of authentic cadystins after pyridylethylation, these peptides were treated with an excess amount of 4-vinylpyridine in a basic medium under a nitrogen atmosphere. After removing the unreacted reagent by repeated extraction with ether, each remaining aqueous solution was analyzed by RP-HPLC. Pyridylethylated P-2 was eluted at 54 min from a Develosil ODS-5 column (0–10% CH_3CN in 0.1% TFA, 60 min, 0.5 ml/min), and the retention time was the same as that of authentic pyridylethylated cadystin $[\gamma\text{-EC}]_2\text{G}$ (data not shown). Similarly, pyridylethylated P-5 was eluted at the same retention time (67 min) as that of authentic pyridylethylated cadystin $[\gamma\text{-EC}]_3\text{G}$ from the Develosil ODS column (0–10% CH_3CN in 0.1% TFA, 60 min, and then 10–20% CH_3CN in 0.1% TFA, 30 min, 0.5 ml/min; data not shown). These results strongly suggested that P-2 and P-5 were cadystins $[\gamma\text{-EC}]_2\text{G}$ and $[\gamma\text{-EC}]_3\text{G}$, respectively.

To confirm the structures of P-2 and P-5, their molecular weights were measured by secondary ion mass spectrometry (SIMS). The observed protonated molecular ion peaks for P-2 and P-5 were correct for cadystins $[\gamma\text{-EC}]_2\text{G}$ ($[\text{M} + \text{H}]^+ = 540$) and $[\gamma\text{-EC}]_3\text{G}$ ($[\text{M} + \text{H}]^+ = 772$).

The spectra and chromatographic behavior led us to the conclusion that P-2 and P-5 were cadystins $[\gamma\text{-EC}]_2\text{G}$ and $[\gamma\text{-EC}]_3\text{G}$, respectively, and that the cadystins were produced from GSH by the action of CPase Y.

To investigate the structures of the other products, their molecular weights were measured by SIMS. The $[\text{M} + \text{H}]^+$ peaks were observed at 483 and 715 for P-3 and P-6, respectively. The results suggested that P-3 and P-6 were $[\gamma\text{-EC}]_2$ and $[\gamma\text{-EC}]_3$, which were the degraded products of cadystins $[\gamma\text{-EC}]_2\text{G}$ and $[\gamma\text{-EC}]_3\text{G}$. The structure of P-1 was speculated to be $[\gamma\text{-EC}]$ by comparing the chromatographic behavior of P-1 with that of authentic $[\gamma\text{-EC}]$ by RP-HPLC. Although the mass spectrum of P-4 ($[\text{M} + \text{H}]^+ = 465$) suggested that it could be a dehydrated product of $[\gamma\text{-EC}]_2$, no further structural analysis was attempted.

Considering the structures of the products, the conversion pathway for glutathione by CPase Y is proposed as shown in Fig. 3. At first, the amide carbonyl group of cysteine in the substrate is attacked by the nucleophilic amino group of the other GSH molecule. Glycine of the substrate is then substituted by the

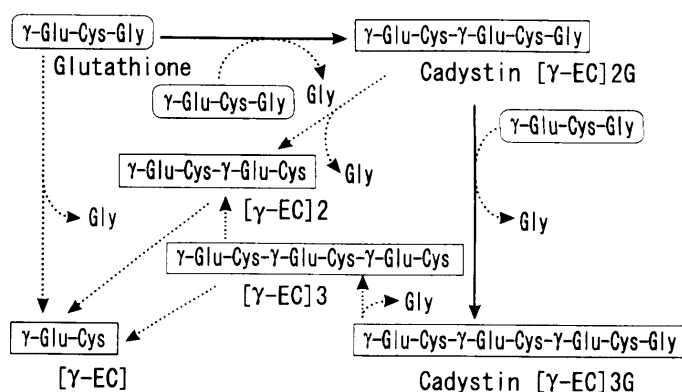


Fig. 3. Possible Transformation Pathway for Glutathione by Carboxypeptidase Y.

attacking GSH molecule to afford cadystin $[\gamma\text{-EC}]_2\text{G}$. Then, the same reaction takes place on the primary product, cadystin $[\gamma\text{-EC}]_2\text{G}$, and gives rise to cadystin $[\gamma\text{-EC}]_3\text{G}$. The products and the substrate are gradually hydrolyzed to afford $[\gamma\text{-EC}]_3$, $[\gamma\text{-EC}]_2$, and $[\gamma\text{-EC}]$. From $[\gamma\text{-EC}]_3$ and $[\gamma\text{-EC}]_2$, the dipeptide unit is successively cleaved out to afford $[\gamma\text{-EC}]$ as the final product.

Although the yields of cadystins were not satisfactory (6.7% for $[\gamma\text{-EC}]_2\text{G}$ and 1.6% for $[\gamma\text{-EC}]_3\text{G}$ after their isolation), the present research proves that carboxypeptidase Y catalyzed the conversion of GSH into cadystins and their analogs.

Acknowledgments. The authors acknowledge the financial support by grants for Scientific Research from Ministry of Education, Science, and Culture of Japan and by Okasan-Kato Foundation.

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