Cerium(IV)–Cyclodextrin Complex for Peptide Hydrolysis in Neutral Homogeneous Solutions

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Di- and tri-peptides are efficiently hydrolysed by the cerium(IV)–γ-cyclodextrin complex in neutral and homogeneous solutions.

Molecular design of catalysts for site selective hydrolysis of peptides is an important subject in biochemistry and biotechnology as well as in the related fields. Many attempts to hydrolyze peptides using transition metal ions or complexes have been reported.[1–4] Some of the most active catalysts are heterogeneous lanthanide hydroxide gels: Up to 70% of dipeptide is hydrolyzed at pH 8.6, 70 °C, on the basis of the paper chromatographic analysis.[1] However, in order to achieve the site selective hydrolysis of peptides, and to clarify the reaction mechanism in more detail, homogeneous catalysts are definitely required. We now wish to report that Ce^{4+} ions solubilized by γ-CyD (CyD = cyclodextrin) effectively hydrolyze peptides in homogeneous states under neutral conditions.

First, the lanthanide salts-induced hydrolyses of dipeptides[1] were reinvestigated more quantitatively by HPLC† and ^1H NMR spectroscopy. Gly-Phe is efficiently hydrolyzed into Gly and Phe at pH 7 at 70 °C. Hydrolysis with $Ce^{IV}(NH_4)_2(NO_3)_6$, the most active salt, is nearly complete after 48 h. Other lanthanide ions showed moderate activities. The scission is totally hydrolytic and no side reaction occurs.‡ Furthermore, no deuterium replacement of α-proton is observed in D_2O, indicating the absence of racemization during the reaction. Apparently, lanthanide ions are potentially useful for the hydrolyses of peptides. However, precipitates of Ce^{4+}–hydroxide gels are readily formed under the conditions employed, which is a significant obstacle for further application. Thus, we have investigated the catalysis of the homogeneous Ce^{4+}–γ-CyD complex for peptide hydrolysis. As recently found by us,[5] the solubility of Ce^{4+} ion is dramatically increased by CyD over a wide pH range due to the formation of a noninclusion type complex.

Di- or tri-peptide (10 mmol dm^{-3}) was dissolved in an aqueous solution of $Ce(NH_4)_2(NO_3)_6$ (10 mmol dm^{-3}) and γ-CyD (50 mmol dm^{-3}), and the mixture was heated at 60 °C for 24 h. The mixture was totally homogeneous throughout the reaction, in contrast to the prompt precipitation of cerium(IV)–hydroxide in the absence of γ-CyD.

The results at pH 8 are listed in Table 1. Each peptide is hydrolyzed by Ce^{4+}–γ-CyD to the corresponding amino acid fragments. The reaction rate is not remarkably dependent on the nature of the amino acid residues (runs 1–4). The rate for the hydrolysis of Gly–Phe shows a maximum at pH 8. Tripeptides are also hydrolyzed (runs 5 and 6), providing mixtures of the dipeptides and the amino acids. The product distributions indicate that the amide bond near the *N*-terminus is hydrolyzed more preferentially relative to the one near the *C*-terminus.

Table 1 Extent of hydrolysis of di- or tri-peptides by Ce^{4+}–γ-CyD at 60 °C, pH 8.0 after 24 h[a]

Run	Substrate	Conversion (%)
1	Gly–Phe	39
2	Phe–Gly	36
3	Gly–Tyr	29
4	Gly–Trp	29
5	Gly–Gly–Phe	20
6	Phe–Gly–Gly	17
7	Gly–Phe–NH_2	4
8	Ac–Gly–Phe	8

[a] 0.1 mol dm^{-3} Tris buffer. $[Ce(NH_4)_2(NO_3)_6]_0 = [peptide]_0 = 0.01$ mol dm^{-3}, $[\gamma\text{-CyD}]_0 = 0.05$ mol dm^{-3}.

The ^1H NMR shifts of Gly–Phe (20 mmol dm^{-3}) induced by Pr^{3+} (20 mmol dm^{-3}) are summarized in Fig. 1. α-Protons both in the Gly and Phe units show marked induced shifts at pD 6.8. However, the induced shift for the Gly moiety considerably decreases with decreasing pD (0.41 at pD 6.8 and 0.16 ppm at pD 5.7). In contrast, those for the Phe moiety are less affected by this pD change. Apparently, Gly–Phe coordinates to the lanthanide ion with the amino group of the *N*-terminus along with the carboxyl group of the *C*-terminus, and the coordination of the amino group is largely promoted upon increasing the pD from 5.7 to 6.8. The increase of the hydrolysis rate from pH 5 to 8 indicates that the coordination of both of the amino group and of carboxyl group is required for effective hydrolysis.§

Consistently, terminal protection of dipeptides has a significant effect on the hydrolysis. The hydrolysis of both *C*- and *N*-protected Gly–Phe (Gly–Phe–NH_2, run 7; Ac–Gly–Phe, run 8) are considerably lower than that for Gly–Phe itself (run 1).

The remarkably high activities of lanthanide compounds for hydrolysis under neutral conditions can be ascribed to their characteristic coordination behaviour. It is known that amide groups in peptides coordinate to transition metal ions with both nitrogen and oxygen. The former mode promotes deprotonation of the amide nitrogen, and renders the peptide inactive towards hydrolysis. Because lanthanide ions preferentially coordinate to oxygen rather than to nitrogen, the inactive-type coordination would be minimized under neutral conditions, resulting in high activities for hydrolysis.¶

Furthermore, coordination numbers of lanthanide ions are higher than those of d-transition metal ions, and the geometries are flexible. Therefore, both the hydroxide ion and the chelated peptide can occupy the internal coordination sphere of the lanthanide ion, giving rise to intramolecular attack of the hydroxide ion to the peptide.‖ A plausible mechanism is shown in Fig. 2.

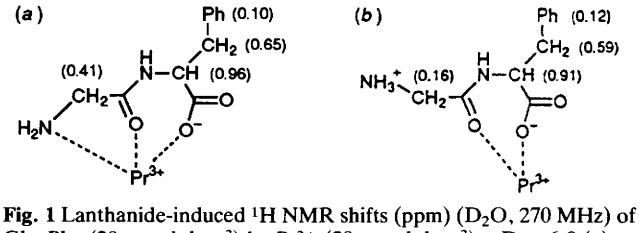

Fig. 1 Lanthanide-induced ^1H NMR shifts (ppm) (D_2O, 270 MHz) of Gly–Phe (20 mmol dm^{-3}) by Pr^{3+} (20 mmol dm^{-3}). pD = 6.8 (*a*) or 5.7 (*b*)

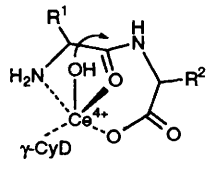

Fig. 2 A plausible mechanism for the hydrolysis of dipeptides by Ce^{4+}–γ-CyD

In conclusion, an efficient hydrolysis of peptides is accomplished with the homogeneous Ce^{4+}–γ-CyD complex under neutral conditions. The complex system will be useful as an active site of artificial peptidases.

This work was partially supported by the Kawakami Memorial Foundation.

Received, 26th April 1994; Com. 4/02481H

Footnotes

† HPLC analyses were performed by using an ODS column (Lichrospher 100 RP-18(e) 5 μm, 4 mm i.d. × 250 mm), eluting with H_2O–MeCN (9/1, v/v) buffered with acetate (pH 3.5), and detected by UV absorption.

‡ Complex products, probably due to oxidation by Ce^{4+}, were observed only when the reaction was conducted below pH 1.

§ Since pK_a of coordinated water on Ce^{4+} is *ca.* 0,[6] the Ce^{4+}–OH^- species, which attacks the peptide as a nucleophile, would be provided in the pH range investigated. Therefore, the pH dependence of the reaction cannot be explained by this factor.

¶ The inactive-type coordination would occur at pH higher than 8, and this is one of the factors for the decrease in the hydrolysis rate above this pH. In contrast, Cu^{2+} is essentially inactive for hydrolysis of peptides at pH > 6, because the inactive-type coordination is dominant under such conditions.[2]

‖ Such intramolecular attack of coordinated water to the chelated peptide has been ruled out for the mechanism of peptide hydrolyses by complexes of Co^{3+},[4] Pt^{2+}, and Pd^{2+},[3] since the coordination numbers and the steric geometries are more restricted for these systems.

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