Metal Species for Amide Hydrolysis

Literature Seminar, 20130511
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§3 Protein-Selective Hydrolysis of Amides
§1 Introduction
Characteristics of Amide Bond

- stability toward hydrolytic cleavage

\[
\text{Me} \text{N} \text{H} \text{O} \text{H} \text{N} \text{O} \text{OH} \xrightarrow{t_{1/2} = 500 \text{ years}} \text{Me} \text{N} \text{H} \text{O} \text{H} \text{N} \text{O} \text{OH} + \text{H}_2\text{N} \text{COOH}
\]

pH = 6.8, 25 °C


- This stability derives from resonance effect.

\[
\begin{align*}
\text{R}^1 & \quad \text{R}^2 \\
\text{NH} & \quad \text{O} \\
\end{align*}
\]

resonance effect

\[
\begin{align*}
\text{R}^1 & \quad \text{R}^2 \\
\text{N} & \quad \text{OH} \\
\end{align*}
\]

inactive toward nucleophilic attack
Characteristics of Amide Bond

- planar structure due to the resonance effect

○ twist angle distribution of tertiary amides

Destabilizing Amide Bond

- activation by Lewis acid

\[
\begin{align*}
\text{Acid} & \quad \xrightarrow{\text{Lewis acid}} \quad \delta^+ \\
\text{R}_1\text{N}\text{R}_2\text{H} & \quad \xrightarrow{\text{cleavage}} \quad \text{R}_1\text{CO}_\text{R}_2 + \text{H}_2\text{N}\text{R}_2
\end{align*}
\]

- activation by bond distortion

\[
\begin{align*}
\text{R}_1\text{N}\text{R}_2\text{H} & \quad \xrightarrow{\text{bond distortion}} \quad \delta^+ \\
\text{III} & \quad \xrightarrow{\text{cleavage}} \quad \text{H}_\text{N}\text{R}_1 + \text{HO}_\text{R}_2
\end{align*}
\]
§1 Introduction

Precedents: Activation by Lewis Acid

- **hydrolysis by cerium(IV)-cyclodextrin complex**

  \[
  \text{Ce(NH}_4\text{)}_2(\text{NO}_3\text{)}_6 \text{ (1 eq.)} + \gamma\text{-CD (5 eq.)} \\
  0.1 \text{ M Tris buffer (0.01 M)} \\
  \text{pH 8.0, 60 °C, 24 h} \\
  \rightarrow \\
  \text{H}_2\text{N} - \text{CH}_2 - \text{COOH} + \text{H}_2\text{N} - \text{CH}_2 - \text{COOH}
  \]

  39% conversion


- **Zinc-catalyzed solvolysis**

  \[
  \text{Cbz} - \text{H}_2\text{N} - \text{CH}_2 - \text{COOMe} \\
  \text{Zn(OTf)}_2 \text{ (5 mol%)} + \text{diethylcarbonate (2 eq.)} \\
  n\text{-BuOH (2 M)} \\
  \text{reflux, 45 h} \\
  \rightarrow \\
  \text{Cbz} - \text{H}_2\text{N} - \text{CH}_2 - \text{CON} - \text{Bu}
  \]

  84% yield

§1 Introduction

Precedents: Activation by Bond Twisting

- molecules containing twisted amide

- total synthesis of
    (tetrafluoroborate salt)

- characters

<table>
<thead>
<tr>
<th></th>
<th>twist angle</th>
<th>bond length C-N</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-quinoclidone</td>
<td>2.5°</td>
<td>1.325 Å</td>
</tr>
<tr>
<td>1-aza-2-adamantanone (Kirby’s Amide)</td>
<td>90.5°</td>
<td>1.475 Å</td>
</tr>
<tr>
<td></td>
<td>90.9°</td>
<td>1.526 Å</td>
</tr>
</tbody>
</table>

(Values are cited from the above 2 papers.)
Precedents: Activation by Bond Twisting

- hydrolysis of twisted amides

\[ \text{NH}_4\text{OBF}_4 \quad t_{1/2} < 15 \text{ s} \quad \text{D}_2\text{O, r.t.} \]

\[ \text{CO}_2\text{D} \]

\[ \text{N} \quad \text{H} \quad \text{O} \quad \text{BF}_4^- \]

\[ \text{H}_2\text{O} \quad \text{over a few minutes} \]

\[ \text{NH}_2\text{COO}^- \]

§1 Introduction

In This Seminar...

- activation by Lewis acid

\[
\begin{align*}
\text{O} & \quad \text{N} & \quad \text{R}_1 & \quad \text{R}_2 \\
\text{H} & \quad \text{N} & \quad \text{R}_1 & \quad \text{H} \\
\text{O} & \quad \text{R}_2 & \quad \text{H} & \quad \text{N} \\
\end{align*}
\]

Lewis acid

\[
\begin{align*}
\delta^+ & \quad \text{O} & \quad \text{N} & \quad \text{R}_1 & \quad \text{R}_2 \\
\text{H} & \quad \text{N} & \quad \text{R}_1 & \quad \text{H} \\
\text{O} & \quad \text{R}_2 & \quad \text{H} & \quad \text{N} \\
\end{align*}
\]

cleavage

\[
\begin{align*}
\text{R}_1 & \quad \text{OH} & \quad \text{N} & \quad \text{R}_2 \\
\text{H} & \quad \text{N} & \quad \text{R}_1 & \quad \text{OH} \\
\text{H} & \quad \text{N} & \quad \text{R}_2 & \quad \text{OH} \\
\end{align*}
\]

- activation by bond distortion

\[
\begin{align*}
\text{O} & \quad \text{N} & \quad \text{R}_1 & \quad \text{R}_2 \\
\text{H} & \quad \text{N} & \quad \text{R}_1 & \quad \text{H} \\
\text{O} & \quad \text{R}_2 & \quad \text{H} & \quad \text{N} \\
\end{align*}
\]

bond distortion

\[
\begin{align*}
\delta^+ & \quad \text{O} & \quad \text{N} & \quad \text{R}_1 & \quad \text{R}_2 \\
\text{H} & \quad \text{N} & \quad \text{R}_1 & \quad \text{H} \\
\text{O} & \quad \text{R}_2 & \quad \text{H} & \quad \text{N} \\
\end{align*}
\]

cleavage

\[
\begin{align*}
\text{H} & \quad \text{N} & \quad \text{R}_1 & \quad \text{OH} \\
\text{H} & \quad \text{N} & \quad \text{R}_1 & \quad \text{OH} \\
\text{H} & \quad \text{N} & \quad \text{R}_1 & \quad \text{OH} \\
\end{align*}
\]
§2 Residue- or Sequence-Selective Hydrolysis of Amides
§2 Residue- or Sequence-Selective Hydrolysis of Amides

**Palladium(II) Complexes**

\[
\begin{align*}
[H_2O]_2PdOH_2 & \quad [H_2O]_2PdNH_2OH_2 & \quad [MeS\beta-CD]_2PdOH_2H_2O
\end{align*}
\]

\[
[Pd(H_2O)_4]^{2+} \quad [Pd(en)(H_2O)_2]^{2+}
\]

\[
\beta-CD = \text{structure}
\]
§2 Residue- or Sequence-Selective Hydrolysis of Amides

Acidic Hydrolysis with $[\text{Pd(H}_2\text{O)}_4]^{2+}$

- substrate: Met-peptide

\[
\text{Ac-Ala-Lys-Tyr-Gly-Gly-Met-Ala-Ala-Arg-Ala-OH}
\]

- hydrolysis condition

\[
\text{Met-peptide} \xrightarrow{[\text{Pd(H}_2\text{O)}_4]^{2+} \text{ (1 eq.)}} \text{fragments}
\]

aqueous media (1 mM)

pH 2.3, 60 °C

24 h

§2 Residue- or Sequence-Selective Hydrolysis of Amides

Acidic Hydrolysis with \([\text{Pd(H}_2\text{O)}_4]^{2+}\)

- experiment procedure

60 °C

5 min: \([\text{Pd(H}_2\text{O)}_4]^{2+}\) was bound to the peptide. (checked by HPLC and MALDI MS)

24 h: two components were observed.

§2 Residue- or Sequence-Selective Hydrolysis of Amides

Acidic Hydrolysis with $[\text{Pd(H}_2\text{O)}_4]^{2+}$

- HPLC charts

2 peaks were separated by HPLC and MALDI mass spectroscopic identification was conducted.

§2 Residue- or Sequence-Selective Hydrolysis of Amides

Acidic Hydrolysis with $[\text{Pd(H}_2\text{O)}_4]^{2+}$

- composition of the fractions

Ac- Ala- Lys- Tyr- Gly- OH

H- Gly- Met- Ala- Ala- Arg- Ala- OH

Ac- Ala- Lys- Tyr- Gly- Gly- Met- Ala- Ala- Arg- Ala- OH

§2 Residue- or Sequence-Selective Hydrolysis of Amides

Acidic Hydrolysis with $[\text{Pd(H}_2\text{O)}_4]^{2+}$

- TOCSY $^1\text{H}$ NMR spectrum

○ SMe singlet: 2.12 ppm $\rightarrow$ 2.45 ppm

$\Rightarrow$ Pd(II) complex was bound to the Met6 side chain.

§2 Residue- or Sequence-Selective Hydrolysis of Amides

Acidic Hydrolysis with $[\text{Pd(H}_2\text{O)}_4]^ {2+}$

- TOCSY $^1\text{H}$ NMR spectrum

- **Met6**$_{\text{NH-CH}}$ signal disappeared: deprotonated
  = complete coordination of amide to the Pd(II) ion

Residue- or Sequence-Selective Hydrolysis of Amides

Acidic Hydrolysis with $\text{[Pd(H}_2\text{O)}_4]^{2+}$

• TOCSY $^1$H NMR spectrum

Doctors derived from Gly5 and Met6 disappeared.

= Amide cleavage occurred at Gly4-Gly5.

Acidic Hydrolysis with $[\text{Pd(H}_2\text{O)}_4]^{2+}$

- equilibrium among Pd(II) complexes

○ 2 and 3 exists at pH 2.3, and 3 is major.

• **2** is hydrolytically **active**, but **3** is hydrolytically **inactive**.

Coordination of 2 amide nitrogen atoms → Lewis acidity of Pd(II) ion was quenched.

Acidic Hydrolysis with \([\text{Pd(en)}(\text{H}_2\text{O})_2]^{2+}\)

- **substrate:** oxidized chain B of bovine insulin

\[
\text{H-Phe-Val-Asn-Gln-His-Leu-Cys}^{\text{OX}}-\text{Gly-Ser-His-Leu-Val-Glu-Ala-Leu-Tyr-Leu-Val-Cys}^{\text{OX}}-\text{Gly-Glu-Arg-Gly-Phe-Phe-Tyr-Thr-Pro-Lys-Ala-OH}
\]

- **hydrolysis condition**

  oxidized chain B of bovine insulin  \[\text{[Pd(en)(H}_2\text{O})_2]^{2+} \text{ (5 eq.)}\]

  aqueous media (1 mM)  pH 2.0, 60 °C  4 days  fragments

Acidic Hydrolysis with $[\text{Pd}(\text{en})(\text{H}_2\text{O})_2]^{2+}$

- cleavage site

H-Phe-Val-Asn-Gln-His-Leu-Cys$^{\text{OX}}$-Gly-Ser-His-Leu-Val-
-Glu-Ala-Leu-Tyr-Leu-Val-Cys$^{\text{OX}}$-Gly-Glu-Arg-Gly-Phe-
-Phe-Tyr-Thr-Pro-Lys-Ala-OH

- $\text{Pd(II)}$ reagent promoted cleavage of the second bond upstream from the $\text{His5}$ and $\text{His10}$ anchor.

(characterized by MALDI mass spectroscopic identification and amino acid analysis: N-terminal Edman degradation)

Acidic Hydrolysis with $[\text{Pd(en)}(\text{H}_2\text{O})_2]^{2+}$

- equilibrium among Pd(II) complexes

$\text{NH}_2 \text{Pd} \text{NH}_2$[Pd(en)(H$_2$O)$_2$]$^{2+}$

$\text{[Pd(en)}(\text{H}_2\text{O})_2]^{2+}$

$\text{[Pd(en)}_2]^{2+} + 3\text{H}_3\text{O}^+$

$\text{2 and 3 exists at pH 2.0}$

1 active

2 inactive

Neutral Hydrolysis with $[\text{Pd}(\text{H}_2\text{O})_4]^{2+}$

- substrates & cleavage site


N. M. Kostić et al., J. Am. Chem. Soc. 2003, 125, 781.
Neutral Hydrolysis with $[\text{Pd}(\text{H}_2\text{O})_4]^{2+}$

- dependence on pH of the 1st-order-rate const.

reaction: peptide $\xrightarrow{[\text{Pd}(\text{H}_2\text{O})_4]^{2+} \ (1 \text{ eq.})}$ fragments

aqueous media (1 mM) 60 °C

<table>
<thead>
<tr>
<th>pH</th>
<th>ProMet-peptide</th>
<th>SarMet-peptide</th>
<th>GlyMet-peptide</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>587(40)</td>
<td>146(8)</td>
<td>28(2)</td>
</tr>
<tr>
<td>2.5</td>
<td>150(2)</td>
<td>93(3)</td>
<td>5(2)</td>
</tr>
<tr>
<td>3.0</td>
<td>67(3)</td>
<td>57(2)</td>
<td>0</td>
</tr>
<tr>
<td>4.0</td>
<td>47(2)</td>
<td>39(4)</td>
<td>0</td>
</tr>
<tr>
<td>5.0</td>
<td>43(2)</td>
<td>24(4)</td>
<td>0</td>
</tr>
<tr>
<td>6.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.0</td>
<td>41(3)</td>
<td>21(4)</td>
<td>0</td>
</tr>
</tbody>
</table>

Neutral Hydrolysis with \([\text{Pd(H}_2\text{O)}_4]^{2+}\)

- Difference between GlyMet- (left) and ProMet- (right) peptide in equilibrium among Pd(II) complexes

\(\text{NH} - \text{CO} \quad \text{NH} - \text{CO} \quad \text{OH}_2 \quad \text{OH}_2\)

1. active (minor*)
2. active

3. inactive (major*)
4. inactive (major*)

(* at pH 2.3)

N. M. Kostić et al., J. Am. Chem. Soc. 2003, 125, 781.
Neutral Hydrolysis with $[\text{Pd(H}_2\text{O)}_4]^{2+}$

- **TOCSY $^1\text{H NMR spectrum (ProMet-peptide)}**

  - signals derived from Met$_7\text{NH}$ disappeared.
  - Met$_7\text{NH}$ group coordinates to Pd(II) ion
  - NH signals of other residues persisted.
    - Other amide groups were not bound either.

Neutral Hydrolysis with $[\text{Pd(H}_2\text{O)}_4]^{2+}$

- external attack vs internal delivery

(a) no cleavage  
(b) cleavage by external attack  
(c) cleavage by internal delivery

○ in this case...

N. M. Kostić et al., J. Am. Chem. Soc. 2003, 125, 781.
Neutral Hydrolysis with $\text{[Pd(H}_2\text{O)}_4\text{]}^{2+}$

- external attack vs internal delivery

○ ROESY $1^H$ NMR spectrum showed the cross peak between the $\text{Gly5}_\alpha$-CH and $\text{Pro6}_\delta$-CH

→ external attack (trans-Gly-Pro)

N. M. Kostić et al., J. Am. Chem. Soc. 2003, 125, 781.
Neutral Hydrolysis with Pd(II)-β-CD complex

- substrate: Met-peptide

\[ \text{Ac-Lys-Gly-Gly-Phe-Ser-Pro-Phe-Phe-Ala-Ala-Arg-Ala-OH} \]

- hydrolysis condition

\[
\begin{align*}
\text{peptide} & \xrightarrow{\text{aqueous media (1 mM)}} \text{fragments} \\
& \leftarrow \text{pH 7.5, 60 °C, 24 h} \\
& \text{(10 eq. PdOH2H2O, MeS-Pd-S-β-CD)}
\end{align*}
\]
Neutral Hydrolysis with Pd(II)-β-CD complex

- cleavage site

Ac-Lys-Gly-Gly-Phe-Ser-Pro-Phe-Ala-Ala-Arg-Ala-OH

- Pd(II)-β-CD complex promoted cleavage of the first one upstream from the Pro6-Phe7 sequence.

(characterized by MALDI mass spectroscopic identification)
Neutral Hydrolysis with Pd(II)-β-CD complex

- possible interaction toward sequence selectivity

CD cavities bind hydrophobic substrates in aqueous media.
Summary of This Section

- **Residue-selective hydrolysis**

  \[ \text{AcLys-Tyr-Gly} + \text{Gly-Met-Ala-Ala-Gly} + \text{Pro-Met-Ala-Ala-Arg-Gly} \]

  \[ [\text{Pd(H}_2\text{O})_4]^{2+} \quad \text{pH 2.0} \]

  \[ \text{AcLys-Tyr-Gly-Gly-Met-Ala-Ala-Gly-Pro-Met-Ala-Ala-Arg-Gly} \]

  \[ [\text{Pd(H}_2\text{O})_4]^{2+} \quad \text{pH 7.0} \]

  \[ \text{AcLys-Tyr-Gly-Gly-Met-Ala-Ala-Gly} + \text{Pro-Met-Ala-Ala-Arg-Gly} \]

- **Sequence-selective hydrolysis**

  \[ \text{AcLys-Tyr-Gly-Gly-Met-Ala-Ala-Gly} \]

N. M. Kostić et al., J. Am. Chem. Soc. 2003, 125, 781.
§3 Protein-Selective Hydrolysis of Amides
Mb-Selective Artificial Protease

- catalyst design
  - catalyst site: Cyc-metal complex
  - binding site: peptide nucleic acid (PNA)

Based on this design, the library of Cyc-containing PNA oligomers was constructed.

- n=7, 8: no activities* toward Mb (Cu(II)-complex)
- n=9: active* toward Mb (Cu(II)-complex)

(* checked by SDS-PAGE)

§3 Protein-Selective Hydrolysis of Amides

Mb-Selective Artificial Protease

- their own combinatorial library


Cu(II)1 was the best.
Mb-Selective Artificial Protease

• changing metal center from Cu(II) to Co(III)

○ Metal transfer to metal-abstracting materials in living body should be slower for Co(III) complexes because of the exchange-inertness of Co(III)

○ Furthermore, Co(III) shows high reactivity than Cu(II).

\[
\begin{align*}
  k_0(\text{Cu}) &= 5.3 \times 10^{-3} \text{ h}^{-1} \\
  k_0(\text{Co}) &= 9.4 \times 10^{-3} \text{ h}^{-1}
\end{align*}
\]

(pseudo-1st-order kinetics)


Mb-Selective Artificial Protease

- Affinity of Co(III)\(^{1}\) complex to Mb

  - Two straight lines intersect at \([\text{Mb}]_0 = 4.7 \, \mu\text{M}\).
  - \(K_m < 4.7 \, \mu\text{M} < 5.0 \, \mu\text{M}\)
  (cf. \(K_m\) of fumarase = 5.0 \(\mu\text{M}\))

→ Affinity of Co(III)\(^{1}\) complex to Mb was high enough.

→ \(k_{\text{cat}}\) (not \(k_{\text{cat}}/K_m\)) should be considered!!

Mb-Selective Artificial Protease

- $k_{\text{cat}}$ comparison between Co(III) complexes

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>$k_{\text{cat}}$ (10^{-3} h^{-1})</th>
<th>Optimum pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co(III)1</td>
<td>22</td>
<td>7.5</td>
</tr>
<tr>
<td>Co(III)3</td>
<td>2.8</td>
<td>7.5</td>
</tr>
<tr>
<td>Co(III)6</td>
<td>2.4</td>
<td>7.5</td>
</tr>
<tr>
<td>Co(III)7</td>
<td>2.4</td>
<td>7.5</td>
</tr>
<tr>
<td>Co(III)9</td>
<td>4.4</td>
<td>7.5</td>
</tr>
<tr>
<td>Co(III)10</td>
<td>8.9</td>
<td>8.0</td>
</tr>
</tbody>
</table>

→ Co(III)1 was the best!

- pH dependence of $k_{\text{cat}}$ for cleavage of Mb by Co(III)1 complex

→ most active at physiological pH!

Mb-Selective Artificial Protease

- **possible cleavage mechanism**
  - This mechanism consists with the pH dependence of Co(III)1 complex.
  - The following two would reduce the reactivity of Co(III)1 complex.
    - protonation of the hydroxo (T) or oxo (T') ligand at low pH
    - deprotonation of the ammonium ion (T') at high pH

---

Mb-Selective Artificial Protease

- cleavage site characterization

Table 2. Molecular weights of protein fragments disclosed by MALDI-TOF MS and cleavage sites proposed to account for the protein fragments

| Catalyst   | m/z of fragments       | Cleavage site proposed on the basis of m/z values
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Co(III)1</td>
<td>7074, 9891, 8045, 8909</td>
<td>Leu$<em>{89}$-Ala$</em>{90}$, Leu$<em>{72}$-Gly$</em>{73}$</td>
</tr>
<tr>
<td>Co(III)3</td>
<td>7066, 9897</td>
<td>Leu$<em>{89}$-Ala$</em>{90}$</td>
</tr>
<tr>
<td>Co(III)6</td>
<td>7066, 9898</td>
<td>Leu$<em>{89}$-Ala$</em>{90}$</td>
</tr>
<tr>
<td>Co(III)7</td>
<td>7068, 9888</td>
<td>Leu$<em>{89}$-Ala$</em>{90}$</td>
</tr>
<tr>
<td>Co(III)9</td>
<td>7068, 9884</td>
<td>Leu$<em>{89}$-Ala$</em>{90}$</td>
</tr>
<tr>
<td>Co(III)10</td>
<td>7075, 9896</td>
<td>Leu$<em>{89}$-Ala$</em>{90}$</td>
</tr>
</tbody>
</table>

- N-terminal sequencing (Edman degradation) was conducted to product solution of Co(III)$_3$.

→ Leu$_{89}$-Ala$_{90}$ was the cleavage site.

Mb-Selective Artificial Protease

- selectivity (control experiment)

albumin, γ-globulin, elongation factor P, gelatin A, gelatin B

- Above 5 proteins were incubated with Cu(II) or Co(III), but protein cleavage was not observed.

Target selectivity can be expected!

PDF-Selective Artificial Protease

- PDF: peptide deformylase
  - involved in deformylation of the formyl-methionyl derivative of proteins formed in the prokaryotic translational systems.
  - Its inhibitors are searched as candidates for new antibiotic drugs.

way toward “catalytic drugs”

PDF-Selective Artificial Protease

- construction of a library of catalyst candidates
  - synthesized by the Ugi reaction (product was racemic mixture)

\[
\begin{align*}
    R^1\text{OH} + H_2N-R^2 + R^3\text{CH} + R^4\text{NC} & \rightarrow R^1N\tilde{N}R^3\text{CH}O
\end{align*}
\]

- analysis of the result (PDF-cleaving activity)
  - examined by MALDI-TOF mass spectrum

PDF-Selective Artificial Protease

- property of screening

\[
\text{PDF (5 \, \mu M)} \xrightarrow{\text{cat. (1.5-3 \, \mu M)}} \text{fragments} \quad \text{overnight, pH 7.5, 37 °C}
\]

- the result of screening
  - most active complex:

- examined by MALDI-TOF mass spectrum

PDF-Selective Artificial Protease

- cleavage site

- **orange**: PDF / **green**: fragment

- *C*-terminal sequencing by carboxy peptidase A
  \[ \text{Glu152-Arg153 was the cleavage site.} \]

- optimum pH = 7.5 / lower limit of \( k_{\text{cat}} = 0.05 \text{ h}^{-1} \)

---

PDF-Selective Artificial Protease

- docking study result

  - S-isomer of the catalyst with PDF was more stable than with the R-isomer.
  - Central acyclic chain of the catalyst interacted with the C-terminal α-helix, while the 3 aromatic tails made contact with the helical and the loop structures residing above the active site.

selectivity (control experiment)

- 15 other proteins were examined, but none of them were cleaved by the Co(III) complex.

Application to Drug is possible!

list:
1. human cyclin-dependent kinase 2
2. human kinase insert domain receptor
3. human farnesyl transferase
4. hepatitis C virus protease
5. YacM
6. N-acetylglucosamine 1-phosphate uridylyltransferase
7. UDP-N-acetylmuramoyl-L-alanine: D-glutamate ligase
8. non-structural protein 5B of hepatitis C virus
9. human protein-tyrosine phosphatase 1B
10. human retinoic X receptor α
11. human peroxisome proliferator activated receptor α
12. human peroxisome proliferator activated receptor γ
13. human liver X receptor α
14. human liver X receptor β
15. human Caspase-8

AmPs-Selective Artificial Protease

- AmPs: amyloidogenic peptides or proteins
  - lacking active sites
  - Conventional approaches can’t be applied.
  - related to diseases

Peptide-cleaving catalysts is suitable for their drug!

- target AmPs of this research
  - amyloid β protein (Aβ): Alzheimer’s Disease
  - human islet amyloid polypeptide (h-IAPP): type 2 diabetes

construction of a library of catalyst candidates

synthesized according to the route shown below

Aromatic moieties were employed as auxiliary binding components in the combinatorial library.

AmpS-Selective Artificial Protease

- property of screening

\[
\begin{align*}
A\beta \ (4.0 \ \mu M) & \xrightarrow{\text{cat.}} \text{fragments} \\
\text{pH 7.5, 37 °C} & \quad 24 \text{ h}
\end{align*}
\]

- 4 complexes were selected.

\begin{align*}
A\beta_{40}: & \text{active} \\
A\beta_{42}: & \text{active} \\
A\beta_{40}: & \text{active} \\
A\beta_{42}: & \text{active} \\
A\beta_{40}: & \text{inactive} \\
A\beta_{42}: & \text{active} \\
A\beta_{40}: & \text{inactive} \\
A\beta_{42}: & \text{active}
\end{align*}

AmPs-Selective Artificial Protease

- property of screening

h-IAPP (4.0 μM) → cat. → fragments
pH 7.5, 37 °C, 24 h

○ examined by MALDI-TOF mass spectrum

8 complexes were selected.

$A_{\beta 40}$: active

$A_{\beta 42}$: active

$h$-IAPP: active

$A_{\beta 40}$: inactive

$A_{\beta 42}$: inactive

$h$-IAPP: active

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AmPs-Selective Artificial Protease

- selectivity (control experiment)
  - horse heart myoglobin, bovine serum γ-globulin,
    bovine serum albumin, human serum albumin,
    chicken egg white lysozyme, chicken egg ovalbumin,
    bovine pancreas insulin

  - Above 7 proteins were incubated with from A to H, but protein cleavage was not observed.

  "Application to Drug is possible! (Drug for all AmPs may be possible...?)"

Summary of This Section

- Mb- or PDF-selective artificial protease
- AmPs-selective artificial protease
  - Conventional methods can’t get these fruits.