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Site-specifically Hydrolytic Cleavage of Oxidized Insulin B Chain With Cu(II) Ion

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Abstract: Electrospray mass spectrometry investigation shows that denatured oxidized insulin B chain can be selectively cleaved by simple Cu(II) ion and the site of cleavage is at Gly8 – Ser9 bond which is second amide bond left from His 10 in the sequence of oxidized insulin B chain.

Keywords: Oxidized insulin B chain, hydrolytic cleavage, electrospray mass spectrometry, Cu^{2+}.

Selectively chemical cleavage of peptides and proteins is one of the most important reactions in both chemical and biochemical processes. Over the past decade, the interaction of palladium(II) complexes with methionine, cysteine and histidine-containing peptides and proteins and the hydrolytic cleavage of the corresponding amide bond by Pd(II) complexes have been extensively studied[1-13]. However, reports concerning the directly selective hydrolysis of peptides and proteins with other simple transition metal ions are very rare[14,15]. Therefore, there is a huge scope in the development of metal-based cleaving agents, which could selectively hydrolyze peptides and proteins. For this purpose, oxidized insulin B chain that contains two histidine residues of His 5 and His 10 as potential binding sites of Cu(II) ion is chosen to carry out this kind of study.

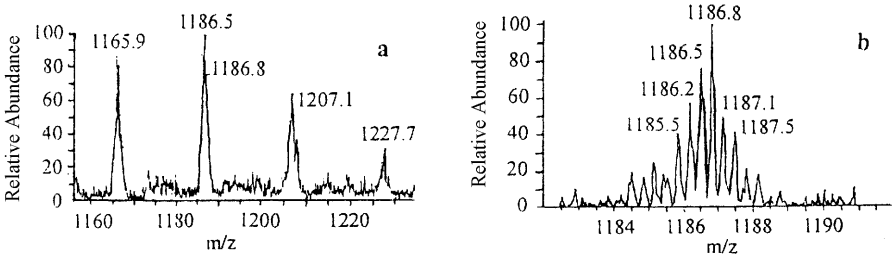
The oxidized insulin B chain (**B**) was prepared and purified according to the published procedure.[16] 1.0 g of insulin was oxidized to yield 0.12 g of oxidized insulin B. The purified **B** was stored in aqueous solution of H_3PO_4 at pH 2.5, with concentration of 20 mg mL^{-1}. Its sequence is as follows: H_2N·Phe-Val-Asn-Gln-His-Leu-Cys(-SO_3H)-Gly-Ser-His-Leu-Val-Glu-Ala-Leu-Tyr-Leu -Val-Cys(-SO_3H)-Gly-Glu-Arg-Gly-Phe-Phe-Tyr-Thr-Pro-Lys-Ala.

LCQ electrospray mass spectrometry (ESMS, Finnigan MAT) was applied to determine the *m/z* of the polypeptide and its complexes of Cu(II) ion. The sample was dissolved in water and diluted to 100 μmol L^{-1}. 1.0 μL of such solution was loaded into the injection valve of the LCQ unit and then injected into the mobile phase solution (50% aqueous methanol containing 1% acetic acid), and carried through the electrospray interface into the mass analyzer at a rate of 200 μL min^{-1}. The employed voltage at the electrospray needles was 5 KV and the capillary was heated to 200°C. A maximum ion injection time of 200 msec along with 10 scans was set. Positive ion mass spectra were obtained. Zoom Scan was used in these experiments. Predicted isotope distribution

patterns for each of complexes were calculated using IsoPro 3.0 program.

The oxidized insulin B chain obtained was analyzed by SDS gel electrophoresis and only one band appeared. The *m/z* of the **B** was determined by ESMS, and the molecular mass determined is 3496.0, precisely equal to 3495.9 calculated by IsoPro3.0 program for $C_{157}H_{232}N_{40}O_{47}S_2$.

Figure 1 ESMS spectrum measured 4 hours after mixing **B** with $CuCl_2$ at room temperature and pH 2.5 (a), and a zoom scan of the peaks with m/z = 1185.1~1187.8 (b). The three groups of peaks, separated by 0.3 m/z each, are attributed to $[Cu\mathbf{B}+H^+]^{3+}$, $[Cu_2\mathbf{B}-H^+]^{3+}$ and $[Cu_3\mathbf{B}-3H^+]^{3+}$, respectively.



An ESMS spectrum (**Figure 1**a) was measured 4 h after mixing $CuCl_2$ with **B** (molar ratio 2:1) in aqueous solution at room temperature and pH 2.5. Besides free **B,** the species $[Cu\mathbf{B}+H]^{3+}$, $[Cu_2\mathbf{B}-H^+]^{3+}$ and $[Cu_3\mathbf{B}-3H^+]^{3+}$ were detected which correspond to the highest *m/z* values of 1186.5, 1207.1 and 1228.1, respectively. The same species were observed in the presence of 6 mol L^{-1} urea. The favored binding sites of the **B** to Cu^{2+} in three species are *N*-terminal NH_3^+, two imidazoles of His 5 and His 10, and deprotonated amide nitrogens from Val 2, His 5 and His 10.

After mixing 10 μL of the stored solution of **B** with 1.5 μL of Cu(II) ion (with concentration of 100 mmol L^{-1}) in a molar ratio of 2 : 1, 11.0 mg of urea was then added. The total volume of the mixture solution was made to be 30 μL and then it was incubated at 40 ± 0.5°C and pH 2.5 for 10 days (a precipitate was formed during one day of incubation without urea). Two groups of peaks in **Figure 2** are produced by double-charged positive ions: one of them with *m/z* = 1296.1~1297.6 fits to ion $[\mathbf{B2}+2Na^+]^{2+}$(**B2** = fragment of Ser9 – Ala30), while the other one with *m/z* = 1304.5~1308.0 corresponds to the ion $[Cu\mathbf{B2}]^{2+}$.

Scheme 1

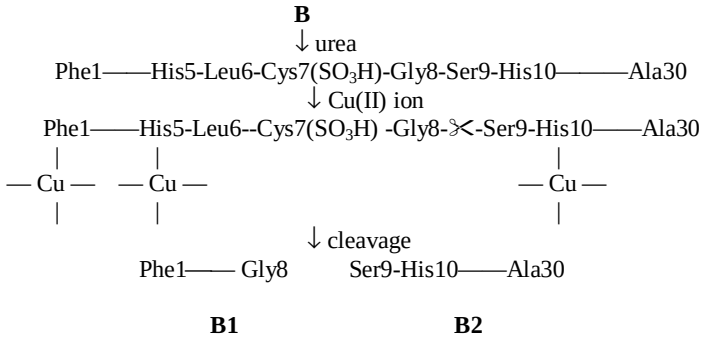
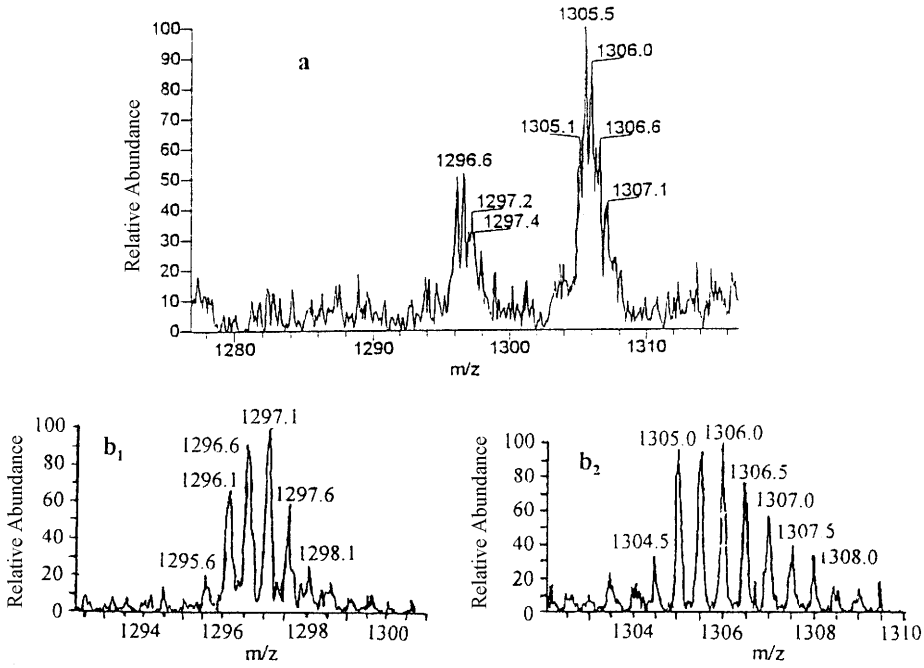


Figure 2 ESMS spectrum of the fragment of **B** cleaved by $CuCl_2$ (a) and zoom scans of two groups of peaks in A (b_1 and b_2) which correspond to calculated isotope distribution patterns of $[\mathbf{B2}+2Na^+]^{2+}$ and $[\mathbf{B2}+Cu(II)]^{2+}$



Therefore, Cu(II) ion only selectively cleaves **B** at Gly8 – Ser9 bond which is second amide bond left from His10, as shown in **scheme 1**. Control experiments with and without urea of 6 mol L^{-1} were carried out at 40°C and pH 2.5 for 32 days and monitored by ESMS. There is no any cleaved fragment observed. This clearly confirms that the selective hydrolysis is promoted by simple Cu(II) ion. G. Allen found the similar site of cleavage in the study of cleavage of Ser-His-containing short peptide with Cu(II) ion. The cleavage of peptide containing Ser-His sequence at the second amide bond left from His demonstrates a specificity of 10-50-fold higher than for other histidine-containing peptides and over 100-fold higher than for peptides lacking histidine residue[15]. Therefore, this report provides new evidence of Cu(II) ion promoted specific cleavage of long peptide containing Ser-His sequence. This site of cleavage which was also observed in the selective hydrolysis of myoglobin with Pd(II) complexes[9] may have potential application in cleavage chemistry of peptides and proteins.

Acknowledgments

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References

1. L. Zhu, N. M. Kostic, *J. Am. Chem. Soc.*, **1993**, *115*, 4566.
2. L. Zhu, L. Qin, T. N. Parac, N. M. Kostic, *J. Am. Chem. Soc.*, **1994**, *116*, 5218.
3. T. N. Parac, N. M. Kostic, *J. Am. Chem. Soc.*, **1996**, *118*, 5946.
4. X. Chen, L. Zhu, H. Yan, X. You, N. M. Kostic, *J. Chem. Soc. Dalton Trans*., **1996**, 2653.
5. T. N. Parac, N. M. Kostic, *J. Am. Chem. Soc.*, **1996**, *118*, 5946.
6. X. Chen, L. Zhu, X. You, et al., *J. Biol. Inorg. Chem.*, **1998**, *3*, 1.
7. T. N. Parac, N. M. Kostic, *Inorg. Chem*., **1998**, *37*, 2141.
8. G. B. Karet, N. M. Kostic, *Inorg. Chem.*, **1998**, *37*, 1021.
9. L. Zhu, R. Bakhtiar, N. M. Kostic, *J. Biol. Inorg. Chem.*, **1998**, *3*, 383.
10. X. Chen, X. Luo, Y. Song, et al., *Polyhedron*, **1998**, *17*, 2271.
11. X. Luo, X. Chen, L. Zhu, *Chemistry Letters*, **1998**, 1079.
12. T. N. Parac, G. M. Ullmann, N. M. Kostic, *J. Am. Chem. Soc.*, **1999,** *121*, 3127
13. X. Luo, W. Huang, Y. Mei, S. Zhou, L. Zhu, *Inorg. Chem*., **1999**, *32*, 1474.
14. E. L. Hegg, J. N. Burstyn, *J. Am. Chem. Soc.*, **1995**, *117*, 7015.
15. G. Allen, R. O. Campbell, *Int. J. Peptide Protein Res.*, **1996**, *48*, 265.
16. F. Sanger, *Biochem. J.*, **1949**, *44*, 126.

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