CATALYTIC ACTIVITIES AND COORDINATION ENVIRONMENTS OF THE COPPER IONS IN THE IMIDAZOLE CLUSTERS OF HISTIDINE-PEPTIDES, HIS(HIS)$_n$GLY AND N-ACETYL-HIS(HIS)$_n$GLY (n = 3, 8, and 18)

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The copper ions in the imidazole-clusters of histidine-oligopeptide, His(His)$_n$Gly (n=18) and N-acetyl-His(His)$_n$Gly(n=8 and 18), showed the d-d transition bands at 520 nm and 630 nm and oxidase-like activities. Those with the d-d bands at 480 nm and 550 nm did not have the activity.

KEY WORDS copper; histidine oligopeptide, imidazole cluster; ascorbate

The importance of histidyl residue, as well as cysteinyl residue, as metal-binding sites in biological systems has been well recognized. Many of those systems which are enzymes and proteins contain copper and iron ions as prothetic groups and perform many functions including electron transfer, dioxygen binding and activation of dioxygen toward substrate oxygenation. X-ray crystallographic studies of the copper-containing proteins have provided much information on the coordination environment of active centers; the metal ions are located in a histidine-rich environment, and active sites consist of more than one metal center.[1-3]

The copper ion in the oxidase functions to mediate electron transfer from the substrate i.e., electron donor to dioxygen i.e., electron acceptor. Copper ion and complexes are shown to be capable of catalyzing the autoxidation of ascorbic acid and thiols of biological relevance.[4-7] Of great interest are the coppers in histidine-containing peptides.[8-10] We had suggested the presence of at least two types of copper, which were classified as catalytically active or inactive/inhibitory ions, in the poly-L-histidine (PLH) complex.[10] The active copper, which seemed labile, was proposed to be located in the imidazole clusters constructed by histidyl side chains. In this paper were communicated the coordination environments of the active and inactive/inhibitory copper ions coordinated to histidine-oligopeptides, His(His)$_n$Gly (n = 3, 8, 18), N-acetyl-His(His)$_n$Gly (n = 3, 8, and 18), and N-acety-His(His)$_n$HisNHCH$_3$(n = 2, 3, and 4), which were newly synthesized.

The peptides were synthesized either by a liquid-phase method using Boc-amino acids or by a solid-phase technique using fmoc-amino acids, and purified by liquid-chromatography.[11] The autoxidation was measured at 25°C and pH 4.5 in 0.02 M acetate buffer. Ascorbate solutions and copper complex solutions were mixed with a UNION MX-7 rapid-mixing apparatus, and subsequent changes in optical absorbance at 260 nm were monitored on a UNION SM-401 spectrophotometer. The activity was expressed by the initial rate of ascorbate oxidation. Circular dichroism(CD) spectrum was obtained on a JASCO J-40 spectropolarimeter.

The catalytic activity of the copper obviously depended on molecular weight and concentra-

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tion, relative to Cu(II), of the peptide. Addition of the peptides with high molecular-weight such as $His(His)_8Gly$, $His(His)_{18}Gly$ and N-acetyl-$His(His)_8Gly$ dramatically enhanced the autoxidation, and the catalytic activity varied depending on the amounts of the peptides added as shown in Fig. 1.

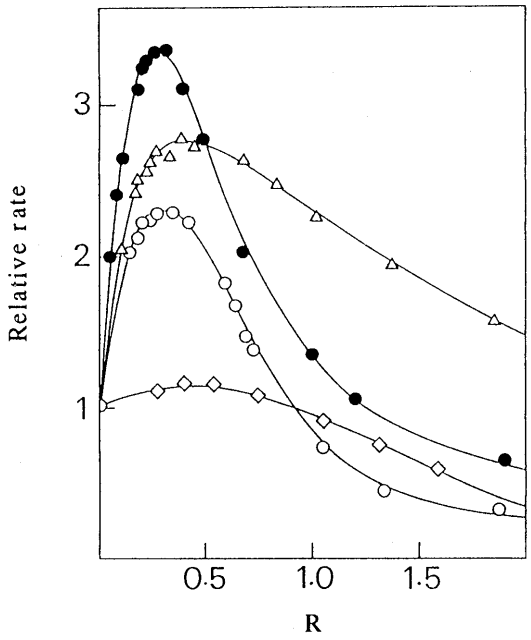

Fig. 1. Dependence of the Rate of Autoxidation on R,[Peptide]/[Cu(II)]
$His(His)_3Gly$: (◇), $His(His)_8Gly$: (○), $His(His)_{18}Gly$: (●), N-acetyl-$His(His)_8Gly$:(△), $[Cu(II)]_0 = 4.1 \times 10^{-6}$ M, $[\text{ascorbic acid}]_0 = 8.0 \times 10^{-5}$ M.

The rate increased with the molar ratio, R=[peptide]/[Cu(II)], reaching a maximum at R = 0.3-0.35, and decreased on addition of those peptides over the optimal molar ratio. Of great significance was that the activity was elevated by acetylation of the peptides at the terminal amino-nitrogen, as observed in $His(His)_8Gly$ and N-acetyl-$His(His)_8Gly$. Low molecular-weight peptides such as $His(His)_3Gly$ and N-acetyl-$His(His)_2$-$HisNHCH_3$ enhanced the reaction a little.

The catalytically active Cu(II) in the complex with high molecular-weight peptides displayed two d-d transition bands: one positive at 520 nm and another negative at 630 nm as measured by circular dichroism spectroscopy, while the Cu(II) inhibiting the autoxidation showed two transition bands at 480 nm(positive) and at 550 nm(negative).[12] As shown in Fig. 2, the bands at 520 nm and 630 nm shifted respectively to 480 nm and 550 nm on addition of increasing amounts of the peptide. High molecular-weight $His(His)_nGly$ probably have two types of the copper-binding site; one is at the N-terminal as observed in GlyHis-X- and GlyGlyHis-X-, where X represents amino acid residues, and another is in the imidazole clusters formed by histidyl residues. In contrast, the 520/630 nm band in both N-acetyl-$His(His)_8Gly$ and N-acetyl-$His(His)_{18}Gly$ complexes did not undergo the hyperchromic shift on addition of large excess of the peptides. Since N-acetyl-$His(His)_nGly$ does not have the Cu(II)-binding site at the N-terminal, the 520/630 nm band is assignable to the d-d transition of the Cu(II) in the histidyl cluster.

The complexes of N-acetyHis$(His)_8Gly$ and N-acetyl-$His(His)_{18}Gly$ contained respectively two and three copper equivalents to one molar peptide, as estimated by the CD titration at λ =

520 nm. The $His(His)_{18}Gly$ complex contained 3.2 Cu(II)s; one Cu(II) was at the N-terminal, and the others were in the clusters. The Cu(II) coordinated with low molecular-weight N-acetyl-$His(His)_nHisNHCH_3$, where n=2 or 3, did not display the 520/630 nm band, while the Cu(II) coordinated with N-acetyl-His-$(His)_4$His-$NHCH_3$ showed the 520/630 nm band. Thus, it is suggested that a sequence of at least six histidyl residues is necessary for the construction of the catalytically active sites involving multi-Cu(II) ions with the d-d transition bands at 520 nm and 630 nm.

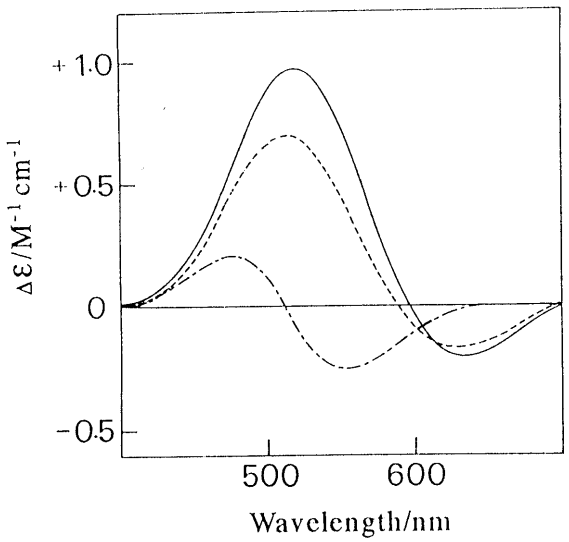

Fig. 2. Circular Dichroism Spectra of Cu(II)-$His(His)_{18}Gly$ Complexes
[Peptide]/[Cu(II)] ; 0.37 : ——, 0.62 : - - -, 1.9 : —·—, [Cu(II)] = 8.1 x 10^{-5} M.

REFERENCES AND NOTES

1) B. Linzedn N.M. Soeter, A. F. Riggs, H.-J. Schneider, W. Schartau, M. D. Moore, E. Yokota, P. Q. Behrens, H. Nakashima, T. Takagi, T. Nomoto, J. M. Verreijken,H. J. Bak, J. J. Beintema, A. Volbeda, W. P. J. Gaykema, W. G. J. Hol, *Science*, **229**, 519 (1985).
2) A. Messerschmidt, A. Rossi, R. Ladenstein, R. Huber, M. Bolognesi, G. Gatti, A. Marchesini, R. Petruzzelli, A. Finazzi-Agro, *J. Mol. Biol.*, **206**, 513 (1989).
3) "Metal Clusters in Proteins," ed by L. Que, Jr., ACS Symposium Series 372, Am. Chem. Soc., Washington, DC (1988).
4) A.Hanaki, *Chem. Pharm. Bull.*, **17**, 1839 (1969); *idem, ibid.*, **17**, 1964 (1969).
5) H. Gamp, A. D. Zuberbuhler, *Met. Ions Biol. Syst.*, **12**, 133 (1981).
6) Li Mi, A. D. Zuberbuhler, *Helv. Chim. Acta*, **75**, 1547 (1992).
7) R. F. Jameson, N. J. Blackburn, *J. Inorg. Nucl. Chem.*, **37**, 809 (1975); *J Chem. Soc., Dalton*, **1976**, 534; *idem, ibid.*, **1976**, 1596; *idem, ibid.*, **1982**, 9.
8) I.Pecht, M. Amber, *Nature*, **207**, 1386 (1965); I. Pecht, A. Levitzki, M.Amber, *J. Am. Chem. Soc.*, **89**, 1587 (1967).
9) A. Levitzki, I. Pecht, A. Berger, *J. Am. Chem. Soc.*, **94**, 6840 (1972).
10) J.-I. Ueda, A. Hanaki, *Bull. Chem. Soc. Jpn.*, **57**, 3511 (1984).
11) J.-I. Ueda, A. Hanaki, T. Yasuhara, T.Nakajima, S. Soufuku, *Anal. Sci.*, **8**, 487 (1992).
12) The Cu(II)-PLH complex at pH 5.0 shows two bands, one positive at 536 nm and another negative at 650nm.[9)]

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