Chem. Pharm. Bull. 16(4) 763—764 (1968)

UDC 612.124-08

An Oxidative Effect of Cytochrome c Heme Peptide

We have investigated several biological properties of the peptide fragments obtained by chemical and enzymatic cleavages of peptide bonds of cytochrome c.[1,2] In cytochrome c, in contrast with other heme proteins, the heme and protein are coupled *via* the two thioether linkages, and the cleavages of cytochrome c permit the isolations of heme combining fragments (heme peptides). A remarkable peroxidative activity was proved by Paléus, *et al.*[3] in the fragments. Now, in addition, we have found a cytochrome c oxidative activity. In this communication we present some preliminary results of the studies on this effect.

Two heme peptides were prepared from *Saccharomyces oviformis* M_2 cytochrome c[4] according to the procedures for equine heart cytochrome c described by Harbury and Loach[5]: an undecapeptide (peptic heme peptide) and an octapeptide (peptic–tryptic heme peptide) were obtained by the enzymatic digestions with pepsin and trypsin. The amino acid analysis data for the prepared heme peptides were given as follows: peptic heme peptide—Thr 1.86, Glu 2.00, Val 1.00, Leu 1.04, Lys 0.77, His 0.86, Arg 0.77: peptic–tryptic heme peptide—Thr 1.04, Glu 2.00, Val 0.88, Leu 1.18, His 0.88. (Cysteines contained in the heme peptides were not estimated on account of the decomposition during the acid hydrolysis for amino acid analysis.)

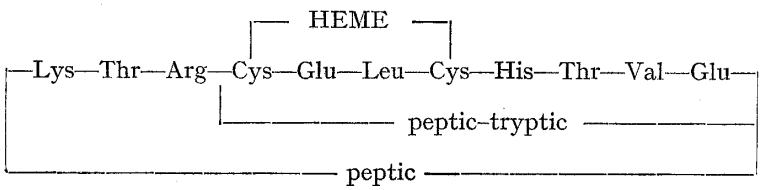

Fig. 1. Amino Acid Sequences of Heme Peptides

The oxidation of ferrocytochrome c by the heme peptides was determined by the decrease in absorbancy at 550 mμ of ferrocytochrome c. Ferrocytochrome c shows a characteristic absorption band (α–band) at 550 mμ, while ferricytochrome c lacks in it. The difference in absorbancies at 550 mμ of ferro– and ferri–types of the *Saccharomyces* cytochrome c (Δ_{550}) is 22.0 mM^{-1}.[6] The reactions were carried out in spectrophotometer cell, the light path of which were 1 cm. To a solution of ferrocytochrome c in 0.15 M phosphate buffer pH 7.0 (2.9 ml), heme peptide dissolved in the same buffer (0.1 ml) was added and the decrease in absorbancy at 550 mμ was recorded. The final concentrations of ferrocytochrome c and heme peptide were 50 μM and 5 μM each.

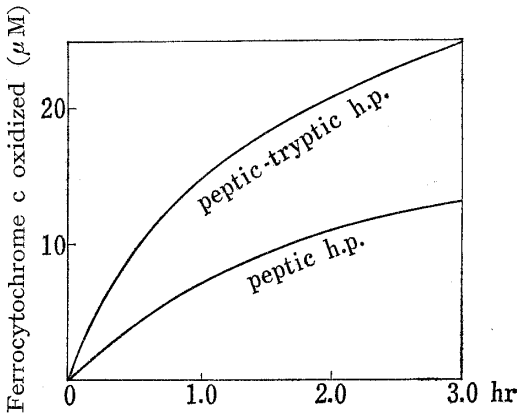

Fig. 2. Time Course Study of the Oxidative Effect

1) Y. Baba, H. Mizushima, A. Ito, and H. Watanabe, *Biochem. Biophys. Res. Commun.*, **26**, 505 (1967).
2) H. Watanabe, M. Murata, K. Kitamura, Y. Baba, and H. Mizushima, 7th International Congress of Biochemistry, H-14 (Tokyo, 1967).
3) S. Paléus, A. Ehrenberg, and H. Tuppy, *Acta Chem. Scand.*, **9**, 365 (1955).
4) K. Narita, *Biochem. Biophys. Acta*, **77**, 688 (1963).
5) H.A. Harbury and P.A. Loach, *J. Biol. Chem.*, **235**, 3640 (1960).
6) M. Shirasaka, private communication.

NII-Electronic Library Service

As shown in Fig. 2 heme peptides are not such simple electron acceptors of cytochrome c as ferricyanide but they show a catalytical property because they oxidize multiple times over an equimolecular quantity of ferrocytochrome c. In this connection, particular attention was directed to the fact that the oxidation was inhibited by cyanide and azide ions (Table I).

Table I. Inhibitions of the Oxidative Effect

Without oxygen		100% inhibition
Azide	(10^{-2} M)	39%
Cyanide	(10^{-2} M)	100%
	(10^{-3} M)	95%
	(10^{-4} M)	72%

Unlike cytochrome c, whose porphyrin–iron is fully and tightly occupied by four pyrrol nitrogens in porphyrin ring and other two ligands in protein moiety, the sixth coordination position of porphyrin iron in heme peptide is not rigidly held—in some cases unoccupied, and in other cases occupied loosely by a nitrogenous group of another heme peptide molecule intermolecularly. This unstable condition of the sixth coordination position gave heme peptides their characteristic properties. Thus, they connect easily with cyanide and azide ions at the sixth position, and, in particular they are extremely autoxidizable because of the interaction with oxygen at the position, hence heme peptides do not remain reduced in aerobic state.

The inhibitions by cyanide and azide ions, which are the strong ligands to the sixth position, indicate that the oxidation reaction proceeds through the functional porphyrin iron of heme peptides. This is also supported by the fact that under anaerobic condition heme peptide exhibited no oxidative effect (Table I). Probably, electron was transferred from ferrocytochrome c to porphyrin iron of heme peptide, followed by the immediate further migration to oxygen in reaction medium.

On the other hand, a marked difference was observed in the activity between the two heme peptides examined (Fig. 2). The variation must be attributed to the peptide moiety of heme peptide.

These problems arouse further interest if heme peptide is considered as an oxidase model. Subsequent investigations are now in progress with several kinds of heme peptides.

Central Research Laboratories,
Sankyo Co., Ltd.
Hiromachi, Shinagawa-ku, Tokyo

Yoshihiko Baba
Hiroshi Mizushima
Hiroshi Watanabe

Received January 19, 1968

[Chem. Pharm. Bull. 16(4) 764—767 (1968)]

UDC 547.717.04 : 547.462.8.04

1,3-Dipolar Cycloaddition Reaction of Aziridinedicarboximide

Recently, many examples of 1,3-dipolar cycloaddition reaction of aziridines onto ethylene or acethylene bonds to form substituted pyrrolidines or pyrrolines have been reported.[1)]

1) A. Padwa and L. Hamilton, *Tetrahedron Letters*, 4363 (1965); *J. Heterocyclic Chem.*, 4, 118 (1967). H.W. Heine and P.E. Peavy, *Tetrahedron Letters*, 3123 (1965). H.W. Heine, P.E. Peavy, and A.J. Durbetaki, *J. Org. Chem.*, 31, 3924 (1966). R. Huisgen, W. Scheer, G. Szeimies, and H. Huber, *Tetrahedron Letters*, 397 (1966).

NII-Electronic Library Service