[Chem. Pharm. Bull. 23(9)2184—2186(1975)]

UDC 547.466.1.04 : 577.15.03

The Role of Single Tyrosine and Histidine Residues in Bovine Neurophysin I

The influence of photooxidation of bovine neurophysin I in the presence of rose bengal on its amino acid composition and binding ability for oxytoxin and arginine-vasopressin were studied.

Single histidine residue was photooxidized very rapidly without any decrease in the hormones-binding ability. On the other hand, single tyrosine residue was found to be photooxidized almost completely after 240 min of irradiation accompanying a decrease in the hormones-binding ability. No significant changes in other amino acid residues were found even after 240 min of irradiation.

Therefore, it is evident that the tyrosine residue has some role in the hormone binding process of bovine neurophysin I.

The bovine pituitary posterior lobe contains two main hormone-binding proteins, neurophysin I and II.[1] On the complex formation between neurophysin (NP) and neurohypophysial hormones, the direct involvement of residue 2 and residue 3 of the hormones has been previously proposed on the basis of binding experiments[2] and spectroscopic studies.[2b,3] However, little is known on the residues in NP molecule which are involved directly in hormones-binding. Neurophysin I (NP-I) and neurophysin II (NP-II) both have single tyrosine residue at position 49 in each molecule and the former possess single histidine and no methionine residue, while the latter possess no histidine and single methionine residue.[4] It is of considerable interest to investigate the role of these single amino acid residues for the hormones-binding ability of NPs.

Our previous findings have shown the possibility of selective photochemical modification of the methionyl and tyrosyl residues in bovine NP-II and also have proposed the possible role of tyrosine-49 residue in the hormone binding process.[5] In this paper, the influence of the photooxidation of NP-I in the presence of rose bengal on its amino acid composition and hormones-binding ability were studied.

NP-I was prepared by the method described previously[5] from acetone-dried powder of bovine pituitary posterior lobes and the purity of this preparation was confirmed by means of electrophoresis and amino acid analysis. Photooxidation was performed as follows: Ten mg of NP-I were dissolved in 10 ml of 0.1M phosphate buffer (pH 7.4) which contained 0.5 mg of rose bengal and irradiated at 25±3° by a 350 W reflecting photoflood lamp at a distance of 15 cm from the reaction mixture. Amino acid analysis and hormones assay were performed as described previously.[5]

Table I shows the amino acid composition of NP-I after 5 and 240 min of irradiation in the presence of rose bengal. After 5 min of irradiation, single histidine residue was found to be photooxidized completely. Single tyrosine residue was found to be photooxidized almost

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Table I. Amino Acid Composition of Neurophysin I Photooxidized in the Presence of Rose Bengal

Amino acid	Reported[a] by E. Breslow, *et al.*[2b]	Found[a] After irradiation (min) of 0	5	240
Tryptophan	0	0.0	0.0	0.0
Lysine	2	1.9	1.9	1.9
Histidine	1	0.82	0.0	0.0
Arginine	4	(4.0)	(4.0)	(4.0)
Aspartic acid	7	7.0	7.2	7.4
Threonine	2	2.1	2.2	2.3
Serine	6	5.5	5.7	5.6
Glutamic acid	10	10.5	10.7	10.4
Proline	9	9.1	9.1	9.1
Glycine	15	15.1	15.4	15.2
Alanine	9	(9.0)	(9.0)	(9.0)
Cystine	7	7.2	7.1	7.0
Valine	3	3.0	2.9	3.0
Methionine	0	0.1	0.1	0.1
Isoleucine	2	2.0	2.0	2.0
Leucine	6	6.2	6.3	6.2
Tyrosine	1	0.98	0.96	0.06
Phenylalanine	3	3.0	3.0	3.2

a) number of mole of residues/mole of protein

completely after 240 min of irradiation. However, there occurred no significant changes in other amino acid residues even after 240 min of irradiation.

To determine the effect of photochemical modification of histidine and tyrosine residues in NP-I on the biological activity of the protein, the hormones-binding ability was tested by equilibrium dialysis. Table II shows the changes of binding ability of the protein for oxytocin and arginine-vasopressin as results of photooxidation of NP-I in the presence of rose bengal. A decrease in the binding ability of the photooxidized protein was found to proceed almost identically for oxytocin and arginine-vasopressin as the ligand.

Table II. Hormones–Binding Ability of Neurophysin I Photooxidized in the Presence of Rose Bengal

Irradiation time (min)	Oxytocin units bound to 0.5 mg of NP–I	Average (±S.D.)	%
0	3.47, 3.44, 3.68	3.53 (±0.11)	100
5	3.41, 3.50, 3.91	3.61 (±0.22)	102.3
240	1.49, 1.87, 1.67	1.68 (±0.16)	47.6

Irradiation time (min)	Vasopressin units bound to 0.5 mg of NP–I	Average (±S.D.)	%
0	3.61, 2.77, 3.30	3.23 (±0.35)	100
5	3.16, 2.93, 3.51	3.16 (±0.25)	97.8
240	1.07, 1.19, 1.12	1.13 (±0.05)	35.0

From the data of Table I and II, it is clear that the histidine residue is nonessential in the hormone binding process since the hormones-binding ability of the protein is almost completely retained when the histidine residue was completely photooxidized. It is also evident that

the photochemical modification of single tyrosine residue has some relevance to the decrease in the hormones-binding ability of NP-I.

Since NP-I and NP-II both possess a very similar amino acid sequence[4] and essentially identical affinity constants for either oxytocin or lysine-vasopressin,[6] it may be considered that their hormone binding sites would consist of similar amino acid residues. This is supported by the finding that the histidine residue in NP-I as well as the methionine residue in NP-II is nonessential for the hormone binding process.

A certain movement of the tyrosine-49 from a hydrophobic environment to a hydrophilic environment upon complex formation between NP and hormones has been reported both in NP-I[7] and in NP-II.[3a,b,d,8] These may strongly suggest an important role of the tyrosine-49 for hormone binding. Our previous findings have suggested that the movement of tyrosin-49 residue in NP-II on the hormone binding would provide a more sterically acceptable binding site for hormones.[5] The decrease in the hormones-binding ability of NP-I by the photodegradation of the tyrosine-49 residue, which was found in the present work may also be explained as the same conception as described for NP-II.

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Received May 26, 1975

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[Chem. Pharm. Bull. 23(9)2186—2188(1975)]

UDC 547.91.04 : 615.356.011.5 : 546.56.04

The Cleavage of Cobalt-Carbon Bond in Methyl-Vitamin B_{12} by Cupric Ions

The cupric ions were found to promote the demethylation of methylcobalamin in solutions only when coordinated with chloride anions.

The cleavage of Co-C bond in methyl-vitamin B_{12} (Me-Co) is a subject of current interest, in which the methyl group is shown to be transferred from Co to the other metal ions such as Hg(II),[1,2,3] Tl(III),[4] Pd(II),[5] Cr(II),[6] Pt(II)·(IV),[4] Au(I)·(III).[4] In this communication

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