THE EFFECTS OF LIGANDS ON ENZYMIC CARBOXYL-METHYLATION OF NEUROPHYSINS

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Summary: The methyl-acceptor activities of bovine neurophysins I and II for the enzyme protein carboxymethylase (EC 2.1.1.24) were found to be similar and as high as for other previously identified, biologically active protein substrates. Effects on the rate of methylation of these neurophysins were investigated with the posterior pituitary hormone ligands, oxytocin and vasopressin, and the hormone-related tripeptide ligand, methionyl-tyrosyl-phenylalaninamide. An increase in the rate of neurophysin II methylation was observed with both oxytocin and tripeptide. This ligand-induced response did not occur with either native neurophysin I or disulfide-scrambled neurophysin II.

INTRODUCTION

Protein carboxymethylase (S-adenosyl-L-methionine:protein-carboxyl O-methyltransferase, EC 2.1.1.24; protein methylase II) catalyzes transfer of the methyl group of S-adenosyl-L-methionine to carboxyl side chains in the formation of protein methyl esters (1-4). The enzyme has been purified from bovine pituitary gland and characterized. A study of the substrate specificity of the enzyme showed that all anterior pituitary hormones were effective methyl acceptors while the posterior pituitary hormones, oxytocin and vasopressin, did not serve as substrates (3). However, investigation of other biologically active proteins showed that the posterior pituitary hormone-binding proteins, the neurophysins, do serve as highly effective substrates for protein carboxymethylase (5,6). Further, a close temporal relationship has been found (7) between the synthesis of neurophysin and the synthesis of endogenous substrate for protein carboxymethylase in cultured guinea pig hypothalamus. This

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relationship is perhaps an expression of in vivo methylation of neurophysin by this enzyme.

Inasmuch as neurophysins are active substrates for protein carboxymethylase and neurophysin hormone-binding activity appears to involve an ionic interaction between a side-chain carboxyl group of neurophysin and the α-amino group of hormone or other peptide ligand (8), a role for methylation in neurophysin function must be considered. This possibility is suggested further by the high specific activity of the enzyme in the posterior pituitary (4). One aspect requiring clarification is the direct relationship, if any, between methylation and hormone binding. Previously (6), it was shown that methylation of neurophysin did not affect qualitatively the binding of the protein to [lysine-vasopressin]-Sepharose by all-or-none affinity chromatographic elution. In the present study, the reverse question was raised, namely, does ligand binding affect methylation. We find that binding of oxytocin and a hormone-related tripeptide increase carboxyl-methylation of neurophysin II.

MATERIALS AND METHODS

Enzyme Purification and Assay-- Protein carboxymethylase was purified from bovine pituitaries by the procedure previously described (3,4). Protein carboxymethylase activity assays (4) were carried out at pH 6.0. In most experiments, neurophysins were preincubated with ligand at 20° for 10 min prior to addition of S-adenosyl-L-[methyl-^{3}H]methionine and purified protein carboxymethylase (more than 2000-fold purified); this enzyme incubation was carried out at 37° for 15 min (the period of linear activity). Specific enzymic activity is expressed as units per mg of protein, where a unit equals 1 pmol [^{3}H]methyl transferred per 15 min.

Neurophysins-- Bovine neurophysins I and II were isolated from acetone-dried posterior pituitaries (Pel-Freeze, Rogers, Arkansas) by the procedure of Hollenberg and Hope (9) with final purification using DEAE-Sephadex A-50 (10). Disulfide-scrambled neurophysin II was prepared by incubation of 0.6 mg of neurophysin II in 1 ml of 0.1 M ammonium bicarbonate, pH 7.2, containing 1.0 mM 2-mercaptoethanol for 45 h at room temperature. The denatured product was used after exhaustive lyophilization.

Neurophysin Ligands and Other Materials-- Synthetic oxytocin (generously provided by Sandoz Pharmaceuticals, as "Syntocinon") was lyophilized, desalted by passing through a Sephadex G-15 column equilibrated with 50% acetic acid, and lyophilized repeatedly from water before use. Native arginine-vasopressin was isolated as a byproduct of the neurophysin isolation and purified further on sulfoethyl-Sephadex C-25 with an elution procedure similar to that described previously (11). Methionyl-tyrosyl-phenylalaninamide, a tripeptide, was prepared by the solid phase synthesis method (12).

S-adenosyl-L-[methyl-^{3}H]methionine, 8.54 Ci/mmole, was purchased from New England Nuclear Corp. Luteinizing hormone (ovine) was a generous gift of Dr. Leo Reichert, Emory University.

Table 1

Comparison of the effects of oxytocin and methionyl-tyrosyl-phenylalaninamide on the carboxyl-methylation of neurophysins.[a]

Protein Substrate	PCM Activity[b] (with no additions) (units)	PCM Activity Ratio with/without added ligand	
		Oxytocin (0.6 mM)	MTP[c] (2 mM)
Neurophysin II			
10 μM	1.62[d]	1.68	1.74
20 μM	3.41[d]	1.14	1.14
Neurophysin II (Scrambled)			
10 μM	0.53[e]	----	1.08
20 μM	1.33[e]	----	0.85
Neurophysin I			
10 μM	2.24	0.75	0.83
20 μM	4.04	0.86	0.99
Luteining hormone			
10 μM	2.29	1.07	----

[a] Unless otherwise indicated, data are from one experiment and are typical of results from at least two other experiments.

[b] Protein carboxymethylase (PCM) activity was assayed as described in Materials and Methods.

[c] Methionyl-tyrosyl-phenylalaninamide.

[d] Average of three experiments.

[e] These data were obtained in an experiment where the control native neurophysin II activities were 1.28 and 2.58 units for 10 μM and 20 μM, respectively.

RESULTS

Effect of ligand binding on methylation of neurophysins-- The posterior pituitary hormones, oxytocin and vasopressin, are not substrates for protein carboxymethylase (3). However, as shown in Table 1, the rate of methylation of bovine neurophysin II was found to be increased by the presence of oxytocin The relative increase in methylation was consistently greater at lower concentrations of neurophysin II, reflecting at least a decrease in K_m. Such a

response was not observed with non-oxytocin binding proteins, i.e., luteinizing hormone (LH). An indication of the specificity of the ligand effect was obtained in the observation that arginine-vasopressin was ineffective in altering significantly the rate of methylation of neurophysin II (data not shown). Also, for neurophysin I, the rate of methylation was little changed or slightly reduced in the presence of oxytocin (Table 1).

Methionyl-tyrosyl-phenylalaninamide, previously shown to bind specifically to neurophysins in a manner similar to oxytocin and vasopressin (13), was examined for its effect on the methylation of neurophysin II (Table 1). Again, an increase in the rate of methylation was obtained, to an extent similar to that found with oxytocin.

In the above studies of ligand effects, changes in methylating activity of 15% or less were considered difficult to interpret based on the errors in the assay and therefore were regarded as insignificant.

Comparison of native and denatured neurophysin II on substrate activity and ligand response-- Disulfide-scrambled neurophysin II was shown previously to be inactive in binding peptide ligands (13). Denaturation of neurophysin II by this reduction procedure caused a marked decrease in substrate activity (Table 1). Furthermore, a concentration of methionyl-tyrosyl-phenylalaninamide which caused an increase in the rate of methylation of native neurophysin II had no significant effect with the scrambled neurophysin (Table 1). Similarly, oxytocin was ineffective in altering the rate of methylation of scrambled neurophysin II (data not shown).

DISCUSSION

The side-chain carboxyl group of neurophysin purported to be involved in hormone binding (8) is a potential site for methylation by protein carboxymethylase. However, since the hormone does not inhibit methylation of neurophysins, hormone binding and carboxyl-methylation probably occur at different sites. This view is consistent with a previous report in which methylation of

neurophysin did not appear to alter the binding of the protein to [lysine-vasopressin]-Sepharose (6).

The data obtained in the present study indicate that certain neurophysin ligands not only do not inhibit methylation but in fact have an opposite effect, i.e., they produce an increase in the rate of reaction. Furthermore, a degree of specificity in this response is indicated by the observation of a significant stimulation of methylation with oxytocin and methionyl-tyrosyl-phenylalaninamide on neurophysin II only, and not on neurophysin I. This phenomenon may be the result of a hormone-induced change in some conformational aspect of the neurophysin, allowing for at least a greater affinity of neurophysin for the enzyme. Aspects of conformation which could be affected by ligand binding might include simply aggregation (13) or perhaps a particular feature in monomeric folding.

The high substrate activity of neurophysins (5), the specificity of the hormone effect on carboxyl-methylation and the high specific activity of protein carboxymethylase in the posterior pituitary and hypothalamus (4) are consistent with the idea of a biological role for methylation in hormone-neurophysin function.

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