STUDIES ON S-ADENOSYLMETHIONINE:PROTEIN-CARBOXYL METHYLTRANSFERASE IN THE HYPOTHALAMO-NEUROHYPOPHYSIAL COMPLEX IN ORGAN CULTURE

Sangduk Kim, David Pearson* and Woon Ki Paik

Fels Research Institute and Department of Biochemistry,
Temple University School of Medicine, Philadelphia, Pa. 19140 and
Roche Institute of Molecular Biology, Nutley, New Jersey 07110

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Summary

The level of protein methylase II (S-adenosylmethionine:protein-carboxyl-methyltransferase, EC.2.1.1.24) activity in the hypothalamo-neurohypophysial complex in organ culture was studied during the period of 21 days. 1. The endogenous enzyme activity, which is a measure of both enzyme and substrate protein levels, in hypothalamus is maximum at the 7th day of culture showing 100% increase, compared to the activity at 0 day. Exogenous enzyme activity in hypothalamus which is a measure of enzyme level only, did not show a peak at 7th day. Thus, this increase of the activity indicates that newly synthesized neurophysin served as endogenous substrate protein. 2. Endogenous enzyme activity of cytosol fraction from anterior pituitary gland gradually increased during the culture period reaching 3-fold increase at 12th day of culture, while the exogenous activity remained unchanged. This specific increase of endogenous enzyme activity also indicates that *in vivo* newly synthesized anterior pituitary peptide hormones serve as substrate.

In 1965, Axelrod and Daly (1) described an enzyme system from the pituitary gland which yielded methanol from S-adenosylmethionine and termed it "methanol-forming enzyme." A few years later, Liss *et al* and Kim and Paik (2,3) described independently protein methylase II (S-adenosylmethionine:protein-carboxyl methyltransferase, E.C.2.1.1.24) which methylates a protein substrate with S-adenosyl-L-methionine as methyl donor. The methyl group incorporated into protein substrate was volatile either in acid or alkali, and the volatile product was identified as methanol by preparing methyl esters of 3,5-dinitrobenzoate and radio-gas chromatography (2,3). The enzymatic product was indicated as protein-methyl ester.

The requirement for S-adenosylmethionine and the formation of methanol by both "methanol-forming enzyme" and protein methylase II led us to compare these two enzymes (4), and their identity was established on the basis of the following

*Department of Animal Science, School of Veterinary Medicine, University of California, Davis, California 95616

experimental results; constant ratio of the two enzyme activities during the various purification steps, similar dependency of the "methanol-forming enzyme" on the specific protein substrates for protein methylase II, and identical sensitivity of the two enzymes to change in pH. A similar conclusion has also been reached by Morin and Liss (5) and Diliberto and Axelrod (6), based on *in vitro* formation of $^{14}CH_3OH$ from the endogenous protein-methyl ester formed with labelled S-adenosylmethionine.

Although various naturally occuring proteins or polypeptides are methyl acceptors for *in vitro* assay of the enzyme (3,7), the nature of the *in vivo* substrate is not known. Our recent study on the endogenous substrate in different parts of rabbit brain (8) and the initial work by Axelrod and Daly (1) indicated that the pituitary gland contains a significant amount of endogenous substrate. Therefore, organ culture of the guinea pig hypothalamo-neurohypophysial complex which synthesizes polypeptide hormones in culture (9) was thought to be an excellent system to study the status of protein methylase II and its endogenous substrates. The present communication deals with results obtained during the organ culture of hypothalamo-neurohypophysial complex of guinea pig.

Materials and Methods

Materials: S-Adenosyl-L-(methyl-^{14}C)methionine (specific activity; 60 mCi/mmole) was purchased from New England Nuclear Corp. and γ-globulin was obtained from Sigma Chem. Corp. Other reagents were obtained from various local sources and were of analytical grade.

Enzyme Assay: An enzyme assay was carried out as described previously (3,8) at pH 6.0 with 10 mg of γ-globulin as exogenous substrate. Specific activity of the enzyme is expressed as picomoles of methyl groups transferred per minute per mg of protein at 37°.

Concentration of protein was determined by the method of Lowry *et al* (10) with bovine serum albumin as standard protein.

Culture of the hypothalamo-neurohypophysial complex: Organ culture were prepared from adult male guinea pigs (300 g) as described previously (11). The ex-

plants were maintained in Dulbecco's Modified Eagle Medium (Grand Island Biological Company), 1% of volume; sodium penicillin G, 100 U/ml; and fetal calf serum, 10% of volume. The medium was changed within 24 hours after the explants were made, and every 48 hours thereafter. The cultures were maintained on a 1 cm^2 piece of lens paper on a stainless steel grid formed to be held on the surface of the medium in the 1 ml well of an organ culture dish (Falcon Plastics). The gas phase was 95% air and 5% CO_2; incubation temperature was 35^0.

Preparation of cultured sample: Supernatant and precipitate fractions were prepared after homogenization of the tissue in 0.28 M sucrose, 0.02 M phosphate buffer, pH 7.2 in the cold and by centrifugation at 105,000 x g for 1 hour.

Results

Protein methylase II activity in hypothalamus, posterior and anterior pituitary gland in organ culture: Both endogenous (without added substrate) and exogenous (with added substrate) enzyme activities were measured in hypothalamus, posterior and anterior pituitary during the organ culture of hypothalamo-neurohypophysial complex at 0,2,7,14 and 21 days. Fig 1A illustrates the changes of the endogenous enzyme activity in the hypothalamus. This endogenous activity is a measure of the enzyme level with the endogenous substrate present in that preparation. Highest endogenous enzyme activities in both whole homogenate and cytosol fractions were shown at the 7th day of culture, while the exogenous enzyme activity does not show any peak during 21 days of organ culture. These peak activities in the change of protein methylase II activity are closely correlated with the biosynthetic pattern of vasopressin and neurophysin in which the peak synthesis of these polypeptides were also observed at 7-10th day of the organ culture (12). Since Axelrod (13) and Edgar and Hope (14) found that neurophysin is a good substrate for the protein methylase II activity in vitro, the result in Fig. 1 strongly indicates that the natural substrate for protein methylase II in hypothalamus is neurophysin.

Although the synthesis of vasopressin has been shown to be coupled with that of neurophysin (15), the former is not likely to be a natural methyl acceptor substrate, since this octapeptide does not contain aspartyl or glutamyl resi-

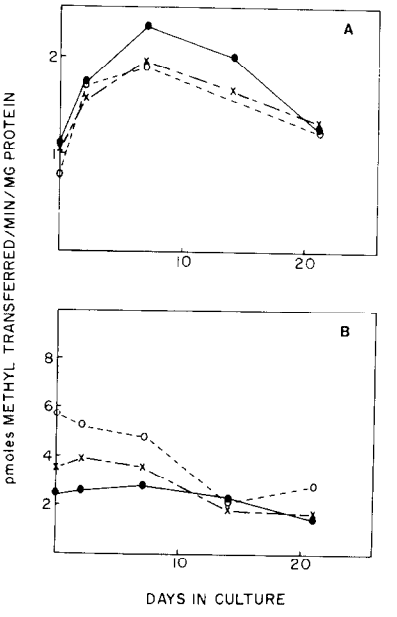

Fig. 1. Protein methylase II activity of hypothalamus during the organ culture. Protein methylase II activity was carried out as standard method at pH 6. 10 mg of γ-globulin were used as exogenous substrate protein. A, endogenous enzyme activity; B, exogenous activity; ●——●——● whole homogenate; o---o---o supernatant; x---x---x precipitate.

dues. Indeed, Diliberto and Axelrod (6) have shown that this peptide is a poor substrate for in vitro enzyme activity. The exogenous enzyme activity in the hypothalamus does not show any peak during 21 days of organ culture (Fig. 1B).

Fig. 2A and 2B shows endogenous and exogenous enzyme activities in posterior pituitary in culture. Both enzyme activities are highest at the 0 day of culture and decrease gradually. This decrease in enzyme activity during the culture period might be partly due to (a) a portion of the posterior pituicytes degenerate during the culture as is frequently observed or (b) the transfer of newly synthesized polypeptide (neurophysin) from hypothalamus to posterior lobe was not active under the present culture condition.

Endogenous and exogenous protein methylase II activities in anterior pituitary lobe are generally higher in the supernatant fractions than the activities of whole homogenate or precipitate (Fig. 3A and 3B). However, the endogenous supernatant activity at 0 day is low and increases gradually during the culture period reaching

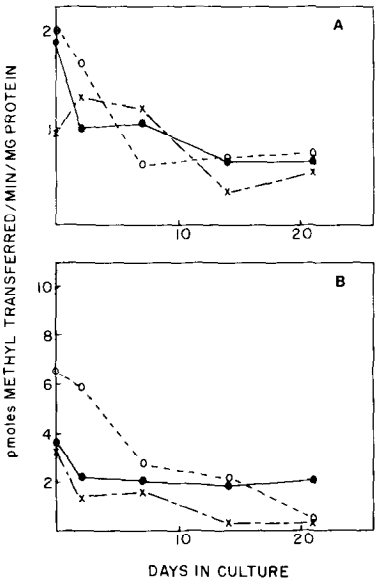

Fig. 2. Protein methylase II activity of posterior pituitary gland during the organ culture. A, endogenous activity; B, exogenous activity; ●—●—● whole homogenate; o---o---o supernatant; x---x---x precipitate. Assay conditions for the enzyme activity are same as Fig. 1.

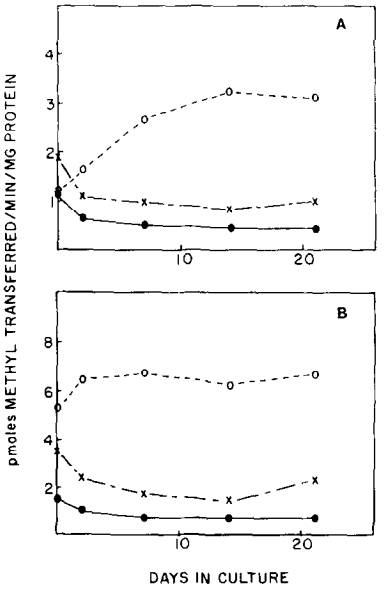

Fig. 3. Protein methylase II activity of anterior pituitary gland during the organ culture. A, endogenous activity; B, exogenous activity; ●—●—●, whole homogenate; o---o---o, supernatant; x---x---x, precipitate. Assay conditions for the enzyme activity are same as Fig. 1.

a steady level of 3-fold increase, at 12th day. This result may be an indication that endogenous methyl acceptor proteins (most likely various anterior pituitary peptide hormones) are synthesized during the culture and served as the acceptor. It should be mentioned that the maximum steady level of endogenous supernatant activity after 12th day of culture is about half of that exogenous activity (specific activity of 3.2 vs. 6.6).

Discussion

Protein methylase II catalyzes the esterification of free carboxyl groups of certain polypeptide substrates. A high content of endogenous substrate for this reaction is found in the bovine and rabbit pituitary gland. Neurophysin from posterior pituitary gland and various polypeptide hormones from the anterior pituitary gland were shown to be good methyl acceptors in the enzyme reaction (13). The present report further extends such findings to hypothalamo-neurohypophysial organ culture, by demonstrating a close temporal relationship between the synthesis of neurophysin and endogenous protein methylase II activity. This finding strongly suggests that the newly synthesized polypeptides are also capable of being esterified.

As to the physiological role of the enzyme, a hypothesis that the enzymatic esterification of neurophysin in posterior pituitary gland may influence its binding capacity between neurophysin and vasopressin, has been suggested, since Asp (Res. 30) and Glu(Res. 31) in the binding protein have been implicated as the binding sites to vasopressin (16). Unfortunately, the ability of agarose-coupled (8-lysine) vasopressin to bind esterified and unesterified neurophysin is similar (14), however, further definitive information is needed before any conclusions can be made

Among the various polypeptide hormones from anterior pituitary gland, luteinizing and follicle stimulating hormones are particularly good methyl acceptors (6). The main target organ in male rat for these gonadotropins is the testis. Recently, protein methylase II was studied in rat testis: (a) The enzyme activity is highest in the organ, when the animal is in puberty (17), (b) decrease of the testis enzyme activity was observed after hypophysectomy and the reduced activity was re-

turned to normal level by testosterone administration (18). These findings together with the presence of the enzyme in the anterior pituitary gland, all support a possible physiological importance of the enzyme in regulation of hormone action.

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