Vol. 82, No. 2, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 30, 1978 Pages 514-517

EVIDENCE FOR PHOSPHORYLATION OF BOVINE ADRENAL TYROSINE HYDROXYLASE BY CYCLIC AMP-DEPENDENT PROTEIN KINASE

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Received April 3,1978

SUMMARY: Direct phosphorylation of bovine adrenal tyrosine hydroxylase with an associated increase in enzyme activity by cyclic AMP-dependent protein kinase was demonstrated by gel filtration on Sephadex G-200.

Tyrosine hydroxylase (EC 1.14.16.2) which catalyzes the conversion of tyrosine to 3,4-dihydroxyphenylalanine (Dopa) is the rate-limiting enzyme in the biosynthesis of catecholamines (1,2). Several recent reports have described adenosine 3':5'-monophosphate (cAMP)-dependent activation of tyrosine hydroxylase in brain extracts (3-5) and adrenal preparation (6). Although tyrosine hydroxylase was activated by exposure to enzymatic phosphorylating conditions, no incorporation of phosphate into the enzyme was observed by either immunoprecipitation studies or by sucrose gradient studies (7,8).

The present study provides evidence indicating that phosphorylation of bovine adrenal tyrosine hydroxylase *in vitro* can be catalyzed by cAMP-dependent protein kinase, with an associated increase in enzyme activity.

MATERIALS AND METHODS

Tyrosine hydroxylase was purified approximately 70-fold with a 18% yield from bovine adrenal medulla cytosol using chromatography on 3-iodotyrosine-substituted Sepharose 4B (9), heparin-substituted Sepharose 4B (10), and phosphocellulose. Tyrosine hydroxylase activity was assayed fluorometrically as described previously (11). The assay mixture contained, in a final volume of 0.5 ml, 100 mM HEPES buffer, pH 6.8, 200 μM tyrosine, 100 μM 2-amino-4-hydroxy-6,7-dimethyl-5,6,7,8-tetrahydropteridine ($DMPH_4$), 40 mM 2-mercaptoethanol, 1mM EDTA, 100 μg of catalase and a suitable amount of the enzyme solution. The reaction was carried out at 30° for 10 min with shaking. Protein was

0006-291X/78/0822-0514$01.00/0

determined by the method of Lowry et al. (12).

RESULTS AND DISCUSSION

The possibility that tyrosine hydroxylase could be directly phosphorylated by the conditions of enzymatic phosphorylation which led to activation of tyrosine hydroxylase was examined by gel filtration experiments as shown in Fig. 1. The single peak of enzyme activity was co-eluted with the peak of radioactivity and the ratios of radioactivity to enzyme activity were almost constant, indicating that bovine adrenal tyrosine hydroxylase was directly phosphorylated after exposure to enzymatic phosphorylating conditions. Tyrosine hydroxylase was activated 3- to 4-fold and both of the activated and control tyrosine hydroxylase showed the similar elution pattern. The recovery of the enzymatic activities of both was approximately 30%. Under the experimental conditions described in Fig. 1(A), about 750 pmol of [^{32}P]phosphate became incorporated into 192 μg of partially purified tyrosine hydroxylase and this amount corresponded to about 5% of the total amount of [γ-^{32}P]ATP used in this experiment. When tyrosine hydroxylase was incubated with [γ-^{32}P]ATP in the absence of protein kinase as shown in Fig. 1(B), only less than 0.5% of the radioactivity was incorporated into tyrosine hydroxylase, indicating that incorporation of [^{32}P]phosphate into tyrosine hydroxylase depended upon the presence of cAMP-dependent protein kinase.

Although there have been a number of studies which showed that phosphorylating conditions activate tyrosine hydroxylase (3-6), attempts to demonstrate direct phosphorylation of the enzyme have failed (7,8). It has been suggested that the enzyme itself is not directly phosphorylated, but that some protein activator is the site of addition of phosphate (8). On the

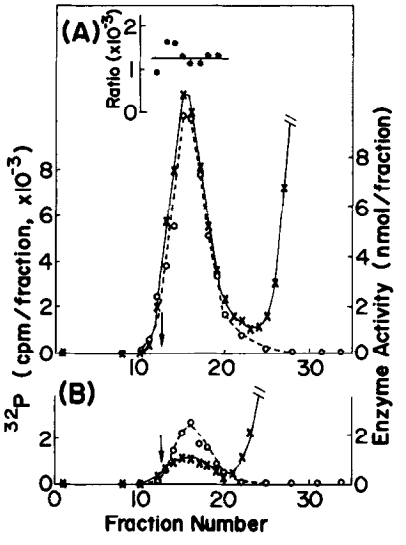

Fig. 1. Gel filtration of ^{32}P-labeled tyrosine hydroxylase on Sephadex G-200. Tyrosine hydroxylase, 192 µg, was incubated at 30° for 5 min in a final volume of 150 µl that contained 20 mM Na-phosphate buffer, pH 6.8, 5 mM $MgCl_2$, 5 µM cAMP, 100 µM [γ-^{32}P]ATP, and 26 µg of cAMP-dependent protein kinase of bovine heart (Sigma Chemical Co.). (A), the complete system; (B), the control system lacking only the kinase. After incubation, the mixtures were subjected to gel filtration on Sephadex G-200 column (1.3 X 44 cm, a total bed volume of 55 ml) equilibrated with 20 mM Tris-HCl buffer, pH 7.3, containing 0.23 M sucrose, 0.2 M ammonium sulfate and 1 mM dithiothreitol. The column was eluted with the same buffer at a flow rate of 5 ml per hour in fractions of 1.6 ml. Aliquots (200 µl) of each fraction were assayed for tyrosine hydroxylase as described under "Materials and Methods." Aliquots (1 ml) of each fraction were withdrawn and ^{32}P radioactivity was measured in 10 ml of water by Cerenkov counting with a liquid scintillation spectrometer. o···o, tyrosine hydroxylase activity; x——x, ^{32}P radioactivity; ●——●, ratio of radioactivity (cpm) to enzyme activity (nmol). The arrows show the void volume of the column determined by blue dextran.

other hand , Letendre et al. have recently reported that radioactive inorganic phosphate was incorporated into tyrosine hydroxylase in organ cultures of rat adrenal medulla and superior cervical ganglia (13). They suggested that phosphate is a normal constitutive part of tyrosine hydroxylase and not a participant in any meaningful regulatory phenomena (14). The present study provides the first evidence for direct phosphoryl-

ation of tyrosine hydroxylase associated with its activation by cAMP-dependent protein kinase.

Acknowledgements: This work has been supported in part by a grant-in-aid for Scientific Research from the Ministry of Education, Science, and Culture of Japan, by grant from the Byotai Taisha Research Foundation for 1976, and by a Naito Research Grant for 1975.

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