amount of hydroxylase activity as did extracts of cells grown on phenylalanine. This observation has been confirmed in studies on the metabolism and incorporation of phenylalanine in whole cells[15]. Other similar examples of the induction of the initial enzyme of a degradative pathway by the product of its action have been reported[16,17].

Data are available to indicate that the tyrosine formed by this inducible system can be used for protein synthesis[15]. A study of the relationship between the alternate routes of tyrosine formation in the induced organism is in progress.

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Received May 27th, 1963

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PN 10062

The involvement of sulfhydryl sites in dopamine-β-hydroxylase activity

The enzyme dopamine-β-hydroxylase catalyzes the β-hydroxylation of dopamine[1] and other structural related phenylethylamines and phenylpropylamines[2–4]. As cofactors, ascorbic and fumaric acids are required. While it was shown that the enzymatic β-hydroxylation of the amines is coupled to a stoichiometrically equivalent oxidation of ascorbic acid, the requirement for fumarate is still unexplained[1]. The enzymatic hydroxylation is stimulated by ATP, and it was suggested that ATP functions as a chelating agent rather than as a donor of high energy[2]. EDTA in-

hibits the enzymatic β-hydroxylation which suggests that a cation is necessary for enzyme activity[5].

In the present study, evidence was obtained that dopamine-β-hydroxylase contains sulfhydryl groups which are essential for enzymatic activity.

The enzyme dopamine-β-hydroxylase was prepared and purified from bovine adrenal glands[1], and the incubation was carried out by the previously described procedure[3]. At the end of the period of incubation, the solution was analyzed for enzymatically formed norepinephrine. [^{14}C]Dopamine was used as a substrate, and after acetylation of the amines, the enzymatically formed [^{14}C]norepinephrine was separated from [^{14}C]dopamine by paper chromatography. The amount of [^{14}C]dopamine which disappeared from the incubation mixture, as well as the amount of [^{14}C]-

TABLE I

THE EFFECTS OF *p*-CHLOROMERCURIBENZOATE ON DOPAMINE-β-HYDROXYLASE ACTIVITY

The incubation mixture contained the following components: 0.1 ml enzyme, 2 μmoles dopamine, 6 μmoles ascorbate, 10 μmoles fumarate, 10 μmoles ATP and 100 μmoles phosphate buffer (pH 6.4) in a total volume of 1 ml. The incubation was carried out for 45 min at 37°. The concentration of *p*-chloromercuribenzoate (PCMB) was 10^{-4} M. The concentration of the cofactors in the preincubation mixture was the same as in the incubation mixture. At the end of the preincubation, in the Expts. 4 and 4a, 2 μmoles of dopamine were added, and in the Expts. 4b and 4c, 10 μmoles of fumarate were added. In the Expts. 4a and 4c, the enzyme was preincubated first with the listed components for 5 min and then with PCMB.

Expt. No.	*Preincubation mixture*	*Preincubation time (min)*	*Norepinephrine formed (μmoles/ml)*	*Relative activity*
1	Enzyme	10	1.20	100
1a	Enzyme, PCMB	10	1.10	91
2	Enzyme	30	1.10	100
2a	Enzyme, PCMB	30	0.55	50
3	Enzyme, 0.2 μmoles Ascorbate	10	1.20	100
3a	Enzyme, 0.2 μmoles Ascorbate, PCMB	10	0.50	42
4	Enzyme, all cofactors	15	0.95	100
4a	Enzyme, all cofactors, PCMB	15	0.30	31
4b	Enzyme, dopamine, all cofactors, fumarate excluded	15	0.95	100
4c	Enzyme, dopamine, PCMB, all cofactors fumarate excluded	15	0.60	63

norepinephrine formed in the incubation mixture, was calculated from the radioactivity of each of these compounds. The amount of enzymatically formed norepinephrine was also determined by a modification of the fluorometric method[6]. The results obtained with both methods were in close agreement. The degree of activation or inhibition of dopamine-β-hydroxylase was determined by comparing the amount of norepinephrine formed in an incubation mixture which contained only the substrate and in an incubation mixture which contained the tested compound and the substrate.

The effects of *p*-chloromercuribenzoate on dopamine-β-hydroxylase activity are shown in Table I. It can be seen that the degree of dopamine-β-hydroxylase inhibition by *p*-chloromercuribenzoate is dependent on the nature and the time of the preincubation mixture. Maximum inhibition is achieved when the preincubation mixture contains catalytic amounts of ascorbic acid (Table I, Expt. 3). This finding suggests that ascorbic acid reduces some disulfide linkages and makes thiol groups accessible for reaction with *p*-chloromercuribenzoate. Thus, ascorbic acid assumes a dual role in the stimulation of the enzymatic β-hydroxylation, it acts as an electron donor[1], and activates the enzyme by protection of the sulfhydryl group from oxidation. Protection against *p*-chloromercuribenzoate inhibition by prior addition of the substrate (Table I, Expt. 4b, c), and reversal of the inhibition by GSH (Table II, Expt. 1), indicates that dopamine-β-hydroxylase contains essential thiol groups which are probably located at the active center. It can also be seen from Table II, that the partially purified enzyme preparations are activated by GSH and that the degree of activation depends on the time which elapsed after the preparation of the enzyme. The activation by GSH is more pronounced with aged enzyme preparations. This suggests that the decrease in the activity of the aged enzyme is due to the oxidation of the essential sulfhydryl groups.

TABLE II

ACTIVATION OF ENZYME WITH GSH

Reaction mixtures and incubation conditions were as described in Table I. The concentration of *p*-chloromercuribenzoate (PCMB) was 10^{-4} M and that of GSH was 10^{-3} M. In Expt. 1b, all cofactors and PCMB were preincubated with the enzyme for 10 min, then GSH was added and the preincubation continued for another 10 min. The preincubated mixture was then dialyzed for 3 h against phosphate buffer (pH 6.8) in order to remove the excess GSH. All cofactors and substrate were then added to the dialyzed enzyme and the mixture was incubated. In all other experiments listed in Table II, the preincubation, dialysis and incubation was carried out in the same manner as in Expt. 1b. In Expt. 2, a 1–8-day old enzyme preparation was used, in Expt. 3, a 30–60-day old enzyme preparation was used.

Expt. No.	*Preincubation mixture*	*Norepinephrine formed (μmoles/ml)*	*Relative activity*
1	Enzyme, all cofactors	0.95	100
1a	Enzyme, all cofactors, PCMB	0.30	31
1b	Enzyme, all cofactors, PCMB, GSH	1.10	116
2	Enzyme**	1.20	100
2a	Enzyme, GSH	1.40	117
3	Enzyme**	0.70	100
3a	Enzyme, GSH	1.40	200

The studies of interaction of substrates and inhibitors with thiol groups of the enzyme may elucidate the mechanism of the enzymatic β-hydroxylation. It has been shown that adrenalone inhibits the enzymatic β-hydroxylation[7], and an attractive assumption would be that the carbonyl group of adrenalone reacts with the essential thiol group of the enzyme. The ease with which thiol groups add to carbonyls to give thiohemiacetals or thiohemiketals, has been described for several thiol enzyme systems[8]. Studies are now in progress to explore this hypothesis.

This work was supported by grant No. MH 02717-05, from the National Institutes of Health.

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Received June 24th, 1963

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* The Research Career Development Awardee of U.S. Public Health Service (Grant No. MH-K3-14, 918).

PN 10065

Chemical evidence for the presence of subunits in glyceraldehyde-3-phosphate dehydrogenase

The fingerprint of a tryptic hydrolysate of crystalline glyceraldehyde-3-phosphate dehydrogenase, (D-glyceraldehyde-3-phosphate: NAD oxydoreductase (phosphorylating), EC 1.2.1.12), isolated from swine muscle, is shown in Fig. 1. It is to be seen from the tracing that only 29 components (16 of them giving an intense colour reaction with ninhydrin and 13 a fainter one) could be separated under the experimental conditions applied. The basic components appeared to be, in addition to free lysine and arginine, mostly small peptides. Among the acidic components the one marked with "30" corresponds to free glutamic acid. The neutral components did not fractionate well under the conditions of fingerprinting. A considerable amount of material remained at the starting point.

From the tryptic hydrolysate 20% of the dissolved material precipitated at 3.3% end-concentration of trichloroacetic acid. The precipitate contained neutral and acidic components, while the basic peptides remained in the supernatant.

The trichloroacetic acid-insoluble fraction was oxidized with performic acid, then gel-filtrated on a Sephadex G-25 column in order to remove components of small molecular weight. The high-molecular-weight fraction could be separated into at least three chief components by chromatography on cellulose powder (Fig. 2). Fraction A was a peptide, containing 2.2% cysteic acid, and had a minimal molecular weight of about 7700. The analysis of this peptide is in progress.