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Conversion of (±)-Synephrine into *p*-Hydroxyphenylacetaldehyde by *Arthrobacter synephrinum*

A NOVEL ENZYMIC REACTION

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A partially purified enzyme from *Arthrobacter synephrinum* was found to catalyse the conversion of (±)-synephrine into *p*-hydroxyphenylacetaldehyde and methylamine. The enzyme is highly specific for synephrine and is distinctly different from monoamine oxidase.

Studies on the metabolism of the naturally occurring phenolic amine (±)-synephrine [(±)-*p*-hydroxy-α-(methylaminomethyl)benzyl alcohol] by *Arthrobacter synephrinum* have revealed that the organism does not adopt the pathway analogous to that operative in mammals for the degradation of catecholamines, involving the initial participation of monoamine oxidase (Abitha Devi *et al.*, 1975). Instead, it converts (±)-synephrine into *p*-hydroxyphenylacetaldehyde, which is subsequently channelled through the phenylacetate pathway utilized by many bacteria for the degradation of phenylalanine and tyrosine (Towers & Subba Rao, 1972). The present paper reports a hitherto unknown enzymic conversion of synephrine into *p*-hydroxyphenylacetaldehyde and methylamine by *A. synephrinum*.

Experimental

A. synephrinum, isolated from citrus-garden soils by enrichment with (±)-synephrine, was grown for 48h on mineral salts medium containing 0.1% (±)-synephrine under the conditions described by Abitha Devi *et al.* (1975). The cells were harvested by centrifugation, washed with 50mM-potassium phosphate buffer, pH7.0, and stored at −20°C.

Preparation of enzyme

Frozen cells of *A. synephrinum* (10g) were homogenized with twice their weight of glass powder in a chilled mortar and extracted with 50mM-potassium phosphate buffer, pH7.0, containing 5mM-2-mercaptoethanol (50ml). The slurry was centrifuged at 20000*g* for 10min. The clear supernatant was applied to a column (20cm×1cm) of DEAE-cellulose (coarse grade) (Sigma Chemical Co., St. Louis, MO, U.S.A.) pre-equilibrated with the same buffer. After the column had been washed with 50ml of 200mM-potassium phosphate buffer, pH7.0, the enzyme was eluted with the same buffer at 500mM concentration (100ml). Active fractions were pooled and used for the present studies.

Enzyme assay

A reaction mixture (1.0ml) containing 15mM-borate/HCl buffer, pH8.0, enzyme (0.1ml), 2mM-(±)-synephrine, 10mM-2-mercaptoethanol and 1mM-$MgSO_4$, was incubated at 30°C for 15min. The reaction was terminated by adding 1ml of 2,4-dinitrophenylhydrazine (0.05% in 2M-HCl). The amount of *p*-hydroxyphenylacetaldehyde formed was determined by the procedure of Green & Hanguton (1961).

Characterization of the reaction products

For the isolation of the enzymic products from (±)-synephrine, a large-scale incubation mixture (50ml), containing 25mM-borate/HCl buffer, pH8.0, 10mM-synephrine, 1mM-$MgSO_4$, 5mM-2-mercaptoethanol and 5ml of enzyme, was incubated for 1h at 30°C.

To a portion of the reaction mixture (25ml), 5ml of 1M-HCl was added, and the mixture was centrifuged at 2000*g* for 10min. The supernatant was extracted three times with equal volumes of peroxide-free diethyl ether. The combined ether extract was shaken with 3×10ml of 5% (w/v) $NaHCO_3$. The ether layer, after shaking with anhydrous Na_2SO_4, was evaporated to dryness under suction to obtain product I, which was redissolved in 1ml of ethyl acetate.

To another portion of the reaction mixture (25ml) 15ml of 10% $Mg(OH)_2$ was added, and product II was steam-distilled into 0.1M-HCl (10ml). A sample of this isolated product (0.5ml) was converted into its dansyl derivative as described by Creveling & Daly (1971), and another sample (0.5ml) was converted into its dinitrophenyl derivative as described by Dubin (1960).

Results

Two products were isolated from the reaction mixture incubated with (±)-synephrine and the partially purified enzyme from *A. synephrinum*. Product I was identified as *p*-hydroxyphenylacetaldehyde by comparing its properties with those of an authentic sample synthesized chemically from (±)-synephrine

Table 1. *Identification of metabolites of synephrine in the presence of an enzyme from A. synephrinum*

The solvents used for chromatography were: A, benzene/acetic acid/water (10:7:3, by vol.; organic phase); B, 2% formic acid; C, benzene/cyclohexane/methanol (85:15:4, by vol.); D, ethyl acetate/cyclohexane (3:2, v/v); E, ethyl acetate/cyclohexane (3:4, v/v); F, benzene/trimethylamine (5:1, v/v). The reagents for the colour reactions were: I, diazotized *p*-nitroaniline (3 vol. of 0.3% *p*-nitroaniline in 2.5 M-HCl + 1 vol. of 5% $NaNO_2$ + 9 vol. of 10% sodium acetate) followed by 10% NaOH; II, 2,4-dinitrophenylhydrazine (0.2% in 2 M-HCl) followed by 10% NaOH. Cellulose-coated plates (0.5 mm) were used for ascending chromatography of product I and authentic *p*-hydroxyphenylacetaldehyde. Silica-gel G-coated plates (0.25 mm), activated at 110°C for 10 min, were used for ascending chromatography of dansyl derivatives of product II and authentic methylamine.

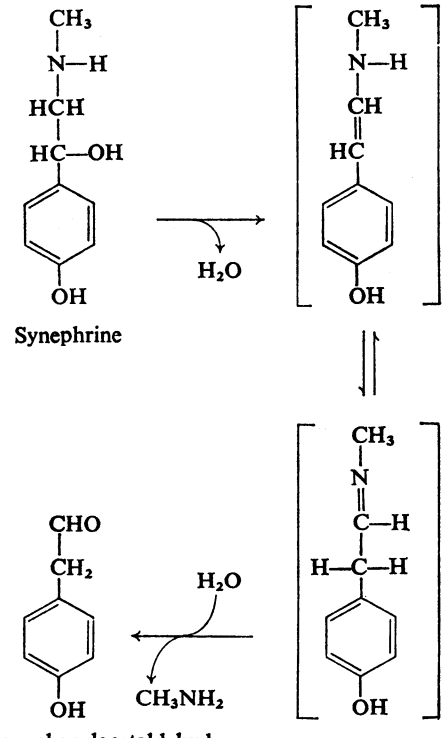
	R_F values						Colour reactions		U.v.-absorption spectrum ($\lambda_{max.}$ nm)		
Compound	A	B	C	D	E	F	I	II	Acid	Alkali	Dioxan
Enzymic product I	0.37	0.65	—	—	—	—	Violet to brown	Brown to yellow	275	285	—
p-Hydroxyphenylacetaldehyde (authentic)	0.37	0.65	—	—	—	—	Violet to brown	Brown to yellow	275	285	—
Dns derivative of enzymic product II	—	—	0.436	0.43	0.63	0.45	—	—	—	—	—
Dns derivative of methylamine (authentic)	—	—	0.436	0.43	0.63	0.45	—	—	—	—	—
Dnp derivative of enzymic product II	—	—	—	—	—	—	—	—	—	—	353
Dnp derivative of authentic methylamine	—	—	—	—	—	—	—	—	—	—	353

(Robbins, 1966). Chromatographically, the enzymic product I was indistinguishable from synthetic *p*-hydroxyphenylacetaldehyde in different solvent systems (Table 1). Further, the synthetic and enzymic products gave similar colour reactions when sprayed with diazotized *p*-nitroaniline followed by 10% NaOH, and with 2,4-dinitrophenylhydrazine followed by 10% NaOH. The u.v.-absorption spectrum of product I was identical with that of synthetic *p*-hydroxyphenylacetaldehyde, showing peaks at 275 nm and 285 nm in acid and alkali respectively (Table 1).

The other enzymic product (II) formed from (±)-synephrine was identified as methylamine by comparing its properties with those of an authentic sample. The reaction product when subjected to ascending paper chromatography (butan-1-ol/acetic acid/water, 4:1:5, by vol.; butanol layer) gave a ninhydrin-positive spot with R_F 0.34. A single spot with the same R_F value was obtained on mixing the enzymic product with authentic methylamine hydrochloride. The dansyl derivatives of synthetic methylamine and enzymic product II behaved identically in various solvent systems (Table 1). The visible spectra of dinitrophenyl derivatives of synthetic methylamine and enzymic product II were also similar, showing a peak at 353 nm (Table 1).

Properties of the enzyme

(±)-Synephrine was the only phenolic amine attacked by the enzyme preparation. Related com-



Scheme 1. *Possible reaction sequence for the conversion of (±)-synephrine into p-hydroxyphenylacetaldehyde*

pounds, such as tyramine, *N*-methyltyramine and hordenine (*NN'*-dimethyltyramine), did not serve as substrates. The enzyme had a pH optimum around 8.0 and showed an absolute requirement for bivalent cations such as Mg^{2+} or Ca^{2+}. 2-Mercaptoethanol or glutathione further enhanced the enzyme activity. The reaction assayed with the substrate and enzyme amounts described was linear up to 30min. The rate of the reaction was not affected when carried out in a Thunberg tube under strictly anaerobic conditions.

Discussion

Blaschko *et al.* (1937) have shown that synephrine could be oxidized by enzyme preparations from various tissues of guinea pig, rat, pig, pigeon, tortoise, frog and trout that oxidize adrenaline. Phenolic amines having a β-phenylethanolamine structure are attacked by monoamine oxidase in higher animals and are converted into the corresponding mandelic aldehyde derivatives. It has been suggested by Pisano *et al.* (1961) that monoamine oxidase may also convert synephrine into *p*-hydroxymandelic aldehyde by a similar reaction. Monoamine oxidase is a flavoprotein and the reaction proceeds under aerobic conditions. The enzyme from *A. synephrinum*, on the other hand, is not oxygen-dependent and catalyses the conversion of (±)-synephrine into *p*-hydroxyphenylacetaldehyde. Further, unlike monoamine oxidase, which oxidizes a variety of phenolic amines, the enzyme from *A. synephrinum* acts on synephrine alone.

The enzymic conversion of synephrine into *p*-hydroxyphenylacetaldehyde is a novel reaction which has not been reported so far. Since the reaction is not oxygen-dependent and since tyramine and *N*-methyltyramine are not intermediates in this reaction, it is likely that the reaction may involve dehydration of synephrine to form an enamine which could readily yield *p*-hydroxyphenylacetaldehyde and methylamine on hydrolysis (Scheme 1). In such a reaction sequence, if the function of the enzyme is simple dehydration, it may be called synephrine hydro-lyase.

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