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Inhibition of dopamine-β-hydroxylase by spinochrome A and echinochrome A, naphthoquinone pigments of echinoids

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THE NATURAL naphthoquinone pigments in echinoids (sea urchins), spinochrome A (2-acetyl-3,6-dihydroxynaphthazarin) and echinochrome A (2-ethyl-3,6,7-trihydroxynaphthazarin), were found to be potent inhibitors of tyrosine hydroxylase.[1] Other naphthoquinone derivatives such as aquayamycin[2,3] and deoxyfrenolicin[4] were also potent inhibitors of tyrosine hydroxylase. Among these naphthoquinones, aquayamycin was also found to be an inhibitor of dopamine-β-hydroxylase,[5] tryptophan hydroxylase and tryptophan 2,3-dioxygenase.[6] Dopamine-β-hydroxylase, a copper-containing enzyme, and tryptophan 2,3-dioxygenase, a heme-containing enzyme, both require ascorbic acid as a cofactor, whereas tyrosine hydroxylase and tryptophan hydroxylase require a tetrahydropterin as a cofactor.

Considering these previous results, we have examined the possibility of inhibition of dopamine-β-hydroxylase by spinochrome A and echinochrome A, and the mechanism of the inhibition. A preliminary report has appeared.[7]

Crystalline spinochrome A and echinochrome A were kindly supplied by Drs. Asashima and Kinoshita. The pigments were dissolved in ethanol in desired concentrations. The concentration of echinochrome A was estimated based on the extinction coefficient of $18{\cdot}9 \times 10^3$ M^{-1}, cm^{-1} at 263 nm in ethanol. Dopamine-β-hydroxylase was purified from fresh bovine adrenal medulla by the method of Friedman and Kaufman.[8] The enzymic activity was measured by two methods as described before.[9] The standard reaction mixture for the enzymic assay contained (μmoles): potassium phosphate buffer (pH 6·5) 200; tyramine or dopamine, 10; fumarate, 10; ascorbic acid, 10; an appropriate amount of the enzyme (5–30 μg of protein), enough catalase to give maximum stimulation and water to 1·0 ml. The reaction mixture was incubated for 30 min at 37°. When tyramine was substrate, the conversion of tyramine to norsynephrine was followed according to the spectrophotometric procedure of Creveling *et al.*[10] When dopamine was used as substrate, norepinephrine formed from dopamine was assayed according to the fluorometric procedure of von Euler and Floding[11] by an Aminco–Bowman spectrophotofluorometer.

When tyramine was used as substrate, both spinochrome A and echinochrome A inhibited dopamine-β-hydroxylase in the presence of ascorbic acid (Table 1). Spinochrome A and echinochrome A inhibited the enzyme by 50 per cent at 5×10^{-6} M and 3×10^{-5} M, respectively.

Nozaki *et al.*[6] have recently reported that aquayamycin at $1{\cdot}5 \times 10^{-6}$ M strongly inhibited the ferric form of tryptophan 2,3-dioxygenase in the presence of ascorbic acid, probably due to a rapid

TABLE 1. INHIBITION OF DOPAMINE-β-HYDROXYLASE BY SPINOCHROME A AND ECHINOCHROME A

Ascorbic acid (1×10^{-3} M)	Inhibitor	Concentration (M)	Activity (% of control)
+	Control*		100
+	Echinochrome A*	3×10^{-6}	103
		1×10^{-5}	81
		6×10^{-5}	31
+	Spinochrome A*	1×10^{-7}	108
		1×10^{-6}	92
		3×10^{-6}	71
		1×10^{-5}	39
		1×10^{-4}	11
−	Control†		100
−	Echinochrome A†	1×10^{-5}	52
		5×10^{-5}	30
		9×10^{-5}	33
		1×10^{-4}	31
+		1×10^{-4}	31
−	Spinochrome A†	5×10^{-5}	103
+		5×10^{-5}	27

* Tyramine was used as the substrate.
† Dopamine was used as the substrate.

oxidation of ascorbic acid by aquayamycin and the resultant exhaustion of oxygen in the reaction mixture. Therefore, the effect of 4×10^{-5} M spinochrome A or 6×10^{-5} M echinochrome A on oxidation of ascorbic acid was examined from the rates of oxygen consumption. Oxygen uptake was measured polarographically by the use of an oxygen electrode at 37°. Rapid oxygen consumption was observed by adding spinochrome A, suggesting the oxidation of ascorbic acid, whereas echinochrome A, did not increase the oxygen consumption by the oxidation of ascorbic acid.

The inhibition of spinochrome A was reversed by oxygen bubbling into the reaction mixture, while that by echinochrome A was not reversed by oxygen bubbling.

As dopamine can function as a reducing agent, dopamine can be used as substrate for the enzyme reaction without ascorbic acid, and 1 mole dopamine is oxidized to an orthoquinone during the hydroxylation of the side chain of another molecule of dopamine to norepinephrine.[12] As shown in Table 1, when dopamine was substrate in the absence of ascorbic acid, dopamine-β-hydroxylase was inhibited by echinochrome A, but not by spinochrome A. However, when ascorbic acid was contained in the reaction mixture with dopamine as substrate, dopamine-β-hydroxylase was inhibited both by spinochrome A and by echinochrome A.

These results indicate that spinochrome A, but not echinochrome A, inhibited the enzyme due to oxidation of ascorbic acid and by the resultant exhaustion of oxygen in the incubation mixture.

The inhibitions by spinochrome A and echinochrome A were reversed by the addition of Cu^{2+}, but the reverse by Cu^{2+} was higher in the case of echinochrome A than that in the case of spinochrome A. In order to see possible interaction of spinochrome A or echinochrome A with Cu^{2+}, changes in ultra-violet difference spectrum of the pigments by the addition of Cu^{2+} were examined. As shown in Fig. 1, the ultra-violet difference spectrum of spinochrome A was not changed significantly by the addition of Cu^{2+}. However, that of echinochrome A was changed significantly; the shoulder at 263 nm disappeared and a new shoulder at 284 nm appeared. The results suggest that these two pigments may interact with Cu^{2+} and that the interaction between echinochrome A and Cu^{2+} is stronger than that between spinochrome A and Cu^{2+}.

From these results, the inhibition of dopamine-β-hydroxylase by spinochrome A may be mainly due to the exhaustion of oxygen in the incubation mixture by a rapid oxidation of ascorbic acid by spinochrome A, whereas the inhibition by echinochrome A may be due to the interaction with Cu^{2+} contained in the enzyme and essential for the enzyme reaction.

This inhibition mechanism of dopamine-β-hydroxylase by echinochrome A and spinochrome A appears to be different from that of tyrosine hydroxylase.

Spinochrome A did not stimulate the oxidation of 6,7-dimethyltetrahydropterin, and inhibited the enzyme by combining with the enzyme probably through enzyme-bound Fe^{2+} and interfering with

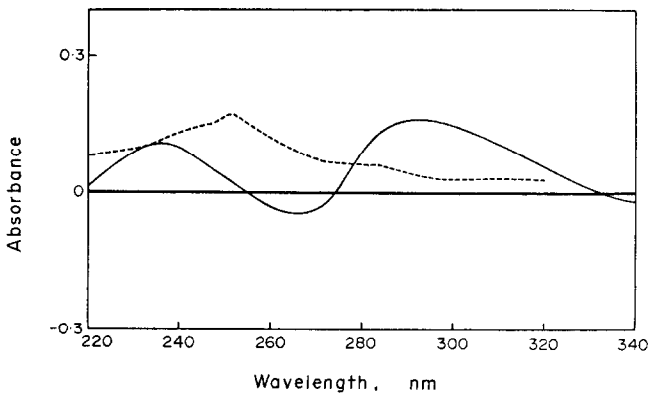

FIG. 1. Changes in the ultra-violet difference spectrum of spinochrome A and echinochrome A by the addition of cupric ion ($CuSO_4$). (——), spinochrome A (0·15 μmol) or echinochrome A (0·15 μmole) in 3·4 ml of ethanol; (– – – –), spinochrome A (0·15 μmole) plus $CuSO_4$ (0·2 μmole) in 3·4 ml of ethanol; (——), echinochrome A (0·15 μmole) plus $CuSO_4$ (0·06 μmole) in 3·4 ml of ethanol.

the binding of the substrate, tyrosine.[1] Echinochrome A appeared to form complexes with exogenous Fe^{2+} and stimulated the oxidation of dimethyltetrahydropterin in the presence of Fe^{2+} to consume the oxygen in the reaction mixture.[1]

Natural naphthoquinone pigments, such as echinochrome A and spinochrome A, and aquayamycin appear to be general inhibitors of hydroxylases like tyrosine hydroxylase and dopamine-β-hydroxylase. However, the inhibition mechanism seems to be complex, and different with the structure of the naphthoquinones, structure of the cofactor and also the enzyme protein. The mechanism of the inhibition may be due to complex formation through the enzyme-bound metal, or due to the non-enzymatic oxidation of the reducing cofactor and the resultant consumption of oxygen in the reaction mixture.

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