INHIBITION OF BRAIN TRYPTOPHAN 5-MONOOXYGENASE BY AQUAYAMYCIN*

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SUMMARY

Aquayamycin inhibits partially purified tryptophan 5-monooxygenase of guinea-pig brainstem about 40 and 80% at 0.1 and 1 μM respectively. The inhibition is not competitive with respect to substrate, $DMPH_4$** or Fe^{++} but is reversed by DTT**. Tryptophan 2,3-dioxygenase was also inhibited almost completely at 2 μM, but other oxygenases including metapyrocatechase, protocatechuate 3,4-dioxygenase, lysine monooxygenase and imidazoleacetate monooxygenase were not inhibited at all at 1 μM.

INTRODUCTION

Aquayamycin is a new antibiotic discovered by Sezaki et al.

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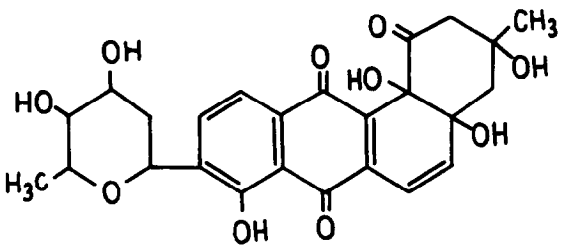
** The abbreviations used are: $DMPH_4$, 2-amino-4-hydroxy-6,7-dimethyl-5,6,7,8-tetrahydropteridine; DTT, dithiothreitol.

from a strain of Streptomyces (1), and its structure was recently established to be 9-(4,5-dihydroxy-6-methyltetrahydro-2-pyranyl)-3,4,4a,12b-tetrahydro-3,4a,8,12b-tetrahydroxy-3-methyl-benz[a]-anthracene-1,7,12(2H)-trione (2). It was reported to be a potent



Structure of Aquayamycin

inhibitor of tyrosine hydroxylase (3), an NADPH-linked pteridine containing monooxygenase and dopamine β-hydroxylase (4), an ascorbate requiring copper containing monooxygenase. These authors suggested that the mechanism of inhibition was probably due to the chelating action of aquayamycin on enzyme bound metal ions. In order to test whether or not aquayamycin inhibits other metal containing oxygenases and to investigate a possibility that this antibiotic may be a general inhibitor of oxygenases acting as a scavenger of active oxygen, we have studied the inhibitory action of aquayamycin on various crystalline or partially purified preparations of both dioxygenases and monooxygenases prepared in our laboratory. Although a number of oxygenases containing metal ions were completely insensitive to aquayamycin, tryptophan 5-monooxygenase of guinea-pig brainstem and tryptophan 2,3-dioxygenase (tryptophan pyrrolase) of Pseudomonas were found to be extremely sensitive to aquayamycin. This communications describes a preliminary account of

the mechanism of inhibition of tryptophan 5-monooxygenase of guinea-pig brain by aquayamycin.

MATERIALS AND METHODS

Crystalline aquayamycin was prepared according to Sezaki et al. (1). L-Tryptophan-(side chain-1-^{14}C) (9 mci per mmole) was purchased from New England Nuclear Corporation and purified by partition chromatography on Sephadex G-25 column (5). $DMPH_4$ and DTT were obtained from Calbiochem. 5-Hydroxy-L-tryptophan-(side chain-1,2,3-^{14}C) was prepared from L-serine-^{14}C (uniformly labeled, 160 mci per mmole) which was obtained from the Radiochemical Centre, Amersham, England and 5-hydroxyindole which was obtained from Sigma using tryptophan synthetase from E. coli, T_3 mutant (6) and purified by charcoal treatment, Dowex 1 column and partition chromatography on Sephadex G-25 column (5).

The homogenate of guinea-pig brainstem in 2 volumes of 10 mM Tris-acetate buffer, pH 8.1 was centrifuged for 30 min at 105,000 X g and the resulting supernatant was used as tryptophan 5-monooxygenase (5). Tryptophan 5-monooxygenase was assayed by measuring the rate of $^{14}CO_2$ evolution from L-tryptophan-(side chain-1-^{14}C) as substrate as described before (5). The standard assay mixture (0.5 ml) contained 50 µmoles of Tris-acetate, pH 8.1, 100 nmoles of pyridoxal phosphate, 5 to 10 units of aromatic L-amino acid decarboxylase which was partially purified from bovine brainstem, 30 µg of catalase, 1 µmole of β-mercaptoethanol, 500 nmoles of $DMPH_4$, 5 nmoles of ferrous ammonium sulfate, 5 nmoles of L-tryptophan-(side-1-^{14}C) (35,000 cpm) and 0.1 ml of the enzyme. The reaction was carried out for 60 min at 37°.

5-Hydroxy-L-tryptophan decarboxylase was determined by measur-

ing the rate of $^{14}CO_2$ evolution from 5-hydroxy-L-tryptophan-(side chain-1,2,3-^{14}C) employed as substrate (5). The assay system contained 20 μmoles of Tris-acetate, pH 8.8, 50 nmoles of pyridoxal phosphate, 2.83 nmoles of 5-hydroxy-L-tryptophan-(side chain-1,2,3-^{14}C) (34,000 cpm) and a suitable amount of enzyme preparations in a final volume of 0.2 ml. The reaction was carried out for 30 min at 37°.

Tryptophan 2,3-dioxygenase was extracted from cells of Pseudomonas fluorescens grown in a medium containing 0.13% L-tryptophan as inducer and purified by ammonium sulfate fractionation, heat treatment and DEAE-cellulose chromatography (7). The enzyme activity was assayed spectrophotometrically by measuring the increase in absorbance at 321 mμ at 24° (7). The standard assay mixture contained 250 μmoles of potassium phosphate buffer, pH 7.0, 20 μmoles of L-tryptophan, 10 μmoles of L-ascorbic acid and a suitable amount of enzyme in a final volume of 2.5 ml. The experiments of tryptophan 2,3-dioxygenase was carried out in collaboration with Miss Hiroko Nakano.

Metapyrocatechase was crystallized from Pseudomonas arvilla and assayed spectrophotometrically by Dr. Mitsuhiro Nozaki (8). Crystalline protocatechuate 3,4-dioxygenase was prepared from Pseudomonas aeruginosa and assayed spectrophotometrically (9). Imidazoleacetate monooxygenase was crystallized from Pseudomonas sp. and assayed spectrophotometrically (10) by Dr. Hiroshi Okamoto. Crystalline lysine oxygenase was prepared from Pseudomonas fluorescens and assayed polarographically (11, 12) by Dr. Teruko Nakazawa.

RESULTS AND DISCUSSION

Tryptophan 5-monooxygenase, first and probably the rate-limit-

Table I

Inhibition of tryptophan 5-monooxygenase by aquayamycin

The reaction mixture contained the standard assay mixture and aquayamycin (concentrations as indicated) in a final volume of 0.50 ml.

Aquayamycin concentration	Tryptophan 5-monooxygenase	
	Activity	Inhibition
M	pmole/hour	%
0	30.6	
10^{-7}	19.3	37
10^{-6}	6.9	78
10^{-5}	0.3	92
10^{-4}	0	100

ing enzyme in the biosynthetic pathway of serotonin, is extremely sensitive to aquayamycin (Table I). Almost 40 and 80% inhibitions were observed at concentrations of 0.1 μM and 1 μM aquayamycin respectively. At concentrations above 10 μM, inhibition was essentially complete. Other quinones, including coenzyme Q_7 and vitamin K_3, were not inhibitory at 1 μM. 5-Hydroxytryptophan decarboxylase activity was inhibited about 10% by 10 μM aquayamycin. Since the effect of aquayamycin on tyrosine hydroxylase (3) and dopamine β-hydroxylase (4) was suggested to be due to chelating activity and ferrous ion appeared to enhance the activity of tryptophan 5-monooxygenase, the effect of ferrous ion was tested. As shown in Fig. 1, ferrous ion itself stimulated the activity by 20 to 30% below 10 μM but is rather inhibitory above 100 μM, but the inhibition by aquayamycin was not affected at all by Fe^{++}. On the other hand, the

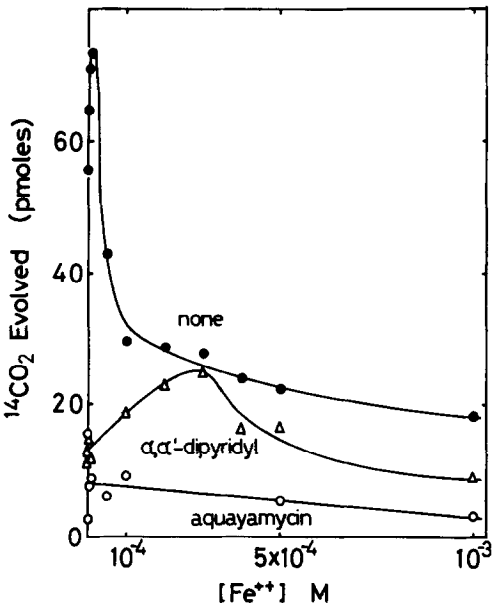


Fig. 1. Effect of Fe^{++} on the inhibition of tryptophan 5-monooxygenase by aquayamycin and α,α'-dipyridyl. The reaction mixture contained the standard assay mixture without ferrous ion and the compounds as indicated. The concentrations of aquayamycin and α,α'-dipyridyl were 1 μM and 40 mM, respectively.

inhibition by α,α'-dipyridyl (about 80% at 400 μM) was partially counteracted by the addition of 300 μM ferrous ion. Crystalline preparations of neither metapyrocatechase, a typical dioxygenase containing Fe^{++}, nor protocatechuate 3,4-dioxygenase, a typical dioxygenase containing Fe^{+++}, were inhibited at all by aquayamycin at a concentration of 10 μM. Crystalline preparations of neither lysine monooxygenase nor imidazoleacetate monooxygenase were inhibited by 10 μM aquayamycin, indicating that this antibiotic is not a general inhibitor of monooxygenase reactions.

The inhibition of tryptophan 5-monooxygenase by aquayamycin was not competitive with respect to substrate and was not affected by increasing the concentration of $DMPH_4$ up to 2 mM with the aquayamycin concentration of 1 μM. However, this inhibition was partially reversed by DTT and other sulfhydryl compounds (Table II). The en-

Table II

Effect of sulfhydryl compounds and ascorbic acid on the aquayamycin inhibition of tryptophan 5-monooxygenase

The reaction mixture contained the standard assay mixture without β-mercaptoethanol and the compounds as indicated. The concentration of sulfhydryl compounds and ascorbic acid was 10 mM.

Additions	Tryptophan 5-monooxygenase activity		
	Without aquayamycin	10^{-6} M aquayamycin	% inhibition
	pmole/hour	pmole/hour	%
None	22.6	6.1	73
β-Mercaptoethanol	52.2	10.1	81
DTT	96.8	89.3	8
L-Glutathione	89.8	48.8	46
L-Ascorbic acid	31.2	7.6	76

zyme activity was stimulated by sulfhydryl compounds and ascorbic acid, but the inhibition of aquayamycin was counteracted most effectively by high concentrations of DTT. In contrast, highly purified preparations of tryptophan 2,3-dioxygenase, heme-containing dioxygenase, was completely inhibited by 2 μM aquayamycin but the latter inhibition was not reversed by DTT and other sulfhydryl reagents. The possibility that the inhibitory action of aquayamycin is due to a chelating action appears unlikely and further studies are now in progress to elucidate the mechanism of inhibition of tryptophan 5-monooxygenase and tryptophan 2,3-dioxygenase by aquayamycin.

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