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N-HYDROXY-2-ACETYLAMINOFLUORENE AS EFFECTIVE INHIBITOR OF ALDEHYDE OXIDASE

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SUMMARY. N-Hydroxy-2-acetylaminofluorene has been found to be an effective inhibitor of aldehyde oxidase. At concentrations of 1 x 10^{-6}M and 1 x 10^{-5}M, 38 % and 88 % inhibition was observed on the oxidase activity towards N^1-methylnicotinamide. The inhibition was of noncompetitive type and had a Ki value of 4.4 x 10^{-6}M. In contrast, little inhibition of the enzyme was observed with 2-aminofluorene, 2-acetylaminofluorene and acetohydroxamic acid even at a concentration of 1 x 10^{-4}M.

The carcinogenic properties of 2-acetylaminofluorene (AAF) was found by Wilson et al. in 1941 (1) and the versatile carcinogenic activity of the compound has been demonstrated by a variety of species and tissues (2). Since the report on the discovery of N-hydroxy-2-acetylaminofluorene (N-hydroxy-AAF), a N-aryl aliphatic hydroxamic acid, as a urinary metabolite of AAF (3), considerable evidence has been presented that N-hydroxy-AAF is a proximate metabolite (4,5) which undergoes further biotransformation to electrophilic reactants in the mechanism of carcinogenesis of AAF (6). However, no information is available concerning the ability of N-hydroxy-AAF and its related compounds to inhibit an enzyme. The present communication provides the first evidence that N-hydroxy-AAF is an effective inhibitor of aldehyde oxidase (EC 1.2.3.1), a well known molybdenum hydroxylase.

MATERIALS AND METHODS

AAF, N^1-methylnicotinamide and acetohydroxamic acid were purchased from Tokyo Chemical Industry Co., Ltd. 2-Aminofluorene,

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menadione, dithiothreitol, cysteine and xanthine were obtained from Nakarai Chemicals, Ltd. Reduced and oxidized forms of glutathione were obtained from Sigma Chemical Co., and bovine serum albumin (Fraction V from bovine plasma) from Amour Pharmaceutical Co., respectively. N-Hydroxy-AAF (3), N-hydroxy-4-acetylaminobiphenyl (7), N-hydroxyphenacetin (8) and N-hydroxy-2-propionylaminofluorene (9) were synthesized by the methods described in the references.

Buttermilk xanthine oxidase (No.X-1875) was purchased from Sigma Chemical Co. Aldehyde oxidase was purified from livers of male albino rabbits (2.0-2.5 kg) by the method of Rajagopalan et al. (10).

Aldehyde oxidase activity was assayed at 25° in 1 ml of 0.1 M phosphate buffer (pH 7.4) containing 0.25 μmol of N^1-methylnicotinamide as a substrate and 0.02 unit of aldehyde oxidase by measuring the increase in absorbance at 300 nm. Xanthine oxidase activity was also assayed at 25° in 1 ml of 0.1 M phosphate buffer (pH 7.4) containing 0.1 μmol of xanthine as a substrate and 0.02 unit of xanthine oxidase by measuring the increase in absorbance at 290 nm.

RESULTS AND DISCUSSION

As shown in Table I, N-hydroxy-AAF was found to exhibit significant inhibition of aldehyde oxidase at relatively low concentrations. In contrast, little inhibition of the enzyme was observed with 2-aminofluorene and AAF even at a concentration of 1×10^{-4}M. N-Hydroxy-AAF possesses the acetohydroxamic acid moiety in its molecule. However, acetohydroxamic acid itself, which is known as an inhibitor of urease (11-13), had little ability to inhibit aldehyde oxidase even at a concentration of 1×10^{-4}M. These facts suggested that the inhibitory activity of N-hydroxy-AAF against aldehyde oxidase is due to the presence in its molecule of two moieties of an aliphatic hydroxamic acid such as acetohydroxamic acid and a N-substituted aromatic ring such as fluorene. In fact, other N-aryl aliphatic hydroxamic acids with such chemical structures, e.g., N-hydroxy-4-acetylaminobiphenyl, N-hydroxyphenacetin and N-hydroxy-2-propionylaminofluorene also exhibited the inhibition of aldehyde oxidase, although their activities were somewhat lower compared with the activity of N-hydroxy-AAF (Table I). With menadione, a well known potent inhibitor of aldehyde oxidase, 56 % and 82 % inhibition of the enzyme was observed at concentrations of 1×10^{-6}M and 1×10^{-5}M, respectively, under the present

Table I. Effect of N-hydroxy-2-acetylaminofluorene and its related compounds on aldehyde oxidase

Compound	Concentration (M)	Inhibition (%)
N-Hydroxy-2-acetylamino-fluorene	1×10^{-6}	38
	1×10^{-5}	88
2-Aminofluorene	1×10^{-5}	0
	1×10^{-4}	3
2-Acetylaminofluorene	1×10^{-5}	0
	1×10^{-4}	8
Acetohydroxamic acid	1×10^{-5}	0
	1×10^{-4}	5
N-Hydroxy-4-acetylamino-biphenyl	1×10^{-6}	15
	1×10^{-5}	51
	1×10^{-4}	70
N-Hydroxyphenacetin	1×10^{-6}	4
	1×10^{-5}	27
	1×10^{-4}	44
N-Hydroxy-2-propionyl-aminofluorene	1×10^{-6}	25
	1×10^{-5}	52

Each value represents the mean of three experiments.

assay conditions. There is the similarity between aldehyde oxidase and xanthine oxidase in some properties. However, the latter enzyme could not be inhibited by these N-aryl aliphatic hydroxamic acids including N-hydroxy-AAF at a concentration of 1×10^{-4}M (data not shown).

Fig.1 showed the double reciprocal plots of N-hydroxy-AAF inhibition of aldehyde oxidase with varying concentrations of N^1-methylnicotinamide. N-Hydroxy-AAF had no effect on the Km value but altered the Vmax value of the aldehyde oxidase activity, indicating that the inhibition is of noncompetitive manner. An appa-

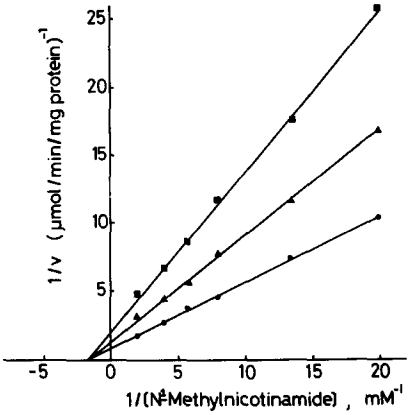

Fig.1. Kinetics analysis of the inhibition of aldehyde oxidase by N-hydroxy-2-acetylaminofluorene using the double reciprocal plot. The assay was performed in the absence (●) or presence of 1 μM (▲) and 5 μM (■) N-hydroxy-2-acetylaminofluorene.

rent Ki value of 4.4×10^{-6}M was obtained from replots of the slopes versus N-hydroxy-AAF concentrations. In contrast to N-hydroxy-AAF herein described, the published analytical data of the effects of aldehyde oxidase inhibitors, i.e., quinacrine (14), arsenite (14), p-mercuribenzoate (14) and 4'-(9-acridinylamino)-methanesulfon-m-anisidide (15) showed that the inhibition was of competitive type. The facts suggest that N-hydroxy-AAF is different from these known inhibitors in the mechanisms for affecting the operation of aldehyde oxidase.

As shown in Table II, the partial protection against the N-hydroxy-AAF inhibition of aldehyde oxidase was achieved in the presence of dithiothreitol, cysteine and glutathione, but not in the presence of oxidized glutathione and bovine serum albumin. In this case, N-hydroxy-AAF added was almost completely recovered from the incubation mixture, ruling out a possibility that the sulfhydryl compounds operated as trapping agents of N-hydroxy-AAF (data not shown).

Aldehyde oxidase is known to catalyze the oxidation of nitrogen-containing aromatic heterocyclic compounds (14,16,17) and aldehydes (16,18). In addition, the enzyme has been shown to

Table II. Protective effect of sulfhydryl compounds on the inhibition of aldehyde oxidase by N-hydroxy-2-acetylaminofluorene

Addition	Aldehyde oxidase activity (% of control)
None	100
N-Hydroxy-2-acetylamino-fluorene*	31
+ Dithiothreitol**	80
+ Cysteine**	88
+ Glutathione (reduced)**	73
+ Glutathione (oxidized)**	31
+ Bovine serum albumin***	30

Each value represents the mean of three experiments.
* 5×10^{-6}M, ** 1×10^{-3}M,
*** 5 mg/ml incubation mixture

have the ability to reduce a variety of xenobiotics (19-25). N-hydroxy-AAF should be useful in studying the in vitro mechanism for such operation of the enzyme.

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