Communications to the Editor

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EVIDENCE FOR REDUCTION OF HYDROXAMIC ACIDS TO THE CORRESPONDING AMIDES BY LIVER ALDEHYDE OXIDASE

Kazumi Sugihara, Shigeyuki Kitamura and Kiyoshi Tatsumi*
Institute of Pharmaceutical Sciences, Hiroshima University, School of Medicine, 1-2-3, Kasumi, Minami-ku, Hiroshima, 734, Japan

Recently, interest in hydroxamic acids has been stimulated because of their important biological activities and their clinical use as novel therapeutic agents. The present paper is the first description of the enzyme responsible for the reduction of hydroxamic acids to the corresponding amides, one of the main metabolic reactions of these compounds. Guinea pig and rabbit liver aldehyde oxidase supplemented with its electron donor exhibited significant reductase activity toward salicylhydroxamic acid. The reduction of nicotinhydroxamic acid and anthranilhydroxamic acid by this enzyme was also demonstrated.

KEYWORDS —— arylhydroxamic acid; enzymatic reduction; reduction product; arylamide; guinea pig; rabbit; liver aldehyde oxidase

In recent years there has been a growing interest in hydroxamic acids and in 1981 the first international symposium on the compounds was held in U.S.A. So far many important biological activities have been discovered in a variety of hydroxamic acids: e.g. urease-inhibitory action, vasodilating action, activity against Trypanosoma, antifungal activity, ribonucleotide reductase-inhibitory action, antineoplastic activity, antiinflammatory and analgesic actions, tuberculostatic activity, serum lipid-lowering activity, mutagenic and carcinogenic activities, and so on.[1] Hydroxamic acids are mainly metabolized by reduction to the corresponding amides or by hydrolysis to the corresponding acids. The *in vitro* metabolism of arylhydroxamic acids has been demonstrated using several liver preparations as follows: conversion of benzohydroxamic acid to benzamide and benzoic acid by sheep liver homogenate,[2] reduction of salicylhydroxamic acid to salicylamide by rat liver homogenate,[3] reduction of nicotinhydroxamic acid to nicotinamide by mouse liver mitochondria,[4] and reduction of anthranilhydroxamic acid to anthranilamide by rat and mouse liver slice.[5] However, little is known about the nature of the enzyme responsible for such metabolic reactions. The present communication describes the first evidence that liver aldehyde oxidase is involved in the reduction of salicylhydroxamic acid and others.

The ability of guinea pig liver 9,000 x g supernatant to catalyze the reduction of salicylhydroxamic acid to salicylamide was examined under both aerobic and anaerobic conditions. As shown in Table I, the liver preparation exhibited, under anaerobic conditions, a significant reductase activity toward the hydroxamic acid in the presence of an electron donor of aldehyde oxidase such as 2-hydroxypyrimidine, N^1-methylnicotinamide or acetaldehyde rather than in the presence of NADPH or NADH.

TABLE I. Reduction of Salicylhydroxamic Acid by Liver 9,000 x g Supernatant, Microsomes and Cytosol of Guinea Pigs

Addition	Salicylamide formed (μmol/30 min/g liver)			
	9,000 x g Supernatant		Microsomes	Cytosol
	Anaerobic	Aerobic	Anaerobic	Anaerobic
None	2.76	2.63	0.23	2.30
NADPH*	4.07	3.28	0.47	3.33
NADH*	5.15	3.15	0.43	4.83
2-Hydroxypyrimidine**	18.80	3.60	0.42	16.30
N^1-Methylnicotinamide**	8.58	2.68	0.23	7.28
Acetaldehyde**	8.85	3.10	0.36	8.30
Hypoxanthine**	2.68	2.70	-	-

Each value represents the mean of three experiments.
*An NADPH-generating system (1 μmol of NADP, 5 μmol of glucose-6-phosphate and 1 unit of glucose-6-phosphate dehydrogenase) or an NADH-generating system (1 μmol of NAD, 50 μl of ethanol and 5 units of alcohol dehydrogenase.
**10 μmol.

TABLE II. Effect of Some Chemicals on Salicylhydroxamic Acid Reductase and Aldehyde Oxidase Activities of the 30-45% Ammonium Sulfate Precipitate from Guinea Pig Liver Cytosol

Addition	Concentration (M)	Salicylamide formed* Control (%)	Aldehyde oxidase activity Control (%)
None	-	100	100
Potassium cyanide	$1x10^{-3}$	13	20
Sodium arsenite	$1x10^{-4}$	10	24
Menadione	$1x10^{-4}$	3	12
p-Chloromercuribenzoic acid	$1x10^{-4}$	0	9
Quinacrine	$1x10^{-4}$	75	60
Chlorpromazine	$1x10^{-4}$	20	12
Tiron	$2x10^{-4}$	62	60
Antimycin A	$1x10^{-4}$	45	28

Each value represents the mean of three experiments.
*The assay was performed in the presence of 10 μmol of acetaldehyde under anaerobic conditions.

When the liver preparation was separated into cytosolic and microsomal fractions, the reductase activity was almost completely associated with the former fraction, but not with the latter. These results suggested that aldehyde oxidase present in the cytosol is a principal enzyme responsible for the reduction of the hydroxamic acid in guinea pig livers.

Previously we showed that when guinea pig liver cytosol was fractionated with ammonium sulfate, aldehyde oxidase was largely precipitated between 30 and 45% ammonium sulfate saturation.[6] Therefore, the ability of the ammonium sulfate precipitate to reduce salicylhydroxamic acid was examined under anaerobic conditions. As a result, the precipitate exhibited a significant reductase activity toward the hydroxamic acid in the presence of electron donors of aldehyde oxidase described above (data not shown). As shown in Table II, the susceptibility of the reductase activity to inhibition by a variety of chemicals was parallel to that of the aldehyde oxidase activity of the precipitate. Next, the ammonium sulfate precipitate was chromatographed on a DEAE-cellulose (DE-52) column. After adsorption of the protein, elution was carried out with 10 mM phosphate buffer (pH 7.4), followed by a gradient of increasing NaCl concentration up to 0.2M concentration. As a result, the elution peak of the reductase was observed at the 0.125M concentration of NaCl, and the peak position was identical to that of the aldehyde oxidase.

As shown in Table III, furthermore, purified rabbit liver aldehyde oxidase supplemented with its electron donor exhibited a significant reductase activity toward salicylhydroxamic acid. Our preliminary study showed that the guinea pig and rabbit liver aldehyde oxidase can also catalyze the reduction of nicotinhydroxamic acid to nicotinamide and that of anthranilhydroxamic acid to anthranilamide. These results led to the conclusion that guinea pig and rabbit liver aldehyde oxidase functions as an enzyme responsible for the reduction of hydroxamic acids to the corresponding amides.

TABLE III. Reduction of Salicylhydroxamic Acid by Rabbit Liver Aldehyde Oxidase

Addition	Salicylamide formed* (μmol/30 min/mg protein)
None	0
2-Hydroxypyrimidine**	4.09
N^1-Methylnicotinamide**	4.00
Acetaldehyde**	2.10
NADPH***	0.50
NADH***	0.86

Each value represents the mean of three experiments.
*The assay was performed under anaerobic conditions.
**10 μmol.
***An NADPH- or NADH-generating system (See the legend of Table I).

EXPERIMENTAL

Salicylhydroxamic acid and salicylamide were purchased from Tokyo Kasei Kogyo Co., Ltd. For enzyme preparation, male guinea pigs (350-400 g) and male albino rabbits (2.0-2.5 kg) were used: Preparation of guinea pig liver microsomes and cytosol (105,000 x g supernatant), and fractionation of the cytosol with ammonium sulfate were performed as described previously.[7] Rabbit liver aldehyde oxidase was purified by the method of Rajagopalan et al.[8]

In the assay of hydroxamic acid reductase, a typical incubation mixture consisted of 1 μmol of salicylhydroxamic acid, an electron donor and an enzyme preparation in a final volume of 2.5 ml of 0.1M K,Na-phosphate buffer (pH 7.4). Incubation was carried out anaerobically for 30 min at 37°C using a Thunberg tube as described previously,[7] or aerobically in an open vessel. Then, the mixture was extracted twice with two volumes of ethyl acetate and the combined extract, after addition of 0.5 μmol of benzoic acid as an internal standard, was evaporated to dryness in vacuo. The residue was subjected to HPLC to determine the reduction product, salicylamide as follows: HPLC was carried out by using a Toyo Soda HLC-803 HPLC apparatus equipped with a LS-410K reversed phase column. The mobile phase was 0.1M KH_2PO_4-CH_3CN (9:1) and the flow rate was 1.2 ml/min. The elution time was 18 min for salicylamide or 9 min for salicylhydroxamic acid. The aldehyde oxidase activity was assayed by the method of Felsted et al.[9]

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