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A novel HPLC procedure for detection and quantification of aminoacetone, a precursor of methylglyoxal, in biological samples

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Abstract

Increase in methylglyoxal is thought to be involved in different pathological conditions. Deamination of aminoacetone by semicarbazide-sensitive amine oxidase (SSAO) leads to production of methylglyoxal. We have synthesized aminoacetone and developed a novel HPLC procedure for its quantitative determination. The urinary excretion of aminoacetone is approximately 20–30 μg/mouse/day, and the concentration is about 0.5 μg/g in mouse liver and small intestine. SSAO inhibitor increases aminoacetone levels in both tissues and urines. Results confirm that aminoacetone is an endogenous substrate for SSAO. However, data also indicate that deamination is not the only catabolic pathway for aminoacetone.

Keywords: Aminoacetone; SSAO; Methylglyoxal; HPLC

1. Introduction

Semicarbazide-sensitive amine oxidase (SSAO, EC 1.4.3.6) is a group of copper containing enzymes present in many mammalian tissues including humans, with relatively high activities present in vascular smooth muscle, cartilage and adipose tissues [1–3]. The enzyme has been independently identified as an endothelial surface adhesion molecule regulating lymphocytes trafficking [4]. Adipose SSAO has also been shown to regulate glucose transport [5]. Methylamine and aminoacetone are thought to be physiological substrates for SSAO [6–8]. SSAO catalyzes the oxidative deamination of these primary amines and lead to production of formaldehyde, and methylglyoxal, respectively, as well as hydrogen peroxide and ammonia [1]. Since these products are quite toxic, the potential pathological implications of deamination of methylamine has been addressed [1,9,10]. It is known that methylglyoxal is increased and related to protein glycation in different pathological conditions, such as diabetes and aging [11,12]. Oxidative deamination of aminoacetone to methylglyoxal has been shown using extracts of human umbilical artery [8] and plasma of goat and bovine [13,14]. Interestingly, administration of aminoacetone was shown not only to increase the urinary excretion of methylglyoxal but also malondialdehyde, an oxidative stress marker, in mice [15]. Methylglyoxal is primarily synthesized by the phosphorylated triose pathway [12]. It is unclear how important is deamination of aminoacetone contributing to methylglyoxal production. Aminoacetone is synthesized from threonine and glycine [16,17]. Although aminoacetone exhibits high affinity for SSAO [15], its presence in tissue has not been reported. Treatment with a SSAO inhibitor reduces the urinary excretion of methylglyoxal in rodents [18]. Investigation on aminoacetone is hampered, since the

Abbreviations: SSAO, semicarbazide-sensitive amine oxidase; AA, aminoacetone; MA, methylamine; MDL-72974A, (*E*)-2-(4-fluorophenetyl)-fluoroallylamine; FMOC-Cl, fluorenylmethyl chloroformate

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compound is not available commercially. In the present study, aminoacetone has been synthesized and an HPLC procedure for its measurement in urine and tissues established. This is the first report showing the presence of aminoacetone in tissues.

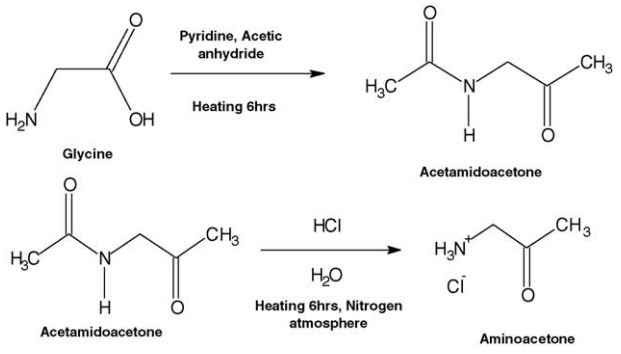
2. Experimental

2.1. Chemicals

Glycine, pyridine, acetic anhydride, acetone, tetramethylammonium chloride, boric acid, sodium hydroxide, phosphorus pentoxide, citric acid, phosphoric acid, hydrochloric acid, acetic acid, sodium acetate, ethanol, diethyl ether and 9-fluorenylmethyl chloroformate (FMOC-Cl) were purchased from Sigma–Aldrich. HPLC-grade acetonitrile, methanol, hexane and hydrochloric acid were obtained from Merck (Darmstadt, Germany). Unless stated otherwise, all reagents were of analytical grade. Potassium borate buffer was prepared from boric acid (0.8 M) adjusted to the desired pH 10 with 5 M potassium hydroxide. (*E*)-2-(4-Fluorophenetyl)-fluoroallylamine MDL-72974A was kindly provided by Marion-Merrell Dow (Cincinnati, Ohio, USA).

2.2. Synthesis and purification of aminoacetone hydrochloride

Aminoacetone hydrochloride was synthesized and purified using a modified procedure according to Hepworth [19] (Scheme 1). Acetamidoacetone was prepared by mixing glycine (37.5 g, 0.5 mol), pyridine (242.5 g, 3.0 mol), and acetic anhydride (595 g, 5.835 mol) heating to boiling, refluxing and stirring for 6 h. The reflux condenser is replaced by one set for downward distillation and the excess pyridine, acetic anhydride, and acetic acid were removed by distillation under reduced pressure. The residues yielded a pale yellow oil (bp 120–125 °C, 1 mmHg). The acetamidoacetone (26 g) was further mixed in concentrated hydrochloric acid (87.5 ml) and water (87.5 ml), and then boiled under reflux in a nitrogen atmosphere for 6 h. The resulting solution containing aminoacetone product is concentrated obtaining a dark red oily residue which is then dried under reduced pressure over phosphorus pentoxide to crystal aminoacetone hydrochloride. Aminoacetone HCl salt was re-crystallized with absolute ethanol and diethyl ether. Yields for acetamidoacetone and aminoacetone after re-crystallization were 45% and 4.1%, respectively. Under a stream of dry nitrogen the solid residues were rapidly filtered through a sintered glass funnel, then washed with ether again and immediately transferred to sample vials, dried again, and sealed in dry nitrogen. The melting point of aminoacetone HCl crystal is sharp at 72–73 °C. Nuclear magnetic resonance (NMR) and HPLC were used to substantiate the identity of aminoacetone. NMR was performed in D_2O solvent using a Varian Germini 400 MHz NMR spectrometer. The data indicate the presence of protons of methyl and methylene groups in acceptable proportion: δH 2.18 (s, 3H), 3.99 (s, 2H). HPLC analysis was conducted as described bellow. Authentic aminoacetone from Dr. Etelvino Bechara was used for quantitative comparison. The synthesized aminoacetone is therefore highly a pure compound. The chemical is kept in a cool and dark place in bottles filled with nitrogen gas. For the preparation of a working standard, aminoacetone was dissolved in absolute alcohol (1 mg/ml) and stored in a freezer (−20 °C), under these conditions it was stable for at least a month.



Scheme 1. Chemical synthesis of aminoacetone from glycine via acetamidoacetone.

2.3. Animal experiments

Male CD1 Swiss white mice weighing 30 g and Wistar rats (260–300 g) were used in the experiments. The animals were housed in hanging wire cages with free access to food and water on 12 h light/dark cycle (lights on at 6 a.m.) at temperature 19–20 °C. The experimental protocol has been designed according with the guidelines of the Canadian Council on Animal Care and approved by University of Saskatchewan Animal Care Committee.

Mice were treated with saline (100 μl, i.p.), SSAO inhibitor (MDL-72974A) (5 mg/kg, i.p.), and subsequently two hours later with aminoacetone (10 mg/kg, i.p.). After the last injection, the mice were placed in metabolic cages for urine collection over a period of 24 h. The collecting vessels were positioned in Styrofoam boxes containing dry ice to freeze the urines immediately after excretion.

Regarding analyses of aminoacetone in tissues animals were killed 2 h after injection of saline or MDL-72974A (5 mg/kg, i.p.) and aminoacetone (10 mg/kg, i.p.). The liver and small intestine were dissected, washed in saline and stored at −70 °C. For the ex vivo catabolism experiments the freshly dissected liver and small intestine were sliced using a McIlwain tissue chopper (Michle Laboratory Engineer, Surry, UK).

Rats were used for the assessment of aminoacetone concentrations in tissues and ex vivo catabolism studies. The collected tissues ($n = 3$) were further divided into three independent parts for independent analyses.