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ORIGINAL ARTICLE

In vitro oxidation of aldehyde oxidase from rabbit liver: Specificity toward endogenous substrates

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Abstract The endogenous vitamins such as pyridoxal (vitamin B6) and all-*trans* retinaldehyde (vitamin A) are metabolized to more or less toxic metabolites by drug-metabolizing enzymes including aldehyde oxidase (AO; EC 1.2.3.1). To better understand this function, the specificity of the rabbit liver aldehyde oxidase enzyme toward endogenous vitamins was quantitatively studied. Therefore, the present study showed the kinetic parameters of AO for the oxidation of vitamin B6 and vitamin A were measured in partially purified rabbit liver fraction. K_m values of AO endogenous vitamin were observed with pyridoxal (21 ± 6.4 μM) and all-*trans*-retinal (46 ± 9.1 μM) respectively for partially purified rabbit liver fraction. AO from rabbit liver fraction showed high V_{max} with vitamin B6 and vitamin A (1.84 ± 0.2 and 1.28 ± 0.1 nmol/min/mg protein, respectively). Therefore, the present study showed the kinetic parameters of AO for the oxidation of vitamin B6 and vitamin A were measured in partially purified rabbit liver fraction. A high affinity and low K_m values of AO endogenous vitamin were observed with pyridoxal (21 ± 6.4 μM) and all-*trans*-retinal (46 ± 9.1 μM), respectively for partially purified rabbit liver fraction. Pyridoxal and all-*trans*-retinal oxidized to their metabolites (25.2 ± 12.7 and 13.3 ± 4.1 nmol/min/mg protein, respectively) by partially purified rabbit liver aldehyde oxidase. These results confirmed that the hydrophobicity enhances affinity of pyridoxal and all-*trans*-retinal (aromatic aldehyde) toward AO as excellent substrates. It is concluded these results presented serve as a guide for predicting the susceptibility of endogenous to oxidation by rabbit liver AO.

1. Introduction

Xenobiotics are compounds that are foreign to the body, which include drugs, pollutants and other substances that are not normally present in the body and are potentially toxic. Xenobiotic metabolism is the series of metabolic reactions that change the chemical structure of xenobiotics; generally acting to detoxify the toxic chemical compounds. Sometimes, however, the product of xenobiotic metabolism can be the cause of toxic effects (Hodgson and Smart, 2001). Of the biotransformations that

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occur in animals oxidation plays a major role in the metabolism of foreign compounds. Although the microsomal cytochrome P-450 mono-oxygenase system is of major importance in this respect, enzymes present in the cytosol also contribute to this process. This study is concerned with the enzyme aldehyde oxidase (AO; EC 1.2.3.1) which is a molybdo-flavoenzyme found in nearly every organism from bacteria to humans (Beedham, 2001; Garattini et al., 2003, 2008, 2009; Garattini and Terao, 2011). AO catalyzes the oxidation of many different N-heterocyclic compounds as well as aliphatic and aromatic aldehydes to their corresponding lactam and carboxylic acids respectively (Beedham, 2001; Garattini et al., 2003, 2008; Garattini and Terao, 2011, 2012). Although AO catalyzes the biotransformation of several endogenous compounds, the absolute primary physiological function of AO is yet to be determined. The physiological importance of aldehyde oxidase's role in aldehyde oxidation is in question due to the fact that the Michaelis constant (K_m) for AO is higher for aliphatic aldehydes than is that of another mammalian enzyme, aldehyde dehydrogenase (ALDH) [EC; 1.2.1.3] (Jakoby and Ziegler, 1990; Panoutsopoulos et al., 2004). Two notable endogenous substrates for AO include retinaldehyde and pyridoxal (Beedham, 2001; Garattini et al., 2003, 2008; Garattini and Terao, 2011, 2012; Huang et al., 1999; Kitamura et al., 2006). Retinaldehyde is the principle component of visual pigments and for this reason it has been suggested that aldehyde oxidase may play an important part in the overall visual process since it catalyzes the biotransformation of this aldehyde to its corresponding carboxylic acid, and retinoic acid, which is the active form of vitamin A (Calzei et al., 1995; Garattini et al., 2008; Garattini and Terao, 2011, 2012; Huang et al., 1999; Stanulovic and Chaykin, 1971). The involvement of AO in all-*trans* retinaldehyde oxidation to all-*trans* retinoic acid was first seen in rabbit liver cytosol, where it was observed that a fraction of the oxidizing activity did not require an addition of NAD^+ and was due to a molybdo-flavoenzyme (Garattini et al., 2008; Tomita et al., 1993; Tsujita et al., 1994). As well as its ability to catalyze the biotransformation of vitamin A metabolite (Fig. 1) AO also converts vitamin B6 (pyridoxal) to 4-pyridoxic acid (Fig. 1) (Tomita et al., 1993). Vitamin B6 is a water-soluble compound that contains a pyridine ring. Vitamin B6 is present in nature in several different forms such as pyridoxal (PL), pyridoxine (PN), pyridoxamine (PM) and their active form pyridoxal 5′-phosphate (PLP) (Fitzpatrick et al., 2007). PLP is the coenzymatically active form of vitamin B6 and plays an important role in maintaining the biochemical homeostasis of the body (Meister, 1990). There are more than 100 PLP-dependent enzymes in a cell that perform essential roles in various metabolic pathways including amino acid metabolism (such as amino acid synthesis and degradation), fatty acid metabolism (such as synthesis of polyunsaturated fatty acids) and carbohydrate metabolism (such as breakdown of glycogen) (Mooney et al., 2009). The preferred degradation route from PLP to 4-pyridoxic acid involves the dephosphorylation of PLP by phosphatase (Jang et al., 2003) followed separately by the actions of aldehyde oxidase and β-nicotinamide adenosine dinucleotide-dependent dehydrogenase (Schwartz and Kjeldgaard, 1951; Stanulovic et al., 1976). In mice Garattini et al. (2008) reported that pyridoxal can be oxidized by purified mouse aldehyde oxidase AOX_1 and AOH1, although it is not an efficient substrate in the case of AOH2. Although a wealth of data is available on endogenous substrates of AO they are still being sought (Garattini et al., 2009).

The activity of AO between animal species varies depending on the substrate considered. Sugihara et al. found the activity of AO in monkeys is higher than in humans when using N^1-methylnicotinamide and benzaldehyde as substrates (Sugihara et al., 2006). Klecker et al. (2006) found the activity of AO is highest in mouse toward zebularine substrate than in monkeys and humans (Klecker et al., 2006). Species differences have also been found when using cinchonidine as a substrate where rabbits have higher AO activity than monkeys (Fukiya et al., 2010).

Therefore, the present study investigates the role of AO in the metabolism of pyridoxal and all-*trans* retinaldehyde in partially purified rabbit liver fractions. The specificity of liver rabbit AO for these substrates was quantitatively explored by determining kinetic constants for a variety of endogenous compounds.

2. Materials and methods

2.1. Chemicals

All chemicals and reagents were obtained from Fisher Scientific and Sigma/Aldrich Chemical Company Ltd., Poole, UK. Mobile phase reagents and solvents were obtained from various companies but were all for HPLC grade purity.

2.2. Preparation of aldehyde oxidase fractions

The New Zealand white male rabbit liver sample was isolated, apportion was taken, weighed, chopped and placed immediately in 3–4 volumes of ice-cold isotonic potassium chloride solution (1.15% KCl w/v) containing 0.1 mM EDTA and homogenized on ice in a homogenizer fitted with a Teflon pestle for 1–2 min at 4 °C. The resulting homogenate was then heated at 55–57 °C for 10 min on a steam bath and ammonium sulfate precipitation as described by Beedham et al. (1995). Rabbit liver fraction was stored in liquid nitrogen until used for spectrophotometric and HPLC analyses.

2.3. Spectrophotometric measurement of enzyme activity

All spectrophotometric aldehyde oxidase assays were conducted using a microplate reader spectrophotometer (BioTek). All assays were carried out in triplicate in 100 µl reaction volumes. All cytosol samples were frozen and thawed only once, and the spectrophotometric data were collected at 5 s intervals for 3–5 min using Gen5™ software on a Windows XP PC connected to the microplate reader spectrophotometer (BioTek). Enzyme activity of partially purified fractions was monitored using all-*trans*-retinal and pyridoxal. Oxidation of 0.1 mM pyridoxal was then monitored at 388 nm and of 0.1 mM all-trans-retinal was monitored at 380 nm as substrates in the presence of molecular oxygen as the electron acceptor using the molar extinction coefficients for pyridoxal and all-*trans*-retinal which are 4900 M^{-1} cm^{-1} and 43,400 M^{-1} cm^{-1}, respectively (Peterson and Sober, 1954; Jäger et al., 1996). All reactions were carried out in phosphate buffer saline, pH 7.4 at 37 °C.