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Involvement of carbonyl reductase in superoxide formation through redox cycling of adrenochrome and 9,10-phenanthrenequinone in pig heart

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Abstract

The effects of adrenochrome, a metabolite of epinephrine (adrenaline), and 9,10-phenanthrenequinone (PQ), a component of diesel exhaust particles, on the stereoselective reduction of 4-benzoylpyridine (4-BP) were examined in pig heart cytosol. PQ was a potent inhibitor for the 4-BP reduction, while adrenochrome was a poor inhibitor. A similar result was observed in the effects of adrenochrome and PQ on the reduction of all-*trans* retinal. Furthermore, although PQ mediated efficiently the formation of superoxide anion radical through its redox cycling in pig heart cytosol, adrenochrome had no ability to mediate the superoxide formation. These may be because the reactivity for adrenochrome, catalyzed by pig heart carbonyl reductase (PHCR), is much lower than that for PQ. The optimal pH for the reduction of PQ in pig heart cytosol was around 5.5. Dicumarol, a potent inhibitor of DT-diaphorase, had little effect on the time course of NADPH oxidation during the reduction of PQ. Therefore, it is concluded that PHCR plays a critical role in superoxide formation through redox cycling of PQ.

Keywords: Adrenochrome; 9,10-Phenanthrenequinone; Carbonyl reductase; Superoxide formation; Pig heart

1. Introduction

Quinones are widely distributed in nature and environment. Quinones cause a variety of toxic effects, including cardiotoxicity, neurotoxicity, immunotoxicity and carcinogenesis [1,2]. The toxic effects of quinones depend mainly on the production of reactive oxygen species through redox cycling mediated by microsomal NADPH-cytochrome P450 reductase (EC 1.6.2.4). The flavin enzyme catalyzes the one-electron reduction of quinones to semiquinones that would result in redox cycling. On the other hand, cytosolic DT-diaphorase (EC 1.6.99.2) and carbonyl reductase (EC 1.1.1.184) have the ability to reduce quinones to

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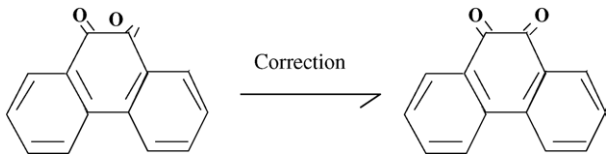

Fig. 1. Chemical structures of adrenochrome and PQ.

hydroquinones through two-electron transfer mechanism [3–7], leading to a detoxification pathway because the resulting hydroquinones serve as substrates for glucuronidation and sulfation reactions. Thus, it is likely that DT-diaphorase and carbonyl reductase play a role in protection against toxic effects of quinones. However, when secondary reactions such as glucuronidation and sulfation become limiting, autoxidation of the hydroquinones could contribute to the generation of reactive oxygen species [8].

Catecholamines can be enzymatically or non-enzymatically oxidized to the corresponding *o*-quinones [9–11]. For example, epinephrine (adrenaline) is biotransformed to adrenochrome (Fig. 1A). Adrenochrome is known to induce cardiotoxicity and neurotoxicity [9,10,12]. We have recently demonstrated that 9,10-phenanthrenequinone (PQ) shown in Fig. 1B, a component of diesel exhaust particles, not only inhibits the reduction of 4-benzoylpyridine (4-BP) and all-*trans* retinal in the cytosolic fraction of pig heart, but also mediates superoxide formation through its redox cycling [13]. That is, PQ disturbs the homeostasis of retinoid metabolism and induces oxidative stress by acting probably as a substrate inhibitor of carbonyl reductase present in pig heart (pig heart carbonyl reductase, PHCR). Since adrenochrome, like PQ, is one of *o*-quinones, it may be reduced to its *o*-hydroquinone by PHCR, and mediate superoxide formation through its redox cycling. The purpose of this study is to examine the involvement of carbonyl reductase in superoxide formation through redox cycling of adrenochrome and PQ in pig heart.

2. Materials and methods

2.1. Materials

The chemicals were purchased from the following sources. Adrenochrome, PQ, all-*trans* retinal and all-*trans* retinol were from Sigma Chemical Co. (St. Louis, MO); 4-BP, cytochrome *c*, superoxide dismutase (SOD, from bovine erythrocyte) and dicumarol were from Wako Pure Chemical Industries (Osaka, Japan); NADPH, $NADP^+$, glucose-6-phosphate and glucose-6-phosphate dehydrogenase were from Oriental Yeast Co. (Tokyo, Japan). *S*(−)-α-Phenyl-4-pyridylmethanol [*S*(−)-PPOL] was synthesized from 4-BP as reported previously [14]. All other chemicals were of reagent grade.

2.2. Preparation of pig heart cytosol

The pig hearts were supplied from a slaughterhouse and stored at −20 °C. The tissues were homogenized in three volumes of 10 mM sodium potassium phosphate buffer containing 1.15% KCl (pH 6.0). The homogenates were centrifuged at 105,000 g for 60 min to obtain the cytosolic fraction.

2.3. Stereoselective reduction of 4-BP

The stereoselective reduction of 4-BP was estimated by measuring *S*(−)-PPOL formed in pig heart cytosol [13]. The reaction mixture consisted of substrate (0.5 mM 4-BP), NADPH-generating system (50 μM $NADP^+$, 1.25 mM glucose-6-phosphate, 50 munits glucose-6-phosphate dehydrogenase and 1.25 mM $MgCl_2$), pig heart cytosol and 100 mM sodium potassium phosphate buffer (pH 6.0) in a final volume of 0.5 ml. In the case of inhibition experiments, adrenochrome or PQ dissolved in dimethyl sulfoxide was added at a concentration of 10 μM. The reaction mixture was incubated at 37 °C for 10 min and boiled for 2 min to stop the reaction. After centrifugation at 5000 rpm, the supernatant (20 μl) was subjected to HPLC for the determination of the reduction product, *S*(−)-PPOL, of 4-BP. HPLC was carried out using a Waters 600E HPLC apparatus (Japan Waters, Tokyo, Japan) equipped with a Daicel Chiralpak AD-RH col-