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Mitochondrial electron transfer chain complexes inhibition by different organochalcogens

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ABSTRACT

Mitochondrial dysfunction plays a pivotal role in the cell toxicology and death decision. The aim of the present study was to investigate the effect of three organocompounds (ebselen [Ebs], diphenyl diselenide [$(PhSe)_2$] and diphenyl ditelluride [$(PhTe)_2$]) on mitochondrial complexes (I, II, I–III, II–III and IV) activity from rat liver and kidney to determine their potential role as molecular targets of organochalcogens. All studied organochalcogens caused a statistically significant inhibition of the mitochondrial complex I activity. Ebs and $(PhTe)_2$ caused a statistically significant inhibition of the mitochondrial complex II activity in both hepatic and renal membranes. Hepatic mitochondrial complex II activity was practically unchanged by $(PhSe)_2$, whereas it significantly inhibited renal complex II activity. Mitochondrial complex IV activity was practically unchanged by the organochalcogens. Furthermore, organochalcogens inhibited the mitochondrial respiration supported by complex I or complex II substrates. The inhibitory effect of Ebs, $(PhSe)_2$ and $(PhTe)_2$ on mitochondrial complex I was prevented by NADH, but it was not prevented by catalase (CAT) and/or superoxide dismutase (SOD). Additionally, the organochalcogens-induced inhibition of complex I and II was completely reversed by reduced glutathione (GSH). In conclusion, Ebs, $(PhSe)_2$ and $(PhTe)_2$ were more effective inhibitors of renal and hepatic mitochondrial complex I than complex II, whereas complexes III and IV were little modified by these compounds. Taking into account the presented results, we suggest that organochalcogen-induced mitochondrial complexes I and II inhibition can be mediated by their thiol oxidation activity, i.e., Ebs, $(PhSe)_2$ and $(PhTe)_2$ can oxidize critical thiol groups from mitochondrial complexes I and II. So, mitochondrial dysfunction can be considered an important factor in the toxicity of Ebs, $(PhSe)_2$ and $(PhTe)_2$.

1. Introduction

Living organisms use a series of integral membrane protein complexes for energy conversion and ATP synthesis (Hatefi, 1985). In addition to their crucial role in energy production and metabolic pathways, the mitochondrial complexes also play key roles in integrating cell death stimuli and executing the apoptotic program (Navarro and Boveris, 2007). Accordingly, several human diseases, such as Alzheimer's disease, Friedreich's ataxia, familial amyotrophic lateral sclerosis, and Huntington's disease, are associated with mitochondrial electron transport chain inhibition, energy metabolism impairment and oxidative stress (Beal, 1998; Nicholls and Budd, 2000). Additionally, biochemical studies indicate a decline of electron transport and in some bioenergetic activities of mitochondria during aging and ischemia–reperfusion (Cadenas and Davies, 2000; Caspersen et al., 2005; Cortopassi and Wong, 1999; Hagen et al., 1998; Hauptmann et al., 2006; Navarro and Boveris, 2007; Nicholls, 2002; Saris and Eriksson, 1995; Sastre et al., 2003). Thus, mitochondrial dysfunction can be associated with different degenerative cellular processes.

Organoselenium and organotellurium compounds have been extensively studied because of their potential antioxidant capacity (Arteel and Sies, 2001; Barbosa et al., 2006, 2008; de Bem et al., 2009; de Freitas et al., 2009; Hort et al., 2011; Moretto et al., 2007; Nogueira and Rocha, 2011; Parnham and Graf, 1991; Prauchner et al., 2011; Prigol et al., 2008; Puntel et al., 2007). In line with this, literature data have indicated that these compounds can provide protective effect against lipid peroxidation induced by a variety of pro-oxidants agents (Barbosa et al., 2006, 2008; Moretto et al., 2007; Nogueira and Rocha, 2010, 2011; Parnham and Graf, 1991; Puntel et al., 2007; Rossato et al., 2002). The antioxidant activity of these organochalcogens has been ascribed either to their glutathione peroxidase-like activity (Maiorino et al., 1988; Santos et al., 2005; Sies, 1993, 1995) or to the fact that they can be substrates

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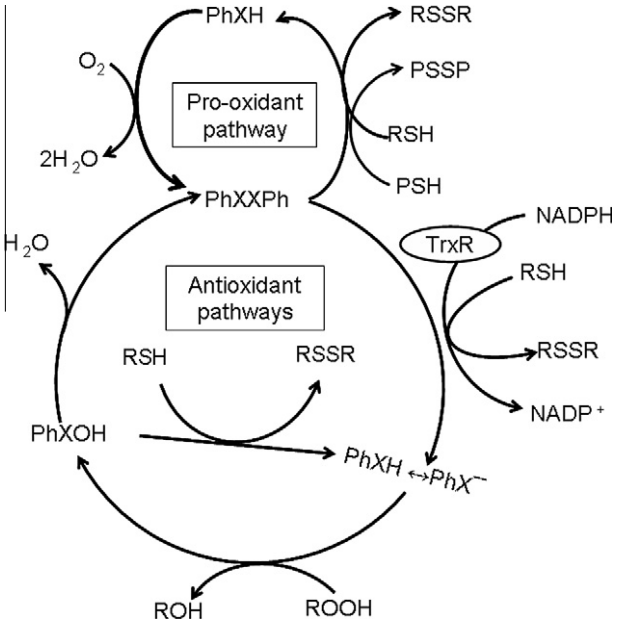
of mammalian thioredoxin reductase (de Freitas and Rocha, 2011; Sausen de Freitas et al., 2010; Zhao and Holmgren, 2002; Zhao et al., 2002). Thus, in order to exert antioxidant properties, the selenium containing compounds have to be metabolized to selenol/selenolate intermediates, a reaction which can be accomplished via reduction of the Se moiety by different types of thiols (Nogueira and Rocha, 2011; Wendel et al., 1984) (Scheme 1). For organotellurium compounds, it has been postulated that the antioxidant activity is linked to changes in the oxidation state of the Te atom (Te(II) ↔ Te(IV)) (Engman et al., 1995; Leonard et al., 1996; You et al., 2003).

Thus, the thiol-peroxidase or thioredoxin-thiol-peroxidase-like activity of organochalcogens (Nogueira and Rocha, 2011; Sausen de Freitas et al., 2010; Zhao and Holmgren, 2002; Zhao et al., 2002) can be of biological and therapeutic significance via artificial modulation of the cellular levels of peroxides. However, the excessive oxidation of thiols, including those in mitochondrial membranes, by organochalcogens without a concomitant reduction of peroxides may be toxic to living cells (thiol-oxidation activity) (Nogueira and Rocha, 2011; Puntel et al., 2010) (Scheme 1). In effect, mitochondrial dysfunction caused by thiol oxidation is closely related to the apoptotic cell death (Morin et al., 2003; Zhao et al., 2006). Accordingly, the organochalcogens should be considered as putative candidates for apoptotic cell death inducer via mitochondrial dysfunction, which may explain, at least in part, their pharmacological/toxicological action (Ardais et al., 2010; Nogueira and Rocha, 2010; Santos et al., 2009a,b). In line with this, recently our group showed that both Ebselen (Ebs) and diphenyl diselenide [$(PhSe)_2$] induced mitochondrial dysfunction via interaction with critical mitochondria thiols (Puntel et al., 2010).

Considering that mitochondrial complexes play a central role in cellular metabolism and in the regulation of apoptotic cell death, we sought to determine whether these mitochondrial complexes could be considered molecular targets for the thiol-oxidation activity of Ebs, $(PhSe)_2$ or diphenyl ditelluride [$(PhTe)_2$]. Specifically, our main objective in this study was to determine whether Ebs, $(PhSe)_2$ and $(PhTe)_2$ could cause mitochondrial complex(es) I, II, I–III, II–III and IV inhibition using mitochondrial membranes prepared from rat liver and kidney. We also determined whether organochalcogens could cause mitochondrial respiration inhibition using intact mitochondria in order to better understand their toxicological site of action at the molecular level.



Scheme 1. Thiol peroxidation and thiol oxidation cycle of dichalcogenides. In the toxic pathway the formation of the selenol/tellurol is associated with a futile oxidation of low-molecular- (RSH) or protein-thiol groups (PSH) causing depletion of GSH levels and/or protein loss of function. In the therapeutic pathway organochalcogens decompose peroxides either as a substrate for TrxR or as a mimic of GPx via the formation of the selenol/selenolate or tellurol/tellurate (PhXH/PhX-) intermediate (X can be Se or Te).

2. Materials and methods

2.1. Chemicals

Chemicals, including NADH, mannitol, rotenone, succinic acid, malonate, potassium cyanide (KCN), sucrose, HEPES and cytochrome c were obtained from Sigma Chemical Company (St Louis, MO, USA). All other reagents were commercial products of the highest purity grade available.

2.2. Animals

Adult male Wistar rats (250–350 g) from our own breeding colony were used in this study. The animals were housed in plastic cages with water and food *ad libitum*, at 22–23 °C, 56% humidity, and 12 h light cycle. The diet of the rats containing (in g/100 g): 52 carbohydrate, 20 crude protein, 5 fat, 6 crude fiber, 5 minerals and 11 moisture. Diet contained 0.1 mg/kg of Se and 30 IU/kg of vitamin E (for complete mineral and vitamin contents, see reference (Puntel et al., 2010)). The protocol was approved by the Institutional Animal Care and Use Committee of Federal University of de Santa Maria (42/2010) and conducted in accordance with the Guide for the Care and Use of Laboratory Animals.

2.3. Isolation of rat liver and kidney mitochondria and mitochondrial membranes preparation

Liver and kidney mitochondria were isolated in a solution containing 0.23 M mannitol, 0.07 M sucrose, 15 mM HEPES (pH 7.2) at a ratio of 1 g of tissue/9 mL of homogenization medium in a Potter homogenizer with a Teflon pestle. The homogenate was centrifuged at 700*g* for 10 min, and the supernatant centrifuged at 8000*g* for 10 min to yield a mitochondria pellet that was washed once in the same buffer. Mitochondrial protein concentration was adjusted to 20 mg/mL (Peterson, 1977) and the samples were immediately frozen and kept at −80 °C. Mitochondria were disrupted and homogenized by twice freezing and thawing and by passage through 15/10 tuberculin needles to produce the mitochondrial membrane preparation according to (Navarro et al., 2002) which were used to the mitochondrial complexes activity assay. In order to study the organochalcogens effect on mitochondrial respiration (oxygen consumption measurements) intact mitochondria were used.

2.4. Mitochondrial complexes activities assay

The activities of complexes I, I–III, II, II–III, and IV were determined spectrophotometrically at 30 °C with mitochondrial membranes (0.5 mg/mL) suspended in 100 mM phosphate buffer (pH 7.4) as previously described (Navarro et al., 2002, 2004) with minor modifications. The mitochondrial membranes were pre-incubated in phosphate buffer in the presence of different organochalcogens concentration (Ebs 0–50 μM; $[(PhSe)_2]$ 0–100 μM; $[(PhTe)_2]$ 0–100 μM, vehicle (DMSO), or the respective classical inhibitor (positive controls) for 10 min. After pre-incubation, the reaction was started with the addition of the corresponding substrate, except for the experiments using reduced glutathione (GSH), as described