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Surfactant and antioxidant properties of an extract from *Chenopodium quinoa* Willd seed coats

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ABSTRACT

Chenopodium quinoa Willd (quinoa) has been a source of food for millennia by the Andes region native population. Because of its bitter taste, quinoa seeds are commercialized without their coat for human consumption. Quinoa coats are surfactant sub-products of the quinoa food industry, which have been only characterized to contain triterpene saponins. We postulated that this coat should also contain antioxidant molecules as part of the defense system of the quinoa seed. We found that a quinoa seed coats hydroalcoholic extract, displayed thiol compounds in addition to polyphenols, recognized antioxidants. Accordingly, it inhibited microsomal lipid peroxidation and the loss of microsomal thiol content, both oxidative phenomena promoted by Cu^{2+}/ascorbate. Microsomal glutathione *S*-transferase (GST) is inhibited by reducing agents, which decrease the content of catalytically active disulfide-linked dimers. The effects of this quinoa extract on microsomal GST are consistent with it displaying disulfide reducing properties. The occurrence of thiol compounds in this quinoa extract is discussed in terms of the potential of their antioxidant properties.

1. Introduction

Chenopodium quinoa Willd (quinoa) belongs to the Chenopodiaceae family and corresponds to a dicotyledonous plant from the Andes region (Mujica, 1984). Quinoa seeds have been traditionally consumed as food by the native population of the Andes region. These seeds contain several vitamins in low concentrations, minerals, oils, antioxidants, high content of proteins, starch, and essential free amino acids (Berti et al., 2004; Mahoney et al., 1975; Repo-Carrasco et al., 2003; Woldemichael and Wink, 2001). Processing of quinoa seeds for human consumption involves the elimination of their coat, due to its bitter taste (Meyer et al., 1990). The coat of quinoa seeds has been only characterized in terms of its high triterpene saponin content (Meyer et al., 1990; Woldemichael and Wink, 2001). This feature allows its commercialization as a surfactant sub-product. Thus, some of quinoa saponins promote hemolysis (Reichert et al., 1985; Woldemichael and Wink, 2001) and changes in intestinal permeability (Estrada et al., 1998). Sunlight is the energy source for plants but it promotes oxidative stress. Thus, it is reasonable to postulate that the quinoa coat, the outmost layer of the seed, contains antioxidants, whose occurrence has yet to be studied in this part of the plant.

Reactive oxygen species (ROS) are produced in all aerobic cells as second messengers and as a byproduct of oxygen metabolism (Droge, 2002). Cells have evolved antioxidant defenses to control ROS generation (Benzie, 2000). If ROS generation overwhelms the cellular antioxidant capacity, oxidative stress ensues. Under these conditions, ROS can oxidize lipids, proteins and nucleic acids, ultimately leading to cell death (Halliwell and Gutteridge, 2007). Polyphenols, the main herbal antioxidants, can protect biomolecules from oxidative damage through different mechanisms that include direct scavenging of oxygen free radicals and chelating transition metal ions (Bors and Michel, 2002); the latter, in their free ionic form may generate ROS *via* Haber–Weiss and/or Fenton reactions (Halliwell and Gutteridge, 2007). Thus, polyphenols are widely used in the treatment of pathologies associated with oxidative stress, such as neurodegenerative disorders (Migliore and Coppede, 2009), cardiovascular diseases (Fearon and Faux, 2009) and cancer (Visconti and Grieco, 2009).

The present work addresses both the surfactant and antioxidant properties of a hydroalcoholic extract of quinoa seed coat. Surfactant activity was measured in terms of the release of hemoglobin from red blood cells. To evaluate the antioxidant activity of the

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quinoa extract, we used rat liver microsomes as a biological system and Cu^{2+}/ascorbate as a ROS-generating system. Antioxidant activity was measured in terms of inhibition of microsomal lipid peroxidation and protection of microsomal thiol content elicited by Cu^{2+}/ascorbate. Polyphenol and thiol contents were also measured in the quinoa extract, which are herbal molecules widely recognized as antioxidant agents.

2. Experimental

2.1. Reagents

The extract from the coat of *C. quinoa* Willd seeds (30% ethanol: H_2O) was kindly donated by Laboratorios Ximena Polanco (Santiago, Chile); aliquots of a freshly prepared extract were stored at −20 °C for up to 3 months. Blood samples from healthy adults were obtained from the Blood Bank of the Clinical Hospital Universidad de Chile, following standard collection and analysis. BSA (Fraction IV), $CuSO_4$, catechin, Folin–Ciocalteau reagent, sodium ascorbate, 5,5′-dithio-*bis* (2-nitrobenzoic) acid (DTNB, Ellman's reagent), 1-chloro-2,4-dinitrobenzene, and GSH were purchased at Sigma–Aldrich. All other chemicals were of the best grade available.

2.2. Animals

Adult male Sprague Dawley rats (200–250 g), maintained at the vivarium of the School of Chemical and Pharmaceutical Sciences, Universidad de Chile (Santiago, Chile) were used. Rats were allowed free access to pelleted food, maintained with controlled temperature (22 °C) and photoperiod (lights on from 07:00 to 19:00 h). All procedures were performed using protocols approved by the Institutional Ethical Committee of the School of Chemical and Pharmaceutical Sciences, Universidad de Chile, and according to the guidelines of the Guide for the Care and Use of Laboratory Animals (NRC, USA).

2.3. Determination of polyphenol and thiol contents of the quinoa extract

Polyphenols were determined as previously described (Letelier et al., 2008), using catechin as standard. Thiols were titrated with DTNB (Ellman's reagent), as formerly described (Letelier et al., 2005).

2.4. Isolation of rat liver microsomes

Microsomal fraction was prepared according to Letelier et al. (Letelier et al., 2005). Protein determinations were performed using the Lowry method (Lowry et al., 1951), using BSA as standard.

2.5. Isolation of red blood cells (RBC)

Fresh blood samples were centrifuged at 1000 g for 10 min at 4 °C. Supernatants were discarded and pellets were washed once with 10 volumes of cold PBS, pH 7.4. Plasma and buffy coat were aspirated and pellets (RBC: 1×10^7 cells/mL) were resuspended in cold PBS, pH 7.4 for analysis.

2.6. Release of hemoglobin from RBC

RBC suspensions were incubated with or without increasing amounts of the quinoa extract (up to 20 μL) for 15 min at 4 °C. Following centrifugation at 500 g for 10 min at 4 °C, absorbance of supernatants was determined at 550 nm. Hemoglobin content was calculated using the $\epsilon 550 = 53{,}412\ M^{-1}\ cm^{-1}$ (Gratzer and Beaven, 1960). For these experiments, 0.1% (V/V) Triton X-100 was used as a positive control.

2.7. Basal microsomal lipid peroxidation assay

Microsomes (1 mg protein/mL) were incubated with increasing volumes of the quinoa extract for 5 min at 25 °C. Afterward, lipid peroxidation was estimated assaying thiobarbituric acid reactive species (TBARS), as previously described (Letelier et al., 2005). For these experiments, 0.05% (V/V) Triton X-100 was used as a positive control, which increased this parameter to 117.6 ± 2.3% ($n = 4$).

2.8. Oxidative conditions

Microsomes (1 mg protein/mL) were incubated with 25 nM $CuSO_4$ plus 1 mM sodium ascorbate for 30 min at 37 °C with constant agitation prior to measuring lipid peroxidation and total thiol content. In other experiments, microsomes (1 mg protein/mL) were incubated with 0.1 mM H_2O_2 for 30 min at 37 °C with constant agitation prior to assaying GST activity.

2.9. Microsomal lipid peroxidation assay

Microsomal lipid peroxidation elicited by Cu^{2+}/ascorbate was estimated assaying TBARS, as described above. The effect of quinoa extract on lipid peroxidation was determined by incubating microsomes (1 mg protein/mL) with or without increasing concentrations of the extract (up to 50 μL) for 5 min at 25 °C, prior to incubation with the Cu^{2+}/ascorbate system. Catechin was used as a positive control.

2.10. Determination of microsomal thiol content

Thiol groups were titrated with DTNB as described previously (Letelier et al., 2005). Microsomes (1 mg protein/mL) were incubated with or without increasing concentrations of the extract (up to 20 μL) for 10 min at 37 °C and then with the Cu^{2+}/ascorbate system for 30 min at 37 °C, prior to titrate thiol content. Catechin was used as a positive control.

2.11. GST activity assay

Conjugation of 1-chloro-2,4-dinitrobenzene, reaction catalyzed by GST, was assayed essentially as previously described (Letelier et al., 2006). Apparition of the conjugate was continuously recorded for 3 min at 25 °C, at 340 nm in a UV3 Unicam UV–VIS spectrophotometer. GST activity was calculated using the $\epsilon 340 = 9.6\ mM^{-1}\ cm^{-1}$ of the conjugate.

2.12. Statistical analysis

Data represent the mean ± SD of at least 4 independent experiments. Statistical significance (ANOVA) and regression analyses were performed using Graph Pad Prism 5.0. Differences were considered as significant when $p < 0.05$.

3. Results

3.1. Characterization of the quinoa seed coat extract

Analysis of polyphenol and thiol compounds content of the quinoa extract showed the occurrence of 9.0 ± 0.33 μmol-equivalents catechin/mL and 1.94 ± 0.10 μmol/mL, respectively.

3.2. Surfactant activity

The quinoa extract promoted the concentration-dependent release of hemoglobin from a suspension of red blood cells (RBC,

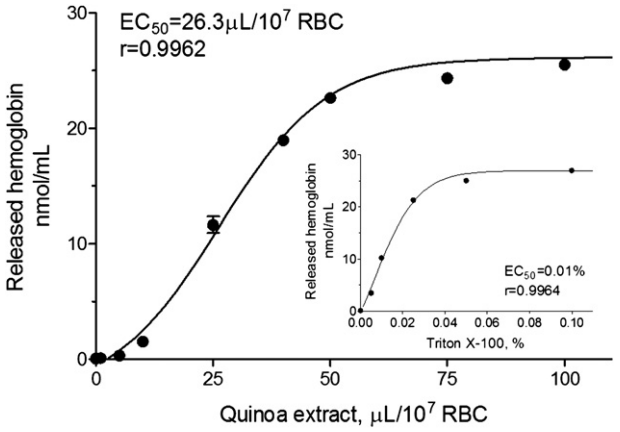

Fig. 1. Hemolytic effect of the standardized quinoa seed coat extract. Following incubation of RBC suspension with increasing volumes of the quinoa extract or % (V/V) Triton X-100 (inset), released hemoglobin was determined spectrophotometrically, as detailed in Experimental.

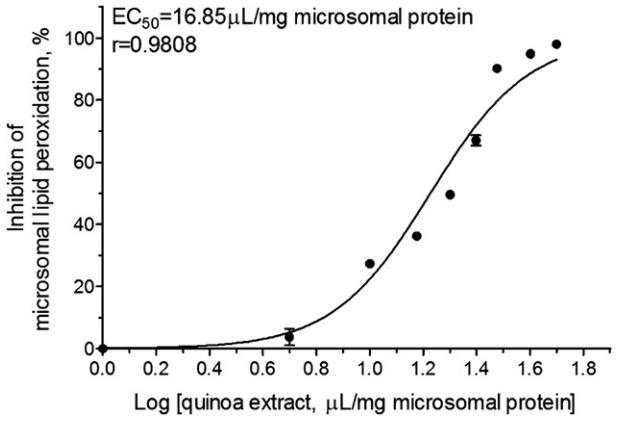

Fig. 3. Effect of quinoa extract on microsomal lipid peroxidation elicited by Cu^{2+}/ascorbate. Microsomal lipid peroxidation was assayed as detailed in Experimental. Data correspond to the % inhibition of the lipid peroxidation elicited by Cu^{2+}/ascorbate without extract pre-incubation (100%).

Fig. 1). Triton X-100, used as a positive control, rendered a similar pattern for hemoglobin release (Fig. 1, inset). The half-maximum effects were obtained with 26.3 μL quinoa extract/10^7 RBC and 0.01% (V/V) Triton X-100. On the other hand, the quinoa extract elicited a biphasic change in basal microsomal lipid peroxidation (Fig. 2); 5–15 μL of this extract/mg microsomal protein increased this parameter in the same percentage; 25 μL of quinoa extract also increased basal microsomal lipid peroxidation, but to a lower extent; 50 μL of quinoa extract however, decreased this phenomenon in approximately 30% compared to the value obtained in the absence of extract (Fig. 2). Triton X-100 (0.05% V/V), used as a positive control, increased this parameter to 117.6 ± 2.3%.

3.3. Antioxidant activity

The quinoa extract inhibited microsomal lipid peroxidation promoted by Cu^{2+}/ascorbate in a concentration-dependent manner, displaying a half-maximum effect at about 17 μL/mg microsomal protein (Fig. 3). Ten and 20 μL quinoa extract also prevented the loss of microsomal thiol content promoted by Cu^{2+}/ascorbate 50% and 100%, respectively (Fig. 4). Catechin (15 nmol/mg protein), used as a positive control, inhibited lipid peroxidation in 45% and protected microsomal thiol content in 14.1%.

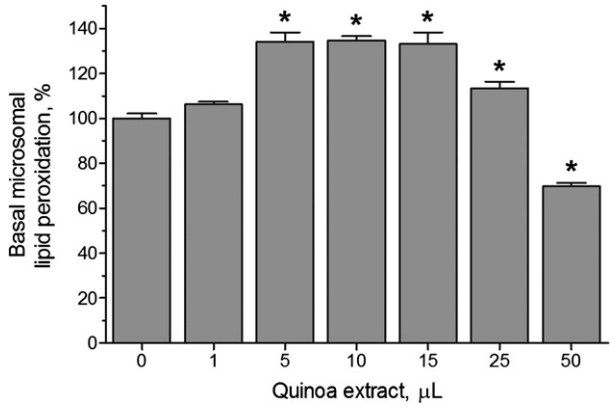

Fig. 2. Effect of quinoa extract on basal microsomal lipid peroxidation. Microsomal lipid peroxidation was assayed as described in Experimental. Data correspond to the % lipid peroxidation considering as 100% the lipid peroxidation determined without extract. *$p < 0.05$ compared to control (without extract).

3.4. Microsomal glutathione S-transferase activity and redox properties of the quinoa extract

Microsomal GST is active in its disulfide dimeric form; hydrogen peroxide promotes its activation while reducing agents decrease its enzymatic activity by altering the relative amounts of GST dimers and monomers (Woldemichael and Wink, 2001; Wu et al., 2004).The quinoa extract (5 μL/mg microsomal protein) completely prevented and notably, reversed the oxidative activation of this enzyme elicited by H_2O_2 (Fig. 5).

4. Discussion

Extracts from plants contain several different compounds which can elicit additive and/or synergic effects on biological systems; thus, their uses in folk medicine are being validated by scientific research worldwide (Newman and Cragg, 2007). Rat liver microsomes contain the main xenobiotic biotransformation enzymes, and also they are one of the main sources of ROS in mammalian cells (Venkatakrishnan et al., 2001). Because herbal compounds are xenobiotics to mammals, rat liver microsomes are an advantageous

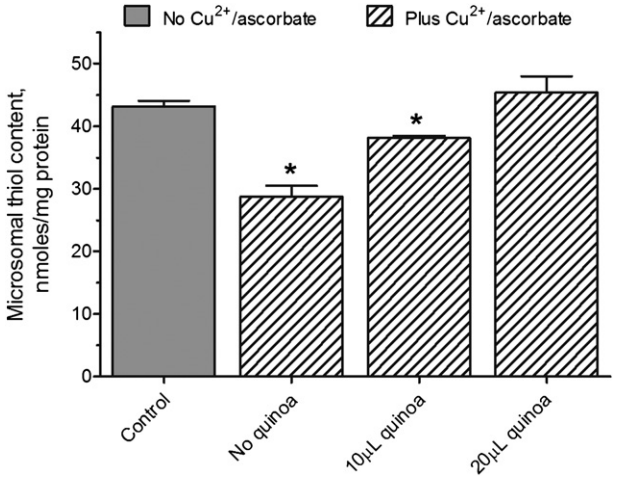

Fig. 4. Effect of quinoa extract on the loss of microsomal thiol content promoted by Cu^{2+}/ascorbate. Microsomal thiol content was titrated as described in Experimental. *$p < 0.05$ compared to control (without extract or Cu^{2+}/ascorbate).

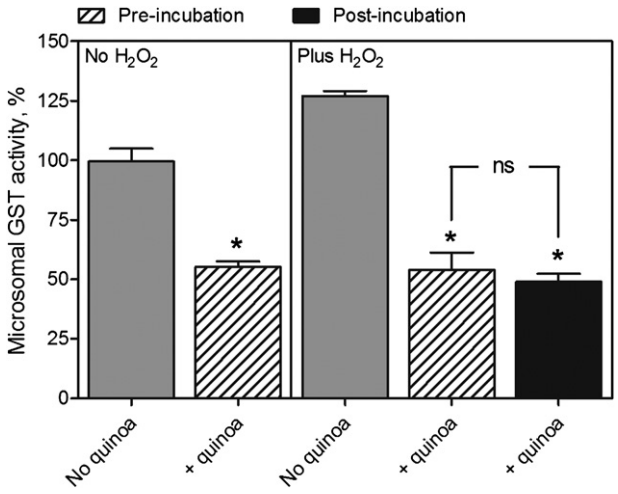

Fig. 5. Effect of quinoa extract on the oxidative activation of microsomal GST elicited by H_2O_2. Microsomes were incubated with 5 μL of the quinoa extract or buffer (gray bars) before (hatched bars) or after (black bar) incubation with 0.1 mM H_2O_2 for 30 min at 37 °C. Microsomal GST activity was assayed as detailed in Experimental. Data correspond to the % microsomal GST activity considering as 100% the activity assayed in the absence of quinoa extract and H_2O_2. *$p < 0.05$ compared to the control (without incubation with extract or H_2O_2); ns, not significant.

system to address the antioxidant properties of herbal compounds (Letelier et al., 2009). Quinoa seed coats have only been characterized in terms of triterpene saponin content (Meyer et al., 1990; Woldemichael and Wink, 2001), which are responsible for its surfactant activity (Reichert et al., 1985; Woldemichael and Wink, 2001). In agreement with this, we found that the extract of quinoa seed coats promoted the release of hemoglobin from red blood cells (Fig. 1), an effect that was mirrored by Triton X-100, a detergent used as a positive control (Fig. 1, inset). The extract also produced changes in the exposition of microsomal lipids, evaluated as an increase in basal microsomal lipid peroxidation (Fig. 2). This effect was also mimicked by Triton X-100. Notably, the highest volume tested of the extract (50 μL) inhibited this phenomenon. This apparent paradox may be due to the occurrence of both surfactant and antioxidant properties at high concentrations of the quinoa extract, while displaying only surfactant activity at low concentrations.

Potential antioxidant activity of the quinoa extract is in agreement with the finding of both polyphenol and thiol compounds in its composition (Bors and Michel, 2002; Wu et al., 2004). Consequently, the quinoa extract inhibited, in a concentration-dependent manner, both microsomal lipid peroxidation and loss of microsomal thiol content, phenomena promoted by Cu^{2+}/ascorbate (Figs. 3 and 4). The quinoa extract appeared to better protect thiol groups than lipids occurring in microsomes (EC_{50} values: 16.85 and 10 μL, respectively). Catechin, in a concentration equivalent to the half-maximum inhibitory effect of the quinoa extract on microsomal lipid peroxidation, was used as a positive control in lipid peroxidation and microsomal thiol content protection assays. In contrast to the quinoa extract, catechin appeared to better protect microsomal lipids than thiol groups (45% and 14.1%, respectively, at the concentration tested). Presence of thiol compounds in the quinoa extract in addition to polyphenols may explain the difference found in the protection of microsomal thiol groups by the quinoa extract and catechin.

The main non-enzymatic antioxidant molecules in mammalian cells are thiol compounds (e.g. GSH, which occurs in mM concentrations in liver cells) (Wu et al., 2004). Moreover, thiol compounds can protect cysteine residues in proteins through direct reduction or the formation of mixed disulfides that are the substrates for enzymes that catalyze their regeneration, such as thioredoxin (Jones, 2008). Redox homeostasis of cysteine residues is paramount for mammalian cell function, which is consistent with GSH being the main non-enzymatic antioxidant (Droge, 2002). Accordingly, most diseases that are associated with oxidative stress processes and/or transition metal overload, including neurodegenerative disorders (Migliore and Coppede, 2009), cardiovascular diseases (Fearon and Faux, 2009), and cancer (Visconti and Grieco, 2009) are also characterized by a depletion of GSH or a decrease in GSH/GSSG ratio (Schafer and Buettner, 2001). Microsomal GST activity is extremely dependent on the formation of its catalytically active −S−S-dimer (Aniya and Anders, 1992). The quinoa extract inhibited this activity (Fig. 5). This extract also prevented and completely reversed the oxidative activation of GST by H_2O_2. This finding supports the idea that the quinoa extract may act as a disulfide reducing agent, which is consistent with the occurrence of thiol compounds in the extract composition. Whether the thiol-reducing property found for the quinoa extract on GST is extensive to other thiol enzymes is a subject of our continuing research.

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