

The Presence of Pyrrolo Quinoline Quinone (PQQ) in Eukaryotic Tissue Extracts

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Abstract: PQQ is a novel quinone, which functions as a cofactor in multiple bacterial dehydrogenases, oxidases and decarboxylases. This quinone has shown antioxidant properties and its presence has been shown in plant and animal products. Enzymes containing PQQ as a cofactor have been called quinoproteins. PQQ promotes growth in plants and animals. Little is known about the presence of PQQ in higher organisms.

Keywords: PQQ, extract, bacterial, eukaryotic, tissue, cycling-redox.

1. INTRODUCTION

The pyrroloquinoline quinone (PQQ) is a novel cofactor of certain bacterial enzymes. It was described for the first time in *Methylobacterium extorquens* associated to ethanol dehydrogenase (Flores-Encarnación *et al.*, 2004; Misra *et al.*, 2012; Shen *et al.*, 2012). So, PQQ has been reported to be associated with dehydrogenases and oxidases, functioning as an enzymatic cofactor in some Gram-negative bacteria (Flores-Encarnación *et al.*, 2014). For example, PQQ-glucose dehydrogenase was isolated from *Gluconobacter suboxydans* and *Klebsiella aerogenes*. This enzyme was found to be associated to the cytoplasmic membrane and functionally linked to respiratory chain (Meyer *et al.*, 2013). In more recent years, in animal cells it has been reported that PQQ has a protector effect in cardiac lesions; it also prevents liver injury eliminating free radicals due to its antioxidant potent properties (50-100 times more effective than ascorbic acid) (Akagawa *et al.*, 2016; Feng *et al.*, 2014; Jia *et al.*, 2015; Rucker *et al.*, 2009; Tao *et al.*, 2007). PQQ is a polyphenolic compound found in plants, various foods and biological fluids (Flores-Encarnación *et al.*, 2017; Noji *et al.*, 2007). The present study aimed to seek evidence the presence of PQQ in eukaryotic tissue extracts.

2. MATERIAL AND METHODS

2.1. Biological Material

For the present study, 3 female Wistar rats weighing 150 to 200 grams were used. The organs of the rats (spleen, muscle tissue, brain, stomach, lung, heart, liver, kidney) were extracted by dissection in the laboratory under optimal conditions and bioethical guidelines. The biological samples were kept at -30°C in sterile flasks for their conservation.

2.2. Tissue Extracts

Tissue extracts were obtained using 1 gram of each tissue. For this, the tissue was cut into 2 to 3 mm fragments and then mechanically homogenized in 10 mL of lysis solution. The lysis solution contained: 50 mM Tris hydrochloride, 150 mM NaCl, 10% SDS, 0.3 mM PMSF and pH 7.0. For homogenization, 10 to 12 pulses were applied for 30 s, with intervals of 1 min in cold. Then, the homogenate was centrifuged at 3,500 r.p.m. in cold for 10 min. The supernatant was recovered and

the pellet was discarded. The supernatant was centrifuged at 8,000 r.p.m. for 5 min and then it was frozen at -30 °C until use.

2.3. PQQ Quantification

The quantification of PQQ was carried out according to the methodology reported by Paz *et al.* (1991). For this, glycine-borate solution, nitro blue tetrazolium (NBT) and the 'cycling redox' technique were used. The reaction mixture contained glycine-borate solution (pH 10), Triton X-100, NBT and 100 µl of tissue extract. The reaction mixture was incubated at room temperature for 1 hour (in the dark) and the absorbance at 605 nm was measured. PQQ from Sigma-Aldrich Co. as standard was used. The analyses were conducted in triplicate.

3. RESULTS

As mentioned earlier, the spleen, muscle tissue, brain, stomach, lung, heart, liver, kidney were extracted from 3 female Wistar rats weighing 150 to 200 grams. Tissue extracts were obtained by mechanical homogenization and the debris was removed by centrifugation at 3,500 r.p.m. for 10 min. The supernatant obtained was centrifuged again at 8,000 r.p.m. for 5 min. This is how tissue extracts were obtained. The quantification of PQQ was carried out using the 'cycling redox' technique, which is based on the formation of a colorful complex of formazan using nitro blue tetrazolium. So, PQQ at alkaline pH can oxidize glycine in an amino oxidation reaction. The hydroquinones formed react with O₂ to form superoxide radical which is followed by the reduction of nitro blue tetrazolium to formazan. The results are shown in Table 1. As shown in Table 1, using the cycling-redox technique in all the extracts tested, the presence of PQQ was detected. In this study, the liver, spleen and lung contained about 180 nmol PQQ gram⁻¹ of tissue, while muscle tissue, brain and heart contained about 220-240 nmol PQQ gram⁻¹ of tissue. The stomach and liver registered the highest values in the content of PQQ, thus the values found were of 260-280 nmol PQQ gram⁻¹ of tissue. In addition to the above, other tests were carried out to verify the presence of PQQ. Thus, through a spectrophotometric scan between 300 and 400 nm, signals were observed at 324, 329 and 353 nm which suggested the presence of PQQ (data not shown).

4. DISCUSSION

Enzymes containing PQQ as a cofactor have been called quinoproteins (Rucker *et al.*, 2009). Quinoproteins were initially described in bacteria functioning as dehydrogenases, oxidases and decarboxylases. These PQQ-dependent bacterial enzymes directly oxidize sugars, alcohols, and aldehydes, a process that takes place in the bacterial cytoplasmic membrane (Flores-Encarnación *et al.*, 2004; Rucker *et al.*, 2009). Some authors have reported that both vegetable and some animal products contain PQQ (Stites *et al.*, 2000). Thus, it has been described that parsley, green tea, green pepper, papaya and other vegetables contain significant concentrations of PQQ, as well as natto made from fermented soybeans (Kumazawa *et al.*, 1995). The present study shows some data related with the presence of PQQ in extracts of eukaryotic tissue. As mentioned earlier, the tissue extracts were obtained by mechanical homogenization from spleen, muscle tissue, brain, stomach, lung, heart, liver, kidney of Wistar rats. It was shown in Table 1, PQQ was detected in tissue rat extracts using the 'cycling redox' technique. So, liver, spleen and lung extracts contained about 180 nmol PQQ gram⁻¹ of tissue; muscle tissue, brain and heart extracts contained about 220-240 nmol PQQ gram⁻¹ of tissue; stomach and liver extracts contained about 260-280 nmol PQQ gram⁻¹ of tissue. The presence of PQQ was observed also spectrophotometrically registering signals at 324, 329 and 353 nm (data not shown). In addition to vegetables, the presence of PQQ has been reported in high concentrations in products of animal origin, such as eggs and milk (Paz *et al.*, 1991). This suggests that PQQ could be present in eukaryotic cells. This could explain the presence of PQQ in the animal tissue extracts tested in this study, that is, PQQ must come from plant foods and animal products. Another important source of PQQ could be the intestinal bacterial microflora. As reported, various enterobacteria are known to synthesize PQQ. Many bacteria produce it in large quantities and excrete it into the environment. The amount of PQQ excreted can vary between 1 µg mL⁻¹ to mg mL⁻¹, which depends on the composition of the growth medium (McIntire *et al.*, 1994). Apparently, PQQ is a novel quinone that promotes many benefits in the animal organisms in which this substance has been tested. Its neuroprotective role has been reported due it works as a powerful antioxidant (Flores-Encarnación *et al.*, 2014; Huang *et al.*, 2015; Murray, 2018; Ohwada *et al.*, 2008; Rucker *et al.*, 2009). PQQ promotes growth in plants and animals. PQQ is a novel vitamin-like compound that acts as an essential active factor in the

functioning of mitochondria. In addition to its role in mitochondria, PQQ stimulates growth and serves as a cofactor for a special class of enzymes involved in cellular functions including cellular growth, development, differentiation and survival (Flores-Encarnación *et al.*, 2014; Harris *et al.*, 2013; Murray, 2018). It has been reported that the immune system seems particularly sensitive to low PQQ levels: PQQ deprivation is accompanied by multiple defects in immune function and loss of the ability of white blood cells to respond properly (Ikemoto *et al.*, 2017; Murray, 2018; Rucker *et al.*, 2009). PQQ is an effective antioxidant, protecting mitochondria against oxidative stress-induced lipid peroxidation, protein carbonyl formation and inactivation of the mitochondrial respiratory chain (Hwang and Willoughby, 2018). Although PQQ is not biosynthesized in mammals, trace amounts of PQQ have been found in human and rat tissues at picomolar to nanomolar levels, and an especially large amount has been found in human milk. Although the higher organisms do not seem to biosynthesize PQQ, this quinone appears to have a universal function among living organisms. In the present study, it was observed that without exogenous addition of PQQ, this quinone is present in different tissues, so PQQ must perform some function that should be explored later.

Table1. Presence of PQQ in extracts of eukaryotic tissue.

Tissue extract	PQQ (nmol gram ⁻¹)
Kidney	180
Spleen	180
Lung	180
Muscle tissue	220
Brain	240
Heart	240
Stomach	260
Liver	280

5. CONCLUSION

This study showed some evidence of the presence of PQQ in extracts obtained from different rat tissues. Apparently PQQ is a universal molecule that has been present in both bacterial cells and plant and animal cells. Its functions as a bacterial cofactor have been extensive, however its role as an antioxidant in animal cells has provided a neuroprotective role. Further studies are needed to determine the function of PQQ in each of the tissues tested.

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