RESEARCH COMMUNICATION

Levels of pyrroloquinoline quinone in various foods

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The levels of free pyrroloquinoline quinone (PQQ) in various foods were examined by the use of gas chromatography–mass spectrometry. PQQ was extracted from the samples, after addition of [U-^{13}C]PQQ as internal standard, with n-butanol and Sep-Pak C_{18} cartridges. After derivatization of PQQ with phenyltrimethylammonium hydroxide, molecular peaks at m/z 448 and 462 were used for detection of PQQ and [U-^{13}C]PQQ respectively, by selected ion monitoring. Free PQQ could be detected in every sample in the range 3.7–61 ng/g or ng/ml. Since its levels in human tissues and body fluids are 5–10 times lower than those found in foods, it is probable that PQQ existing in human tissues is derived, at least partly, from the diet.

INTRODUCTION

Pyrroloquinoline quinone (PQQ) was identified as a novel cofactor of several prokaryotic dehydrogenases in 1979 [1,2] and was found to be synthesized by, and essential for growth in, some microorganisms [3]. Some years ago, a number of mammalian enzymes, such as lysyl oxidase, dopa decarboxylase, dopamine β-hydroxylase, plasma amine oxidase and diamine oxidase, were suggested to contain PQQ as a cofactor [4–9]; however, this proposal has now been almost disproved [10–12]. Nevertheless, it has been reported that PQQ is nutritionally important as a vitamin or growth factor in mice [13]. Very recently, we have reported that free PQQ exists in tissues and body fluids of humans and rats in the ng/g range, by use of gas chromatography–mass spectrometry (GC–MS) [14]. In the present study, we have examined the levels of free PQQ in various foods in an attempt to discover the origins of PQQ found in mammalian tissues.

EXPERIMENTAL

Chemicals and food samples

PQQ was obtained from Mitsubishi Gas Chemical Company Inc. (Niigata, Japan); phenyltrimethylammonium (PTMA) hydroxide (20–25% methanol) from Tokyo Kasei Kogyo Co. (Tokyo); [U-^{13}C]PQQ was synthesized microbiologically in *Hyphomicrobium methylovorum* as described previously [15]. Other common chemicals were of the highest purity commercially available. Twenty-six kinds of food samples commonly available in Japan were examined.

Extraction and derivatization of PQQ

To 1 g or 1 ml of each of the samples to be analysed, including 50 ng of [U-^{13}C]PQQ as internal standard, were added 4 ml of 1 M HCl solution, 50 μl of 2-mercaptoethanol, 100 μl of 10% (w/v) potassium ferricyanide and 10 ml of n-butanol, and samples were homogenized with a Polytron homogenizer for 5 min. After centrifugation at 800 *g* for 5 min, the organic layer was transferred to another centrifuge tube containing 20 ml of n-heptane, 1 ml of pyridine, 0.1 g of NaCl and 1 ml of distilled water, and shaken for 5 min. The tubes were centrifuged at 800 *g* for 5 min and the aqueous layer was evaporated to dryness *in vacuo*. The residue was dissolved in 10 ml of 0.1 M HCl and applied to a Sep-Pak C_{18} cartridge (Waters Associates, Milford, MA, U.S.A.). The cartridge was washed with 20 ml of 1 mM HCl, and finally 3 ml of 5% (v/v) pyridine solution was passed through it. The eluate was evaporated to dryness *in vacuo*. Derivatization of PQQ was carried out as described previously [14]; a 100 μl aliquot of PTMA hydroxide was added to the residue and heated at 100 °C for 15 min for methylation of PQQ; 1 μl was then subjected to GC–MS analysis.

GC–MS conditions

The analyses were carried out on an HP-5890 gas chromatograph (Hewlett-Packard, Palo Alto, CA, U.S.A.) coupled to a JMS-AX505H mass spectrometer (JEOL, Tokyo, Japan) with a computer-controlled data analysis system. GC separation was achieved with a DB-1 fused-silica capillary column (15 m × 0.32 mm i.d., film thickness 0.25 μm; J & W Scientific, Folsom, CA, U.S.A.). GC conditions were: column temperature, 200–300 °C (20 °C/min); injection temperature, 280 °C; and helium carrier gas flow, 3 ml/min. The samples were injected in the splitless mode and the splitter was opened after 1 min. The MS conditions were: electron energy, 70 eV; accelerating voltage 3.0 kV; ionization current, 300 μA; separator temperature, 280 °C and ion-source temperature, 280 °C. The molecular peaks at m/z 448 and 462 were used for sensitive detection of PQQ and [U-^{13}C]PQQ respectively, by selected ion monitoring (SIM). The details of specificity, quantitativeness and reliability of the present GC–MS method were described in a previous report [14].

RESULTS AND DISCUSSION

Typical SIM profiles for the authentic PQQ and for extracts from wine, kiwi fruit and carrot are shown in Figure 1. The 50 ng of internal standard [U-^{13}C]PQQ, which had been added to each 1 g or 1 ml sample, appeared as a big peak in each SIM at m/z 462. For all samples, a small peak appeared on the channel at

Abbreviations used: PQQ, pyrroloquinoline quinone; PTMA, phenyltrimethylammonium; GC–MS, gas chromatography–mass spectrometry; SIM, selected ion monitoring.

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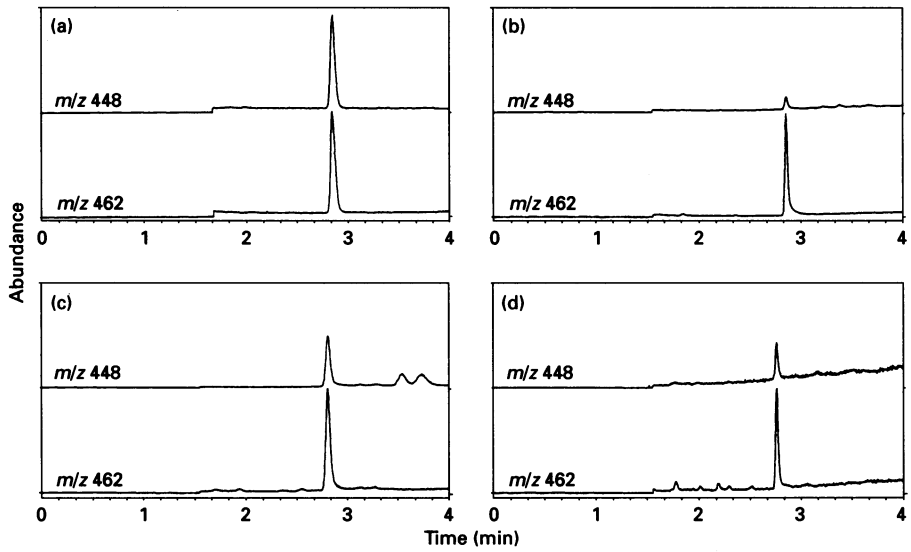

Figure 1 SIM for PTMA derivatives of PQQ, with [U-^{13}C]PQQ as internal standard, extracted from some foods

(**a**) The authentic PQQ (500 pg on column) and [U-^{13}C]PQQ (500 pg on column) without extraction; (**b**) wine; (**c**) kiwi fruit; and (**d**) carrot. The amount of [U-^{13}C]PQQ added to each sample was 50 ng. Typical results are presented in this Figure.

Table 1 Concentrations of PQQ in foods

The amount of [U-^{13}]PQQ as an internal standard added to each sample was 50 ng. Mean ± S.D. are given. The number of samples is given in parentheses.

Sample	PQQ (ng/g wet weight or ng/ml)
Broad bean	17.8 ± 6.78 (4)
Green soybeans	9.26 ± 3.82 (4)
Potato	16.6 ± 7.34 (5)
Sweet potato	13.3 ± 3.72 (5)
Parsley	34.2 ± 11.6 (3)
Cabbage	16.3 ± 3.96 (4)
Carrot	16.8 ± 2.81 (4)
Celery	6.33 ± 2.41 (4)
Green pepper	28.2 ± 13.7 (4)
Spinach	21.9 ± 6.19 (4)
Tomato	9.24 ± 1.82 (4)
Apple	6.09 ± 1.36 (4)
Banana	12.6 ± 3.81 (4)
Kiwi fruit	27.4 ± 2.64 (4)
Orange	6.83 ± 2.20 (4)
Papaya	26.7 ± 8.57 (6)
Green tea	29.6 ± 12.9 (3)
Oolong (tea)	27.7 ± 1.92 (3)
Coke	20.1 ± 3.17 (3)
Whiskey	7.93 ± 1.84 (3)
Wine	5.79 ± 2.73 (3)
Sake	3.65 ± 1.39 (3)
Bread	9.14 ± 3.64 (4)
Fermented soybeans (natto)	61.0 ± 31.3 (4)
Miso (bean paste)	16.7 ± 3.30 (3)
Tofu (bean curd)	24.4 ± 12.5 (5)

m/z 448 at exactly the same retention time as that of the internal standard, showing the presence of PQQ in the samples.

The concentrations of free PQQ in many foods were carefully quantified, as shown in Table 1. Trace amounts of free PQQ could be detected in every sample in the range 3.7–61 ng/g or ng/ml; it was highest in fermented soybeans and lowest in sake (rice wine).

To our knowledge, the present report is the first demonstration of PQQ in vegetables, fruits and beverages. Paz and co-workers reported that high levels of free PQQ (574–16500 ng/ml) were contained in eggs and skim milk, by the use of a redox cycling method [16,17]; we have re-examined PQQ levels in eggs and skim milk by our specific GC–MS method and found that the levels are 3–4 orders of magnitude lower than those measured by the above redox cycling method [18].

Killgore et al. [13] reported that mice fed with a PQQ-deficient diet grew poorly, suggesting nutritional importance of PQQ in mammalian species. In our previous paper, we reported that the levels of free PQQ in human tissues or body fluids are 0.8–5.9 ng/g or ng/ml [14]. However, there are no reports that eukaryotic cells can synthesize PQQ. If mammalian cells cannot synthesize PQQ, two origins of it can be considered: production of PQQ by enteric bacteria and/or dietary origin. In the present study, we have been able to detect PQQ in every food (Table 1); its levels are 5–10 times higher than those obtained in human tissues or body fluids [14]. Thus, it is probable that PQQ existing in human tissues is derived from the diet, at least partly.

In a previous study, we demonstrated that physiological concentrations of PQQ (1–10 ng/ml) are effective in stimulating DNA synthesis in cultured human fibroblasts [19]. Other investigators have suggest that PQQ can modulate immune response in mice [20] and can prevent animals from liver injury [21], cataract formation [22] and lipid peroxidation [23].

In conclusion, PQQ could be identified in many foods in this study, suggesting that a part of PQQ existing in mammalian tissues is of dietary origin, and such exogenous PQQ is bioactive in mammalian tissues physiologically (nutritionally) and also pharmacologically.

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