Alternative biosynthetic pathways of isoprenoid quinones in microorganisms

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Introduction

Isoprenoid quinones are a widespread group of compounds, occurring usually in membranes, where they act as electron carriers and antioxidants. They can also play other roles such as enzyme cofactors. In photosynthetic organisms, plastinoquinones (PQs) and tocopheroquinones (TQs) are found, the latter being regarded as oxidation products of tocopherols (Toc). In higher plants, PQ and Toc biosynthetic pathways share the same head group precursor, homogentisic acid (HGA) synthesized from tyrosine and similar enzymes carrying out condensation reaction (see Fig.1.). Arabidopsis thaliana mutant with disabled biosynthesis of HGA is deficient in both PQ and Toc. Analogous mutant of cyanobacterium Synechocystis sp. PCC 6803 lacks only PQ, whereas PQ level remains unchanged [1]. In many cyanobacteria there is no close homologue of HGA prenyltransferase and no Toc can be found, while PQ is always present, means that cyanobacteria has an additional pathway of PQ biosynthesis. The other question concerns α-TQ, which was reported to be present in many species of microorganisms [2], but PQ biosynthesis pathway. PQ or α-TQ. Also in this case, an alternative biosynthesis of α-TQ was postulated. To verify and extend the results obtained by Hughes and Tove [3] we checked for the presence and abundance of α-TQ (and its reduced form tocopherolquinol, TQH₂) in selected species of microorganisms: yeasts Saccharomyces cerevisiae, Candida utilis, and Pichia pastoris; bacteria Escherichia coli, and Butyryribrobacter fibrisolvens; cyanobacteria Synechococcus sp. PCC6803, Synechocystis sp. PCC7002, Synechococcus elongatus PCC7942 (Anacystis nidulans R2) and Phormidium laminosum.

Results

Bacteria and yeast: HPLC analysis of extracts of E. coli, S. cerevisiae, C. utilis, P. pastoris was carried out in solvent A (for absorbance detection of TQ and fluorescence detection of α-TQ) or system with post-column. TQ was detected only in B. fibrisolvens (Figs. 2 and 3), in E. coli fibrisolvens growing on medium with rumen fluid, mostly TQ was detected. TQH₂ was found in one culture harvested after 20 h of growth, whereas in older cultures there was only TQ detected. α-TQ was not detected in any of the examined microorganisms, but the presence of α-TQ in B. fibrisolvens was confirmed by further experiments.

Cyanobacteria - the following species were analyzed: Synechocystis sp. PCC6803, S. elongatus sp. PCC7942, Synechococcus sp. PCC7002 and R. laminosum. The extracts were analyzed in solvent A. methanol:hexane for measurements of total PQ (oxidized + reduced) and in system with post-column (acting as Zn-postcolumn but is more comfortable in use) with methanol:water 95:5 as a solvent. The content of PQ was similar in all the species examined. The content of TQ was found only for PQ biosynthesis strain (Fig.4 and Table 1).

Table 1

<table>
<thead>
<tr>
<th>Amount of total TQ</th>
<th>Amount of total PQ</th>
<th>Amount of α-TQ</th>
</tr>
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<tbody>
<tr>
<td>In Synechocystis sp. PCC6803</td>
<td>0.23</td>
<td>0.23</td>
</tr>
<tr>
<td>In Synechococcus sp. PCC7002</td>
<td>0.35</td>
<td>0.35</td>
</tr>
<tr>
<td>In Phormidium laminosum</td>
<td>0.73</td>
<td></td>
</tr>
</tbody>
</table>

Conclusions

1. The previous literature data concerning E. coli and yeast were not confirmed. They claimed the presence of α-TQ in most of the examined microorganisms. Based on literature data: E. coli (4-7 nmol of total α-TQ/g FW), S. cerevisiae (8.1 nmol TQ/g FW), C. utilis (9.1 nmol TQ/g FW) [2] we do not expect to find larger amounts of α-TQ in mixed culture concentration about 18 μM, which should be detectable in our system.

2. We have found about 10 times lower α-TQ content in B. fibrisolvens as compared to literature data.

3. The recovery of α-TQ in our system was high as showed control experiments.

4. Even though extraction of lyophilized and fresh cells of E. coli and yeast were used, no α-TQ was detected.

5. HPLC in method described by Hughes and Tove does not allow detect α-TQ. E. coli and yeast extracts high amount of ubiquinones, compounds with similar properties to TQs.

It is worth to mention that Hughes and Tove evaluations of TQ content in rat liver [3] were questioned by other scientists [4,5]. Our results contradict to widespread occurrence of α-TQ in microorganisms, but the presence of α-TQ in B. fibrisolvens still raises questions. Unfortunately, α-TQ biosynthesis gene is not available, it is not possible to look for homologues of the genes of isoprenoid quinones synthesis.

6. Results of analysis PQ in cyanobacteria are comparable to other evaluations of PQ content in photosynthetic organisms (0.4-15 μmol/g DW in tobacco leaves, 30-26 μmol/g DW in Chlorella [7], 0.150±0.04 μmol/g FW in Synechocystis sp. PCC6803 [8], 0.21±0.04 μmol/g FW in Synechocystis sp. PCC6803 [9]).

In Synechocystis sp. PCC6803, HGA prenyltransferase is present, as well as Toc. In Synechocystis sp. PCC7002, HGA prenyltransferase homologue is present and α-Toc was found (although its amounts were very variable depending on the culture). PQ biosynthesis genome has not been sequenced yet, but α-Toc was present. In S. elongatus sp. PCC7949, there is neither HGA prenyltransferase nor Toc, but PQ biosynthesis is very effective in other examined species. The question of alternative PQ biosynthesis in cyanobacteria remains open. Although HGA prenyltransferase enzymes are present only in some of cyanobacteria species with sequenced genome, in all of them homologues of E. coli p-hydroxybenzoate octaprenyltransferase. The question of these enzymes take part in PQ biosynthesis needs to be answered in the future.

Materials and methods

Growth of microorganisms in liquid cultures: E. coli DH5α strain was cultured overnight in LB medium at 37°C on a shaker. Yeast strains (Saccharomyces cerevisiae, strain B4 4741, and Pichia pastoris, strain P. pastoris) were grown in YPD medium at 30°C on a shaker. Synechocystis sp. PCC6803 (strain PCC6803) was grown in N2 medium containing 25 μg/ml thiamine, 30 μg/ml biotin, and 10 μg/ml arginine. Synechocystis sp. PCC6803 (strain PCC6803) was grown for 6 weeks on BG-11 medium in 45°C on a shaker. Synechocystis sp. PCC6803 (strain PCC6803) was grown under high light (1200 μmol/m²/s) for 6 weeks on BG-11 medium in 45°C on a shaker. Harvested cells (for optical density measurements) were centrifuged at 10,000 g for 10 min. Harvested cells (for optical density measurements) were centrifuged at 10,000 g for 10 min. Harvested cells were washed with cold 0.9% NaCl solution.

Extraction: For E. coli, B. fibrisolvens and yeast we used the extraction with chloroform:methanol (2:1 v/v) along with fresh weight (extraction 60 s, 5 times) or from lyophilized (grinding in mortar). For cyanobacteria extraction of lipids was not used.

HPLC analysis of extracts was performed on C-18 column (1 mm; 250x4.6 cm; Teknokroma). The following solvent systems were used: Solvent A (acetonitrile:methanol:water:acetic acid, 70:25:5:0.1) flow rate 1.5 ml/min; Solvent B (acetonitrile:hexane:acetic acid, 340:20:1) 3.5 ml/min; Solvent C (acetonitrile:hexane:acetic acid, 340:20:1) 3.5 ml/min; Solvent C (acetonitrile:hexane:acetic acid, 340:20:1) 3.5 ml/min. The content of PQ was similar in all the species examined. The content of TQ was found only for PQ biosynthesis strain (Fig.4 and Table 1).

Figure captions:


2. HPLC chromatograms of extracts from various microorganisms in system A. A) E. coli 1 ml of broth with 100 μmol/g of PQ (left). B) P. pastoris grown on medium with rumen fluid and fluid on rumen fluid (right). C) C. utilis 1 ml of fluid was obtained from 0.5 g of DW.

3. HPLC chromatograms of extracts from various microorganisms in system A. A) E. coli 1 ml of broth with 100 μmol/g of PQ (left). B) P. pastoris grown on medium with rumen fluid and fluid on rumen fluid (right). C) C. utilis 1 ml of fluid was obtained from 0.5 g of DW.