Biological significance of the side chain length of ubiquinone in *Saccharomyces cerevisiae*

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Received 15 April 1998; revised version received 9 June 1998

Abstract Ubiquinone (UQ), an important component of the electron transfer system, is constituted of a quinone structure and a side chain isoprenoid. The side chain length of UQ differs between microorganisms, and this difference has been used for taxonomic study. In this study, we have addressed the importance of the length of the side chain of UQ for cells, and examined the effect of chain length by producing UQs with isoprenoid chain lengths between 5 and 10 in *Saccharomyces cerevisiae*. To make the different UQ species, different types of prenyl diphosphate synthases were expressed in a *S. cerevisiae COQ1* mutant defective for hexaprenyl diphosphate synthesis. As a result, we found that the original species of UQ (in this case UQ-6) had maximum functionality. However, we found that other species of UQ could replace UQ-6. Thus a broad spectrum of different UQ species are biologically functional in yeast cells, although cells seem to display a preference for their own particular type of UQ.

Key words: Ubiquinone; Side chain; *Saccharomyces cerevisiae*

1. Introduction

Ubiquinone (UQ), 2,3-dimethoxy-5-methyl-6-polyprenyl-1,4-benzoquinone, is an essential component of the respiratory chain which provides energy for aerobically grown organisms. UQ is found in many types of organisms, but the length of the isoprenoid side chain differs depending on the species. Therefore, UQs have been used for the taxonomic study of microorganisms. UQ biosynthesis consists of 10 sequential steps including methylation, decarboxylation, hydroxylation, and isoprenoid transfer [1,2]. In the UQ biosynthetic pathway, *p*-hydroxybenzoate (PHB):polyprenyl diphosphate transferase, which transfers the isoprenoid side chain to the quinone frame PHB, is thought to be a rate-limiting enzyme. PHB:polyprenyl diphosphate transferases have broad substrate specificity, and the length of isoprenoid substrates does not alter their transfer activity [3]. Thus, the length of the side chain is not determined by the substrate specificity of PHB:polyprenyl diphosphate transferase, but, rather, is determined by the nature of the isoprenoids as shown by our previous experiments [4]. The isoprenoid side chain of UQ is supplied by a product whose synthesis is catalyzed by polyprenyl diphosphate synthase (PDS). So far, we have succeeded in producing UQ-8 and UQ-9 in *Saccharomyces cerevisiae*, which naturally produces UQ-6, by simply expressing the octaprenyl and solanesyl diphosphate synthases, respectively. These results suggest that the side chain length of UQ does not affect fundamental UQ activity in *S. cerevisiae*. However, experimental proof of this has been limited to only a few species of UQ.

The present study was carried out to determine if there exists a functional difference between UQ species with isoprenoid side chains ranging in size from 5 to 10 in *S. cerevisiae*. We show that the natural species of UQ in *S. cerevisiae* has maximum activity compared to other species of UQ. However, the species of UQ does not seem to be a phenotypically critical factor for cell growth.

2. Materials and methods

2.1. Materials

Restriction enzymes and other DNA-modifying enzymes were purchased from Takara Shuzo Co. and New England Biolabs. Kiesel gel 60 F_{254} thin-layer plates were purchased from Merck. *Escherichia coli* strain JM109 was used for the general construction of plasmids [5]. *S. cerevisiae COQ1* disrupted strain YKK6 was used as a host strain for the expression of prenyl diphosphate synthase genes. Strains were grown at 30°C in synthetic complete (SC) (0.67% (w/v) yeast nitrogen base, 2% (w/v) glucose or 3% (w/v) glycerol, and appropriate amino acids) minimal medium. Plasmids pUC118 and YEp13 M4 were used as vectors [6].

2.2. Construction of the plasmids

To express various kinds of polyprenyl diphosphate synthases in *S. cerevisiae*, we constructed new plasmids containing the genes for heptaprenyl diphosphate synthase [7,8] and decaprenyl diphosphate synthase [9]. Both of the 1.0 kb *Eco*RI-*Hin*dIII fragments from the HepPP synthase gene [7,8] and DPP synthase gene [9] were cloned into the same sites of pSA1 [4], which has 53 amino acids of the Coq1 mitochondrial import signal, to yield pSH7 and pSD11, respectively. Both of the 1.2 kb *Bam*HI-*Hin*dIII fragments from pSH7 and pSD11 were cloned into the same sites of the yeast shuttle vector YEp13 M4 to yield pYH7 and pYD11, respectively. The plasmid Y-GGPSmut3, which contains the mutated GGPP synthase gene from *Sulfolobus acidocaldarius*, was obtained from Dr. Ohnuma [10]. The plasmid YEpG3ΔSpH contains the hexaprenyl diphosphate synthase gene from *S. cerevisiae* as described previously [4]. Plasmids pYE6 and pYD10 were constructed previously [4,11]. Plasmids used in this study are shown in Fig. 1.

2.3. Ubiquinone extraction and measurement

Ubiquinone was extracted by the method described previously [4,12]. The extracted crude ubiquinone was analyzed by normal phase thin-layer chromatography with standard ubiquinones. Normal phase thin-layer chromatography was carried out on a Kiesel gel 60 F_{254} plate (Merck) with benzene-acetone (93:7, v/v). The UV visualized band containing ubiquinone was removed from the thin-layer chromatography plate, and extracted with chloroform-methanol (1:1, v/v). Samples were dried and the precipitate was redissolved in ethanol. The purified ubiquinone was further analyzed by HPLC with ethanol as the solvent [12].

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Abbreviations: UQ, ubiquinone; PDS, polyprenyl diphosphate synthase; PHB, *para*-hydroxybenzoate

PII: S0014-5793(98)00753-4

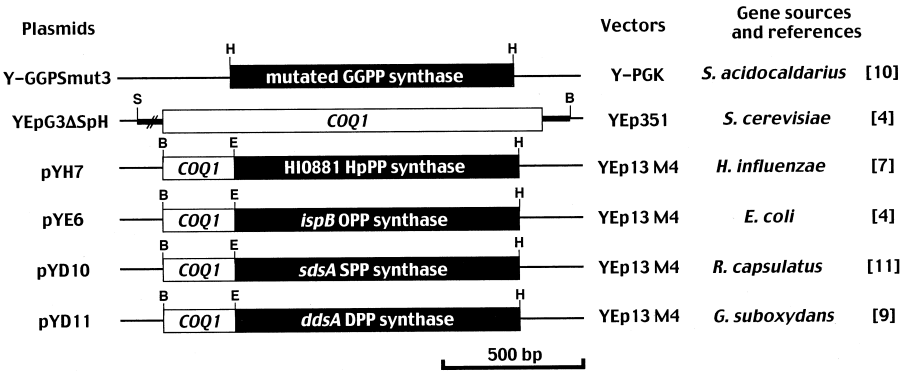

Fig. 1. Plasmid constructions used in this study. pYH7, pYE6, pYD10, and pYD11 have the mitochondrial import signal from the *COQ1* gene followed by the corresponding prenyl diphosphate synthase (PDS) gene. Y-GGPSmut3 expresses the mutated GGPP synthase gene under the control of the phosphoglycerol kinase promoter. YEpG3ΔSpH contains the original *COQ1* gene. The *COQ1-PDS* fused genes were expressed under the control of the native *COQ1* promoter. B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; S, *Sst*I.

3. Results

3.1. Expression of various prenyl diphosphate synthases in S. cerevisiae

To produce different types of UQ in *S. cerevisiae*, we constructed plasmids that express various prenyl diphosphate synthase (PDS) genes. Fig. 1 shows the constructed plasmids used for the production of UQ homologs. These PDS genes on the plasmids were designed to be expressed from the original *COQ1* gene promoter. Two plasmids which express heptaprenyl diphosphate synthase and decaprenyl diphosphate synthase were newly constructed. To target these prenyl diphosphate synthases into mitochondria, the 53 amino acid mitochondrial import signal of hexaprenyl diphosphate synthase (Coq1) from *S. cerevisiae* was fused to the N-terminal side of prenyl diphosphate synthase by gene fusion on the yeast expression vector. This 53 amino acid mitochondrial import signal was previously shown to be functional for the targeting of the fused protein into mitochondria [4]. Y-GGPSmut3 has the mutated GGPP synthase gene from *S. acidocaldarius*, and was expressed under phosphoglycerol kinase promoter without the need for any other targeting signal peptide [10]. This mutated GGPP synthase was altered to give geranylfarnesyl diphosphate synthase activity which produces 5 units of isoprene [10].

To test whether these PDS genes on plasmids function in yeast, we transformed them into the respiratory deficient *S. cerevisiae* mutant, YKK6 (*COQ1::URA3*). YKK6 can grow on the SC minimal medium containing glucose but not on SC minimal medium containing glycerol as sole carbon source, because it does not produce UQ [4]. As shown in Fig. 2, all strains which harbored a PDS gene grew on SC glycerol medium as well as on SC glucose agar medium, indicating that all of the expressed PDS proteins can complement the defect in the *COQ1* gene and restore normal respiration.

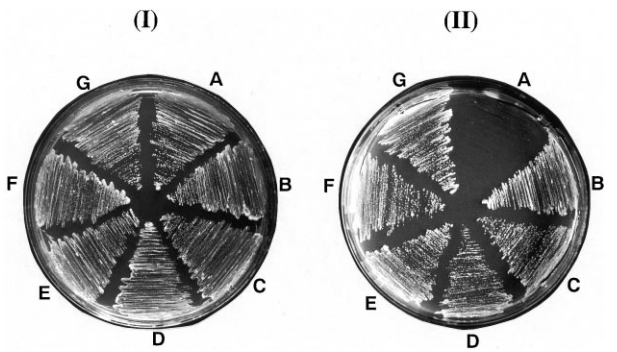

Fig. 2. Complementation of the *COQ1* disruptant by expression of the *COQ1-PDS* fused gene. The YKK6 (*COQ1::URA3*) strain harboring YEp13 M4 (A), YEpG3ΔSpH, which carries the *COQ1* gene (B), Y-GGPSmut3 (C), pYH7 (D), pYE6 (E), pYD10 (F) and pYD11 (G) were grown on SC-Leu-Ura medium with glucose (I) or glycerol (II).

3.2. Determination of UQ species in YKK6

To examine which species of UQ were produced, we extracted UQs from the YKK6 strain harboring the various constructed plasmids. Each strain produced a distinct UQ species, while no UQ was detected in the YKK6 strain (Fig. 3G). In each of the YKK6 strains, Y-GGPSmut3 produced UQ-5, YEpG3ΔSpH produced UQ-6, pYE6 produced UQ-8, pYD10 produced UQ-9 and pYD11 produced UQ-10 as main product (Fig. 3A–F). In all these cases, the expected kind of UQ was detected. However, YKK6 harboring pYH7 heterologously produced UQ-6 and UQ-7. Such heterologous production of UQ is sometimes observed in organisms isolated from the environment [11,13]. Basically, the length of the side chain of UQ was consistent with the isoprenoid product supplied from PDS. These results also suggest that *S. cerevisiae* PHB:polyprenyl diphosphate transferase (Coq2) can transfer isoprenoids ranging in size from C25 to C50 to the quinone frame. The exact amounts of ubiquinone of those strains were quantitated. The strains that produce UQ-5, UQ-6, UQ-7, UQ-8 and UQ-10 produce 29.6, 27.7, 29.4, 31.6 and 12.3 μg/g dry cells, respectively. The amounts were measured at least twice and are represented as averages. The amount of ubiquinone dose not differ much between species except UQ-10.

3.3. Growth curve of S. cerevisiae producing different types of UQs

To test the functionality of each quinone in yeast, we compared the growth rates of strains carrying UQ-5, UQ-6, UQ-7, UQ-8, UQ-9, or UQ-10 in SC minimal medium containing glycerol (Fig. 4). The original strain producing UQ-6 dis-

played the maximum growth rate, with strains expressing other quinones growing a little slower. However, these differences were minor, indicating that although there seems to be a preference for a particular UQ species depending on the species, this does not affect the fundamental growth ability of *S. cerevisiae*.

4. Discussion

In this study, we addressed the biological significance of the side chain length of UQ using *S. cerevisiae* as a model organism. We made *S. cerevisiae* producing UQ-5 to UQ-10 by expressing different types of prenyl diphosphate synthase in a *COQ1* mutant which lacks hexaprenyl diphosphate synthase. The different types of UQ ranging in size between UQ-5 and UQ-10 were successfully produced as main products except for UQ-7. Functional differences in complementation were not observed between the strains producing different UQs on SC glycerol plates (Fig. 2). The amount of UQ production was almost the same between the different strains (Fig. 3). The growth curve of these strains indicated a preference for the original UQ species, but did not change appreciably when the strain produced another type of UQ (Fig. 4). These results seem reasonable in light of observations that show the quinone part to be more important than the side chain part for UQ function. In fact, it has been shown biochemically that the side chain part of UQ is not a critical factor for UQ function [4,11]. The question remains, however, why do different organisms have a preference for a special UQ species, as surveyed by taxonomic researchers [13]? We speculate that there may exist a special affinity between the lipid composition of the membrane of a given organism and the length of its UQ isoprenoid group. In other words, there may be a link between the isoprenoid length of UQ and the membrane hydrophobicity of a particular organism.

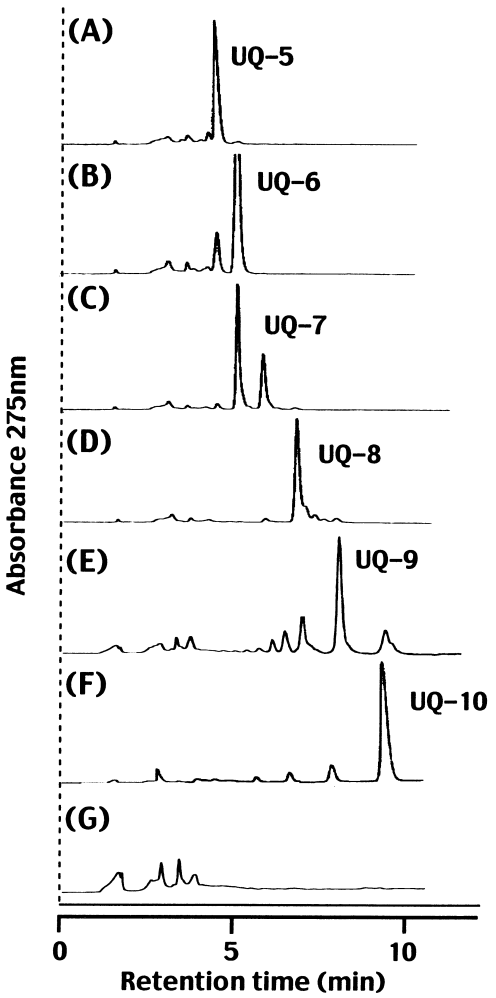

Fig. 3. Detection of UQ species in YKK6 expressing various PDS genes by high-performance liquid chromatography. UQs were extracted from YKK6 (*COQ1::URA3*) harboring Y-GGPSmut3 (A), YEpG3ΔSpH (B), pYH7 (C), pYE6 (D), pYD10 (E), and pYD11 (F) as described in Section 2. No UQ was detected in YKK6 itself (G).

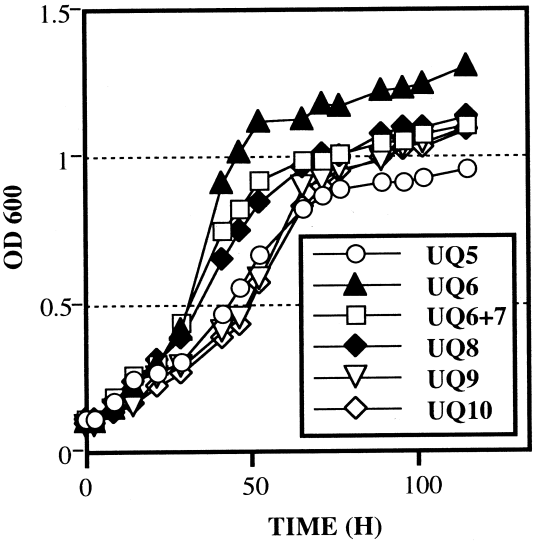

Fig. 4. Growth curves of YKK6 producing different kinds of UQs. YKK6 harboring Y-GGPSmut3 (○), YEpG3ΔSpH (▲), pYH7 (□), pYE6 (◆), pYD10 (▽), and pYD11 (◇) were precultured, diluted 200-fold into fresh SC minimum liquid medium containing glycerol, and then grown for 5 days. OD_{600} values were measured and plotted.

We produced different species of UQ, the isoprenoid groups of which ranged in size between 5 and 10, by simply expressing the appropriate PDS genes in *S. cerevisiae*. However, we were unable to produce UQ-4. We have tried to produce UQ-4 by expressing the GGPP synthase gene from an *Arabidopsis* source with mitochondrial targeting signal, but significant production of UQ-4 was not detected (data not shown). Because geranylgeranyl diphosphate was used for protein prenylation, there may be some limitation regarding the use of shorter units of isoprenoid as a side chain of UQ. In fact, only trace amounts of UQ-4 have been observed in organisms isolated from the environment [12].

Our conclusions drawn from observations of the in vivo function of UQ species in yeast are supported by the results of studies of the in vitro function of UQ [14]. So far, we have not seen a significant correlation between the side chain length of UQ and biological activity. However, considering the fact that most microorganisms use a highly specific UQ species, some unknown role for the length of the isoprenoid group of UQ may still exist.

Acknowledgements: This work was supported by a Grant-in-Aid for Special Scientific Research from the Ministry of Agriculture, Forestry,

and Fisheries. Plasmid Y-GGPS was kindly gifted by Dr. S. Ohnuma, University of Tohoku.

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