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Detection of 6-demethoxyubiquinone in CoQ_{10} deficiency disorders: Insights into enzyme interactions and identification of potential therapeutics

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ABSTRACT

Coenzyme Q_{10} (CoQ_{10}) is an essential cofactor of the mitochondrial oxidative phosphorylation (OXPHOS) system and its deficiency has important implications for several inherited metabolic disorders of childhood. The biosynthesis of CoQ_{10} is a complicated process, which involves at least 12 different enzymes. One of the metabolic intermediates that are formed during CoQ_{10} biosynthesis is the molecule 6-demethoxyubiquinone (6-DMQ). This CoQ precursor is processed at the level of COQ7 and COQ9. We selected this metabolite as a marker substance for metabolic analysis of cell lines with inherited genetic defects (*COQ2*, *COQ4*, *COQ7* and *COQ9*) or siRNA knockdown in CoQ biosynthesis enzymes using ultra-performance liquid chromatography coupled to tandem mass spectrometry (UPLC–MS/MS). In COQ4, COQ7 and COQ9 deficient cell lines, we detected significantly elevated levels of 6-DMQ. This suggests a functional interplay of these proteins. However, additional siRNA studies demonstrated that elevated 6-DMQ levels are not an exclusive marker of the COQ7/COQ9 enzymatic step of CoQ_{10} biosynthesis but constitute a more general phenomenon that occurs in disorders impairing the function or stability of the CoQ-synthome. To further investigate the interdependence of CoQ_{10} biosynthesis enzyme expression, we performed immunoblotting in various cell lines with CoQ_{10} deficiency, indicating that COQ4, COQ7 and COQ9 protein expression levels are highly regulated depending on the underlying defect. Supplementation of cell lines with synthetic CoQ precursor compounds demonstrated beneficial effects of 2,4-dihydroxybenzoic acid in COQ7 and COQ9 deficiency. Moreover, vanillic acid selectively stimulated CoQ_{10} biosynthesis and improved cell viability in COQ9 deficiency. However, compounds tested in this study failed to rescue COQ4 deficiency.

1. Introduction

Coenzyme Q_{10} (CoQ_{10}) is already known for >50 years and belongs to the first organic cofactors to be discovered in biochemical research history [1]. It plays an important role in the mitochondrial respiratory chain where it functions as an electron carrier between complex I/II and complex III. In addition, it is involved in oxidative stress defense and participates in fatty acid oxidation, biosynthesis of pyrimidines and apoptosis regulation [2].

CoQ_{10} is one of the most widely used dietary supplements, ranging from application as an over-the-counter drug for non-medical purposes

Abbreviations: UPLC–MS/MS, ultra-performance liquid chromatography coupled to tandem mass spectrometry; 6-DMQ, 6-demethoxyubiquinone; CoQ, Coenzyme Q; VA, vanillic acid; 2,4-HBA, 2,4-dihydroxybenzoic acid; 2,3-HBA, 2,3-dihydroxybenzoic acid; 2,3,4-HBA, 2,3,4-trihydroxybenzoic acid; 4-HBA, 4-hydroxybenzoic acid; 2,3-DMBA, 2,3-dimethoxybenzoic acid; 2-OH-3-MBA, 2-hydroxy-3-methoxybenzoic acid.

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to more specific administration in the context of neuromuscular disorders. It is both synthesized in the body and obtained from food; however, endogenous biosynthesis is by far the predominant source in humans. Inherited disorders disrupting the CoQ_{10} biosynthesis pathway were identified almost 30 years ago [3]. In the following, genetic defects affecting 9 different enzymes were characterized (*PDSS1*, *PDSS2*, *COQ2*, *COQ4*, *COQ6*, *COQ7*, *ADCK3*, *ADCK4*, and *COQ9*). Clinical phenotypes related to these defects are extremely heterogeneous and range from fatal neonatal presentations with multisystem involvement to adult-onset isolated myopathy [2,4].

Human CoQ_{10} is composed of a benzoquinone ring connected to a polyisoprenoid side chain of 10 isoprene units. The benzoquinone ring is derived from tyrosine whereas the polyisoprenoid side chain is synthesized from acetyl-coenzyme A via the mevalonate pathway. The appropriate length of polyisoprenoid side chain is generated by COQ1 (also known as PDSS1/PDSS2). The condensation of the 4-hydroxybenzoate ring with the polyisoprenoid side chain is presumably mediated by COQ2 (a schematic overview of the CoQ_{10} biosynthesis pathway is depicted in Fig. 1). Subsequently, a decarboxylation step of the ring occurs for which the corresponding enzyme until now remains undiscovered. Next, the monooxygenase COQ6 catalyzes the hydroxylation of 3-decaprenyl-4-hydroxybenzoic acid to 3-decaprenyl-4,5-dihydroxybenzoic acid. In the following, the *O*-methyltransferase COQ3 and *C*-methyltransferase COQ5 are required for methylation reactions of further CoQ intermediates leading to the formation of 3-decaprenyl-2-methyl-5-methoxy-1,4-benzoquinol (6-demethoxyubiquinone/6-DMQ). Additionally, the enzymatic step catalyzed by COQ5 requires the activity of the atypical protein kinase ADCK4. The resulting metabolic intermediate is further hydroxylated at the 6-position of the ring via the hydroxylase enzyme COQ7. In addition, the lipid binding protein COQ9 is required during this metabolic step. The *O*-methyltransferase COQ3 catalyzes the last step of CoQ_{10} biosynthesis and finally COQ10 transports CoQ_{10} from its synthetic site to its functional site [5].

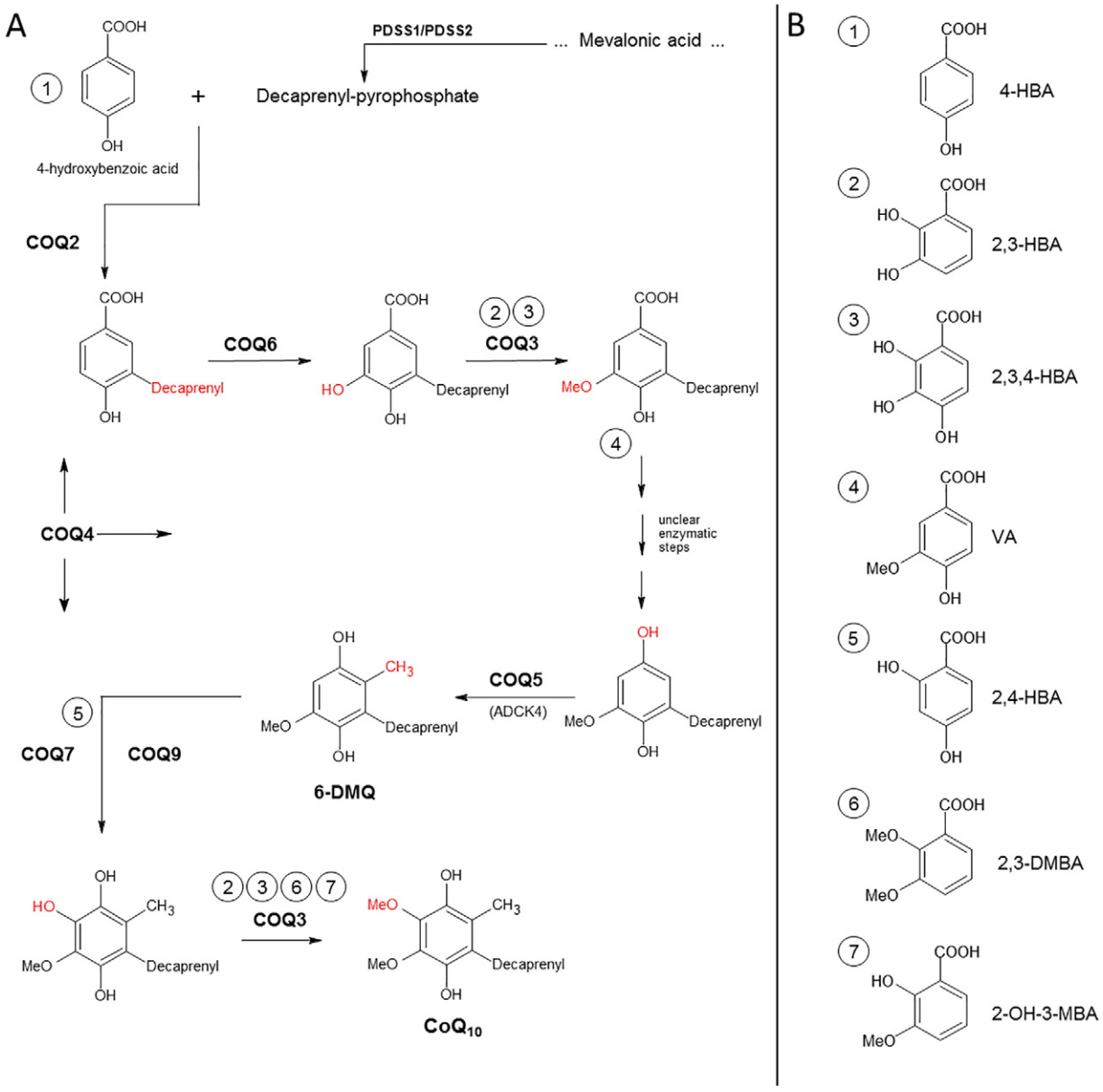

Fig. 1. A) Schematic illustration of the CoQ_{10} biosynthesis pathway in mammalian cells. Metabolic modifications performed during the different enzymatic steps are highlighted in red. B) Structural formulas of CoQ_{10} precursor compounds that could be theoretically used to bypass specific defects within the CoQ_{10} biosynthesis pathway. VA = vanillic acid; 2,4-HBA = 2,4-dihydroxybenzoic acid; 2,3-HBA = 2,3-dihydroxybenzoic acid; 2,3,4-HBA = 2,3,4-trihydroxybenzoic acid; 4-HBA = 4-hydroxybenzoic acid; 2,3-DMBA = 2,3-dimethoxybenzoic acid; 2-OH-3-MBA = 2-hydroxy-3-methoxybenzoic acid. Numerals in A) and B) indicate the level at which the compounds theoretically step into the CoQ_{10} biosynthesis pathway.