Rapid Reports

Escherichia coli QueD Is a 6-Carboxy-5,6,7,8-tetrahydropterin Synthase†

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ABSTRACT: To elucidate the early steps required during biosynthesis of a broad class of 7-deazapurine-containing natural products, we have studied the reaction catalyzed by *Escherichia coli* QueD, a 6-pyruvoyl-5,6,7,8-tetrahydropterin synthase (PTPS) homologue possibly involved in queuosine biosynthesis. While mammalian PTPS homologues convert 7,8-dihydroneopterin triphosphate (H_2NTP) to 6-pyruvoyltetrahydropterin (PPH_4) in biopterin biosynthesis, *E. coli* QueD catalyzes the conversion of H_2NTP to 6-carboxy-5,6,7,8-tetrahydropterin (CPH_4). *E. coli* QueD can also convert PPH_4 and sepiapterin to CPH_4, allowing a mechanism to be proposed.

Pyrrolo[2,3-*d*]pyrimidines, collectively termed deazapurines, are found in a number of antibiotic secondary metabolites produced by species of *Streptomyces* (*1*), as well as the hypermodified tRNA base queuosine. Recent studies have led to identification of many of the genes that appear to be required for biosynthesis of deazapurine-containing secondary metabolites, including queuosine and toyocamycin (*3*−*7*). These results indicate that the biosynthesis of deazapurine-containing compounds as diverse as queuosine and toyocamycin begins with the conversion of GTP to 7,8-dihydroneopterin triphosphate (H_2NTP) by GTP cyclohydrolase type I (GCH I), followed by the action of three additional enzymes: a 6-pyruvoyltetrahydropterin synthase (PTPS) homologue, a protein of the radical SAM superfamily (*8*), and an ExsB family homologue (see Figure 1).

PTPS catalyzes the conversion of H_2NTP, produced by GCH I, to pyruvoyltetrahydropterin (PPH_4) in the second step of tetrahydrobiopterin biosynthesis in mammals (see Scheme S1 of the Supporting Information). PPH_4 is subsequently converted to tetrahydrobiopterin (BH_4) by sepiapterin reductase in an NADPH-dependent reaction (*9*). In mammals, BH_4 serves as a cofactor for such enzymes as phenylalanine hydroxylase and nitric oxide synthase. Curiously, PTPS homologues are widely present in prokaryotes, which are not known to produce biopterin. *E. coli* contains a single PTPS homologue (B2765 in *E. coli* W3110), which is variously annotated as *ygcM*, *queD*, and *sscR*. Since queuosine is the only deazapurine produced by this organism and studies on deazapurine biosynthetic pathways have implicated a PTPS homologue, it is possible that this protein is engaged in the biosynthesis of queuosine.

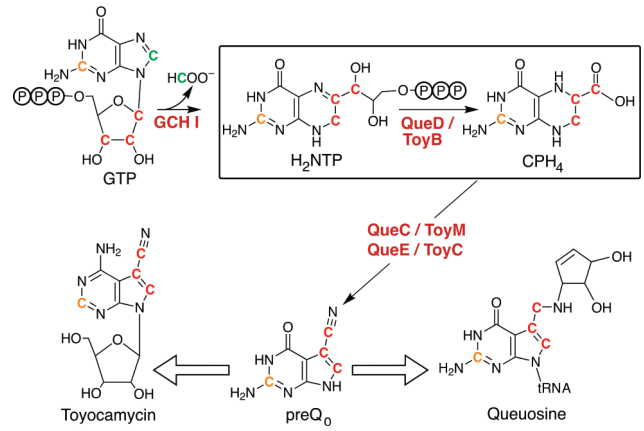

FIGURE 1: Biosynthesis of the deazapurines toyocamycin and queuosine begins with the conversion of GTP to H_2NTP by GCH I. The remaining steps leading to the central precursor, $preQ_0$, are catalyzed by PTPS (QueD/ToyB), radical SAM superfamily (QueE/ToyC), and ExsB family (QueC/ToyM) homologues (protein designations Que and Toy refer to those involved in queuosine and toyocamycin biosynthesis, respectively). In this study, *E. coli* QueD (boxed reaction) was shown to convert H_2NTP to 6-carboxy-5,6,7,8-tetrahydropterin (CPH_4). The coloring scheme of the carbon atoms reflects the results of early radiotracer experiments on deazapurine biosynthesis (*1, 2*).

Studies of *E. coli* QueD by Park and co-workers (*10*) have established that the protein has an alternate activity, which they proposed to be the conversion of sepiapterin to 7,8-dihydropterin. However, these assays were carried out under aerobic conditions, and products were detected by fluorescence after oxidative derivatization. We have re-examined the activity of QueD under strictly anaerobic conditions without derivatization and reveal that the product is 6-carboxy-5,6,7,8-tetrahydropterin (CPH_4).

In all experiments described here, recombinant native QueD was assayed under anaerobic conditions (95% N_2 and 5% H_2) and the substrate (H_2NTP) was generated using the *E. coli* GCH I homologue FolE, in situ. HPLC analysis indicated that under these conditions, H_2NTP could be

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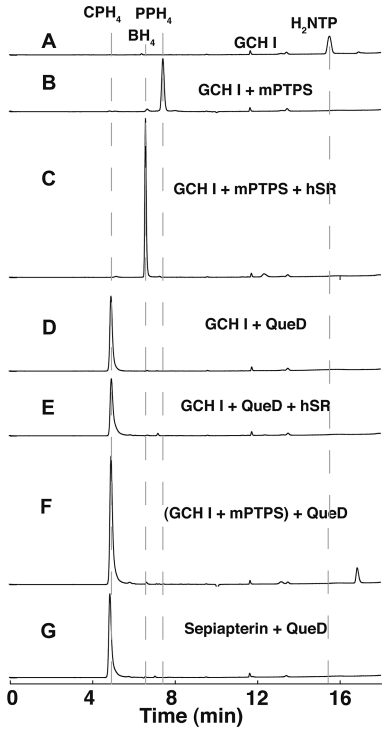

FIGURE 2: HPLC chromatograms of the QueD-catalyzed reaction. The chromatograms correspond to (A) H_2NTP produced from GTP by *E. coli* GCH I (FolE), (B) PPH_4 produced from GTP by FolE and mouse PTPS (mPTPS), (C) BH_4 produced from GTP by FolE and mPTPS, and hSR with NADPH, and (D) CPH_4 produced from GTP with FolE and *E. coli* QueD. Chromatogram E was obtained under the same conditions as chromatogram D but with hSR and NADPH. Chromatogram F was obtained by addition of QueD to a reaction mixture containing PPH_4 prepared as described for panel B. Chromatogram G was obtained with sepiapterin as a substrate for QueD. Refer to the Supporting Information for the experimental procedures.

produced quantitatively from GTP (Figure 2A). As a control, when the GCH I reaction is carried out in the presence of recombinant mouse PTPS (mPTPS), H_2NTP was quantitatively converted to PPH_4 (Figure 2B) which elutes at 7.5 min and also has a UV−visible spectrum consistent with that expected for PPH_4 (Figure S1A of the Supporting Information). Furthermore, when recombinant human sepiapterin reductase (hSR) and NADPH are included, PPH_4 is converted cleanly to BH_4 (Figure 2C), the retention time (6.5 min) of which is identical to that of commercially obtained BH_4 (data not shown). By contrast, when H_2NTP is incubated with QueD, a peak at 4.9 min is observed, and the retention time (Figure 2D,E) and spectral properties (see Figure S1C,D of the Supporting Information) of that peak do not change in the presence of hSR. Interestingly, when PPH_4, generated enzymatically from the combined actions of GCH I and mPTPS, is combined with QueD, the peak for the product has a retention time and spectral properties identical to those observed when H_2NTP is mixed with QueD, suggesting that the identical product is formed (compare panels D and F of Figure 2). Moreover, the same product forms when sepiapterin is the substrate for QueD (see Figure 2G and Figure S1F of the Supporting Information). These experiments clearly establish that the product of QueD differs from PPH_4. On the basis of the UV−visible spectra of the compounds eluting from the HPLC column at 4.9 min, the product of QueD is a tetrahydro-substituted pterin (Figure S1C−F of the Supporting Information).

To identify the molecular formula of the unknown QueD product, we repeated the QueD reaction as in Figure 2D with unlabeled GTP and [U-$^{13}C_{10}$,$^{15}N_5$]GTP and analyzed the reactions by Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) for both positively and negatively charged ions (see Figures S2 and S3 of the Supporting Information). [U-$^{13}C_{10}$,$^{15}N_5$]GTP is 15 amu heavier than unlabeled GTP. Since GCH I removes C-8 of GTP as formate (*11*), a genuine QueD product would be expected to show a mass difference of ≤14 amu with uniformly labeled GTP relative to the unlabeled control. Indeed, we observe an $[M + H]^+$ ion with *m*/*z* 212.0778 and an $[M - H]^-$ ion with *m*/*z* 210.0642, in positive and negative ion modes, respectively. These peaks are absent from the corresponding spectra with labeled GTP. Instead, $[M + H]^+$ and $[M - H]^-$ ions with *m*/*z* 224.0868 and 222.0732 are observed in positive and negative ion modes, respectively. Moreover, the MS of a solution containing samples of both the unlabeled and labeled GTP reaction mixtures shows both ions; a control reaction lacking GTP does not show any of the peaks. The high-resolution mass measurements are consistent with a molecular formula of $C_7H_9N_5O_3$ which, when taken in the context of the UV−visible spectral data, is consistent with identification of 6-carboxy-5,6,7,8-tetrahydropterin (CPH_4) as the product of the QueD reaction.

The identity of CPH_4 was confirmed by tandem MS/MS experiments in which the positively and negatively charged molecular ions for the product of QueD, observed with unlabeled and labeled GTP, were isolated in the quadrupole and underwent collision-induced fragmentation (Figure S4 of the Supporting Information). The ions exhibit loss of HCOOH or CO_2, under positive and negative detection, respectively, further confirming the presence of a carboxy moiety.

While CPH_4 is not commercially available, pterins can be reduced to 5,6,7,8-tetrahydropterin with reducing agents such as $NaBH_4$ (*12*). Commercially available 6-carboxypterin was reduced with $NaBH_4$, and the reaction mixture was subjected to HPLC analysis as described in the Supporting Information. The product of the reduction has a retention time and UV−visible spectrum identical to those of the QueD product; moreover, the two coelute from the HPLC column in a coinjection experiment (Figure S5 of the Supporting Information), confirming formation of CPH_4 by *E. coli* QueD.

Alignment of *E. coli* QueD with eukaryotic PTPS proteins, which catalyze the conversion of H_2NTP to PPH_4, reveals the conservation of amino acid residues that have been shown to be involved in substrate binding and catalysis in the well-characterized rat PTPS homologue (Figure S6 of the Supporting Information) (*13, 14*). These include three His residues that coordinate an essential zinc metal ion. Inductively coupled plasma optical emission spectroscopy (ICP-OES) analysis of purified, recombinant QueD used in the experiments described in this work revealed the presence of 1.1 equiv of Zn per monomer. On the basis of these observations, together with the fact that QueD is capable of utilizing H_2NTP, PPH_4, and sepiapterin as substrates, a plausible scheme for formation of CPH_4 is proposed (Figure 3). In this scheme, we assume that the initial rearrangements involved in the conversion of H_2NTP to PPH_4 by QueD are

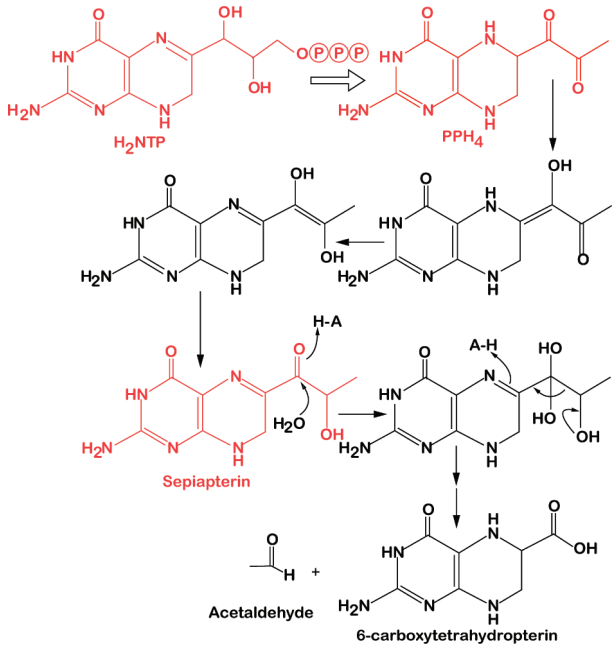

FIGURE 3: Proposed scheme for conversion of H_2NTP to 6-carboxy-5,6,7,8-tetrahydropterin and acetaldehyde. Compounds colored red have been demonstrated to be turned over by QueD to 6-carboxytetrahydropterin.

similar to those proposed for the mammalian PTPS (*13, 14*), and they have not been depicted. PPH_4 can tautomerize to sepiapterin, the hydrate of which undergoes carbon−carbon bond cleavage by an aldolase-like mechanism, followed by a tautomerization, to yield CPH_4. The proposed scheme predicts the formation of acetaldehyde as a leaving group. Indeed, when the QueD reaction is quenched with acidic 2,4-dinitrophenylhydrazine and analyzed by HPLC, a new peak is observed that has the same retention time as the hydrazone adduct of acetaldehyde (see Figure S7 of the Supporting Information).

The side chain cleavage activity of QueD, which results in the formation of CPH_4, fulfills the requirement for loss of carbons C-4′ and C-5′ (GTP numbering) necessary for conversion of GTP to $preQ_0$, a known intermediate in queuosine biosynthesis. The details surrounding two additional transformations required for deazapurine biosynthesis, which are catalyzed by a radical SAM superfamily and an ExsB family homologue (see Figure 1), remain to be elucidated. However, we hypothesize that CPH_4 serves as the substrate in one of them. Another interesting question posed by the results presented here is how prokaryotic PTPS homologues that are involved in deazapurine biosynthesis can catalyze the production of a product which is distinct from that produced by mammalian homologues, even though sequence alignments reveal very few differences between them. Additional studies aimed at addressing the molecular basis for the observed activities as well as mechanistic studies of this fascinating protein are underway and will be reported in due course.

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SUPPORTING INFORMATION AVAILABLE

Materials and detailed experimental procedures, UV−visible spectra of compounds shown in the HPLC traces in Figure 2, FT-ICR MS data, comparison of the QueD reaction product with synthetic CPH_4, multiple-sequence alignment of PTPS homologues, and acetaldehyde assay. This material is available free of charge via the Internet at http://pubs.acs.org.

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