FEBS Letters 580 (2006) 4900–4904

6-Pyruvoyltetrahydropterin synthase orthologs of either a single or dual domain structure are responsible for tetrahydrobiopterin synthesis in bacteria

Jin Sun Kong[a], Ji-Youn Kang[a,1], Hye Lim Kim[a], O-Seob Kwon[b], Kon Ho Lee[c], Young Shik Park[a,*]

[a] *Mitochondrial Research Group, School of Biotechnology and Biomedical Science, Inje University, Kimhae 621-749, Republic of Korea*
[b] *School of Environmental Science and Engineering, Inje University, Kimhae 621-749, Republic of Korea*
[c] *Division of Applied Life Science, Environmental Biotechnology National Core Research Center, Gyeongsang National University, Jinju 660-701, Republic of Korea*

Received 27 July 2006; accepted 2 August 2006

Available online 14 August 2006

Edited by Miguel De la Rosa

Abstract 6-Pyruvoyltetrahydropterin synthase (PTPS) catalyzes the second step of tetrahydrobiopterin (BH4) synthesis. We previously identified PTPS orthologs (bPTPS-Is) in bacteria which do not produce BH4. In this study we disrupted the gene encoding bPTPS-I in *Synechococcus* sp. PCC 7942, which produces BH4-glucoside. The mutant was normal in BH4-glucoside production, demonstrating that bPTPS-I does not participate in BH4 synthesis in vivo and bringing us a new PTPS ortholog (bPTPS-II) of a bimodular polypeptide. The recombinant *Synechococcus* bPTPS-II was assayed in vitro to show PTPS activity higher than human enzyme. Further computational analysis revealed the presence of mono and bimodular bPTPS-II orthologs mostly in green sulfur bacteria and cyanobacteria, respectively, which are well known for BH4-glycoside production. In summary we found new bacterial PTPS orthologs, having either a single or dual domain structure and being responsible for BH4 synthesis in vivo, thereby disclosing all the bacterial PTPS homologs.
© 2006 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.

Keywords: 6-Pyruvoyltetrahydropterin synthase; Tetrahydrobiopterin; Cyanobacteria; Green sulfur bacteria; Bimodular structure; Paralog

1. Introduction

6-Pyruvoyltetrahydropterin synthase (PTPS, EC 4.2.3.12) is an essential enzyme catalyzing the second step of tetrahydrobiopterin (BH4) synthesis [1]. BH4 is ubiquitous in higher animals as an essential cofactor for aromatic amino acid hydroxylation and nitric oxide synthesis. The de novo synthesis of BH4 starting from GTP is catalyzed by at least three enzymes: GTP cyclohydrolase I (GTPCH, EC 3.5.4.16), PTPS, and sepiapterin reductase (SR, EC 1.1.1.153). GTP is converted to dihydroneopterin triphosphate (H2-NTP) by GTPCH and then to 6-pyruvoyltetrahydropterin (PPH4) by PTPS. PPH4 is reduced finally to BH4 by SR.

BH4 was also found as glycosidic forms in some bacterial groups such as cyanobacteria and *Chlorobium* sp., although the function remained unknown [2–7]. Bacterial BH4 synthesis follows the same biochemical steps established in higher animals [8]. The final step of glycosylation is catalyzed by pteridine glycosyltransferase (PGT) [9]. GTPCH is ubiquitous in bacteria because of its involvement in tetrahydrofolate synthesis, while PTPS is expected to be in those producing BH4 or its glycosides. However, the genome sequences of many bacteria, which were known not to produce BH4 or its glycosides, revealed PTPS homologs. The bacterial PTPS homologs, named here bacterial PTPS-Is (bPTPS-Is), of *Escherichia coli* and *Synechocystis* sp. PCC 6803 were shown to synthesize PPH4 from H2-NTP in vitro [10], although the specific activities were much lower than that of human PTPS. Furthermore, bPTPS-Is were identified to have a peculiar catalytic function to cleave the C6-side chain of sepiapterin to yield dihydropterin (SSCR activity) [10]. In order to explore if bPTPS-I is responsible for the synthesis of BH4 in vivo as the genuine PTPS homolog, the encoding gene in *Synechococcus* sp. PCC 7942 which produces BH4-glucoside [5,11] was disrupted by homologous recombination. The mutant, however, showed normal BH4-glucoside production and growth. The result led us to find another PTPS homolog, named here bacterial PTPS-II (bPTPS-II), from the deduced genome sequence of *Synechococcus elongatus* PCC 7942, which had not been available during our initial study on bPTPS-I [10]. The bPTPS-II, consisting of two PTPS domains in tandem organization, was characterized to exhibit high PTPS activity. Computational analyses further revealed bPTPS-II orthologs of a single or double domain structure mostly in green sulfur bacteria and cyanobacteria, respectively. We report here the new finding of bPTPS-II orthologs, which are paralogs of the previously identified bPTPS-Is.

*Corresponding author. Fax: +82 55 336 7706.
E-mail address: mbyspark@inje.ac.kr (Y.S. Park).

[1] Present address: Lundbeck Korea Co. Ltd., 98-4 Garak-Dong, Songpa-Gu, Seoul 138-160, Republic of Korea.

Abbreviations: BH4, tetrahydrobiopterin; GTPCH, GTP cyclohydrolase I; H2-NTP, dihydroneopterin triphosphate; PPH4, 6-pyruvoyltetrahydropterin; PTPS, 6-pyruvoyltetrahydropterin synthase; bPTPS-I, bacterial PTPS-I; bPTPS-II, bacterial PTPS-II; hPTPS, human PTPS; SR, sepiapterin reductase; SSCR activity, sepiapterin side chain releasing activity

0014-5793/$32.00 © 2006 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.febslet.2006.08.006

2. Materials and methods

2.1. Growth conditions

Synechococcus sp. PCC 7942 wild type and mutant strains were grown at 30 °C in BG-11 medium in batch culture under continuous white light (100–120 μmol m^{-2} s^{-1}) and air bubbling. Growth was measured by the optical density at 730 nm.

2.2. Creation of PTPS-I knockout mutant

To construct a knockout vector a 1724 bp DNA fragment of bPTPS-I gene harboring neighbor sequences at both sides was amplified from the total genomic DNA of *Synechococcus* sp. PCC 7942 by using the primer pair, AAGCTTGGGCACTAAAGTCAATCG (forward) and GAGCTCCCGATATTTTGGTCTGTC (reverse). The DNA fragment was cloned into the corresponding site of *Hin*dIII/*Sac*I in pBS SK+ and then the 343 bp *Eco*RI/*Sca*I fragment in the middle of bPTPS-I gene was replaced by the 1115 bp spectinomycin resistant gene. The resistant gene was digested from the pGEM-T-Easy vector harboring the DNA amplified from pAM990K plasmid [12] using a primer pair GAATTCAAGGCACGAACCCAGTG (forward) and AGTACTAAGCGGCGTCGGCTTGA (reverse). *Synechococcus* cells were transformed following the previous publication [9]. Positive colonies were selected on BG11 agar plates containing 40 μg/ml of spectinomycin and restreaked to mono ones with at least five serial transfers to obtain full segregation of the mutation.

2.3. Construction of expression plasmids

The ORF sequences of bPTPS-I and bPTPS-II genes in *Synechococcus* sp. PCC 7942 were amplified by PCR from the genomic DNA using the primer pairs (bPTPS-I, CATATGATGCCGCGTTGGACTCT and AAGCTTTTAATCCTCGTAGGTCGCC; bPTPS-II, CATATGATGAGAGACAGCCAATCACG and AAGCTTTTAGAGCAAAA CGGGTACTG). The N- and C-terminal domains of bPTPS-II (bPTPS-IIA and bPTPS-IIB, respectively, as shown in Fig. 2A) were amplified from the bPTPS-II gene using the primer pairs (bPTPS-IIA, CATATGATGAGAGACAGCCAATCACG and AAGCTTTTAGGCTTGTCCTAGATAGTCG; bPTPS-IIB, CATATGGAAGCCTACCTAACGATTCAGA and AAGCTTTTAGAGCAAA ACGGGTACTG). The amplified DNA was cloned into a pGEM-T vector and subsequently cloned as an *Nde*I/*Hin*dIII restriction fragment into pET-28b (for bPTPS-I, bPTPS-II, and bPTPS-IIB) or pET-15b (for bPTPS-IIA). The plasmids were transformed into *E. coli* BL21(DE3) (Novagen). DNA sequences were confirmed by sequencing. The overexpression plasmid of human PTPS (hPTPS) was prepared previously [10].

PCR amplifications were performed with *Pfu* polymerase (Promega) in 1× reaction buffer, 200 μM dNTPs, 0.5 μM each of primer pairs, and templates, under the following conditions: 4 min at 95 °C, followed by 30 cycles of 95 °C for 1 min, 57–65 °C depending on the primer pairs for 1 min, and 72 °C for 1 min, and a final DNA polymerization at 72 °C for 7 min.

2.4. Expression and purification of recombinant proteins

The transformed cells were grown at 37 °C in LB + Amp broth with vigorous shaking and then induced to overexpress recombinant proteins by 0.5 mM IPTG when A_{600} reached 0.6. The cells were grown overnight and harvested by centrifugation. After being washed and resuspended in lysis buffer (50 mM sodium phosphate, pH 8.0, 300 mM NaCl, 10 mM imidazole), the cells were disrupted by sonication. The crude extract was chromatographed on a column of Ni-NTA gel and His-tag proteins were eluted with 100 mM imidazole in the lysis buffer. The purified protein was dialyzed against 10 mM Pipes (pH 7.5), mixed with glycerol to a final concentration of 10%, and stored at −70 °C until used.

2.5. Activity assay

PTPS activity to catalyze the synthesis of PPH4 from H2-NTP was assayed in the absence or presence of SR as described previously [10]. The reaction mixture consisted of 100 mM Tris–HCl (pH 7.5), 10 mM $MgCl_2$, 10 mM DTT, 0.1 mM H2-NTP, and aliquots of enzymes in a final volume of 50 μl. In a coupled assay with SR, the mixture was supplemented with 0.2 mM NADPH and 5 μg of recombinant mouse SR. The mixture was incubated for 1 h at 37 °C and then mixed with an equal volume of an acidic iodine solution (2% KI and 1% I_2 in 1 N HCl). After 1 h in the dark, the mixture was centrifuged and the supernatant was reduced with ascorbic acid and subjected to HPLC. H2-NTP was prepared from GTP with recombinant *Synechocystis* sp. 6803 GTPCH.

The SSCR activity to convert sepiapterin to 7,8-dihydropterin was measured following the previous method [10]. The reaction mixture contained 10 mM Pipes, pH 7.5, 2 mM sepiapterin, and an aliquot of enzyme in a total volume of 50 μl. The mixture was incubated at 37 °C for 20 min and stopped with an equal volume of cold 10% trichloroacetic acid. After 10 min on ice and centrifugation at $10000 \times g$ for 10 min, the reaction supernatant was 10-fold diluted with water. The amount of remaining sepiapterin in the reaction mixture was measured at 420 nm. The concentration of sepiapterin was calculated from the molar extinction coefficient, 10.4 mM^{-1} cm^{-1} at 420 nm.

The assay of BH4 synthesis by the whole cell protein extract was performed in the reaction mixture of 100 mM Tris–HCl (pH 7.5), 0.1 mM H2-NTP, 10 mM $MgCl_2$, 10 mM DTT, 0.2 mM NADPH, and an aliquot of enzyme in a final volume of 50 μl. The mixture was incubated for 2 h at 37 °C and iodine oxidized as described above for HPLC analysis. To prepare protein extract cells were suspended in 10 mM Pipes, pH 7.5, 1 mM PMSF (4 ml per gram of wet weight) and disrupted by sonication. The homogenate was centrifuged for 30 min at $15000 \times g$ to remove the precipitate. The supernatant was mixed with saturated ammonium sulfate solution to a final concentration of 50% and centrifuged at $15000 \times g$ for 20 min. The protein precipitate was dissolved in 10 mM Pipes, pH 7.5, and desalted by a spin column of Sephadex G-25.

2.6. HPLC analysis of pteridines

HPLC was performed on a Kontron Model 430 equipped with an Inertsil ODS-3 C18 column (5 μm, 150 × 2.3 mm, GL Science, Japan). Pteridines were eluted isocratically with 10 mM sodium phosphate (pH 6.0) at a flow rate of 1.2 ml min^{-1} and monitored at 350/450 nm (excitation/emission) by using HP Model 1046A fluorescence detector as described previously [10]. Standard pteridines were purchased from Schirck's Lab (http://www.schircks.com).

2.7. Miscellaneous methods

SDS–PAGE analysis of proteins was performed on denaturing conditions with a 12.5% polyacrylamide gel. Native-PAGE was performed in 25 mM Tris–192 mM glycine (pH 8.4) buffer with a 7.5% polyacrylamide gel. Protein concentration was measured by the Bradford method using bovine serum albumin as a standard.

3. Results and discussion

3.1. bPTPS-I knockout mutant

The gene encoding bPTPS-I of 128 amino acid residues was disrupted in *Synechococcus* sp. PCC 7942 by insertional inactivation. PCR amplification of the gene from the genomic DNA showed only a 1158 bp DNA fragment containing spectinomycin resistant gene, while wild type copy was 387 bp (Fig. 1A), indicating successful gene targeting of bPTPS-I. However, the mutant was found normal in photosynthetic growth and BH4-glucoside production (data not shown). In order to confirm disruption of the gene the mutant cell extract was assayed for the SSCR activity [10]. As shown in the HPLC chromatogram (Fig. 1B), in contrast to the wild type, the pterin peak disappeared almost in the mutant reaction mixture, verifying the absence of bPTPS-I in the mutant. However, the mutant cell extract assayed for BH4 synthesis exhibited a biopterin peak as much as wild type extract did (Fig. 1C). The results demonstrated that bPTPS-I did not involve in BH4 synthesis in vivo and suggested the existence of another enzyme having a genuine PTPS activity in *Synechococcus* sp. PCC 7942.

3.2. Primary structure properties of bPTPS-II

A homology search through the genome sequence of *S. elongatus* PCC 7942 revealed another PTPS homolog.

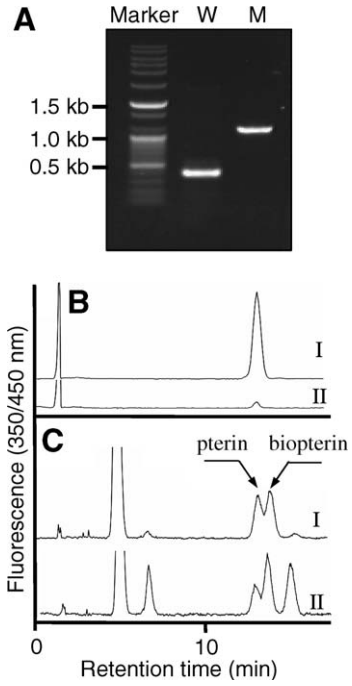

Fig. 1. Molecular and biochemical characterization of *Synechococcus* sp. PCC 7942 mutant disrupted in the gene encoding bPTPS-I. (A) PCR amplification of bPTPS-I gene from the genomic DNAs of wild type (W) and the mutant cells (M). HPLC identification of SSCR activities of wild type (I) and mutant cell extracts (II). HPLC analysis of BH4 synthesis with the cell extracts from wild type (I) and mutant cells (II). The reaction mixtures for (B) and (C) were prepared as described in Section 2. The reaction mixtures were oxidized by acidic iodine solution and chromatographed on a C18 column isocratically with 10 mM sodium phosphate, pH 6.0, at a flow rate of 1.2 ml/min. The pteridines were identified by coinjection with authentic pteridines.

Interestingly, the homolog, named here bacterial PTPS-II (bPTPS-II), composed of 300 amino acid residues having two PTPS domains in tandem (Fig. 2A). Each domain sequence (N-terminal domain; bPTPS-IIA, C-terminal domain; bPTPS-IIB) was so complete as to be aligned well with *Synechococcus* bPTPS-I (128 amino acid residues) and hPTPS (Fig. 2B). The amino acid sequence identity between the domains was 29.5%. The domains were more similar to hPTPS than bPTPS-I and between the domains bPTPS-IIB was more than bPTPS-IIA: the sequence identities were 15.7% (bPTPS-IIA and bPTPS-I), 20.7% (bPTPS-IIB and bPTPS-I), 33.1% (bPTPS-IIA and hPTPS), and 41.4% ((bPTPS-IIB and hPTPS). The critical residues for catalysis, positioning pterin ring, and coordinating with zinc ion, which were identified from the crystal structure of rat PTPS [13], were also conserved in both domain sequences (Fig. 2B). Therefore, the bPTPS-II was highly expected to have PTPS activity.

3.3. Catalytic activities of bPTPS-II and its domains

In order to determine the catalytic activities of the bPTPS-II and its separated domains, the ORF sequences were cloned in bacterial expression vectors and hetereologously overexpressed in *E. coli*. For comparative analyses of PTPS and SSCR activities, the recombinant proteins of *Synechococcus* bPTPS-I and hPTPS were also prepared. The recombinant proteins were obtained to apparent homogeneities as shown in Fig. 3. They were assayed for PTPS activity in the absence or presence of recombinant SR and analyzed by HPLC after iodine oxidation. The reaction mixture of bPTPS-II exhibited pterin in the absence of SR and biopterin in the coupled assay with SR (data not shown). The specific activity of bPTPS-II measured by pterin production was 15.95 ± nmol/min/mg of protein, which was 13-times higher than that of bPTPS-I (1.22 ± 0.03 nmol/min/mg) and even better than human enzyme (13.89 ± 0.45 nmol/min/mg) as shown in Fig. 4A. However, bPTPS-II showed much lower SSCR activity (3.29 ± 0.29 nmol/min/mg), merely 1.73% of bPTPS-I

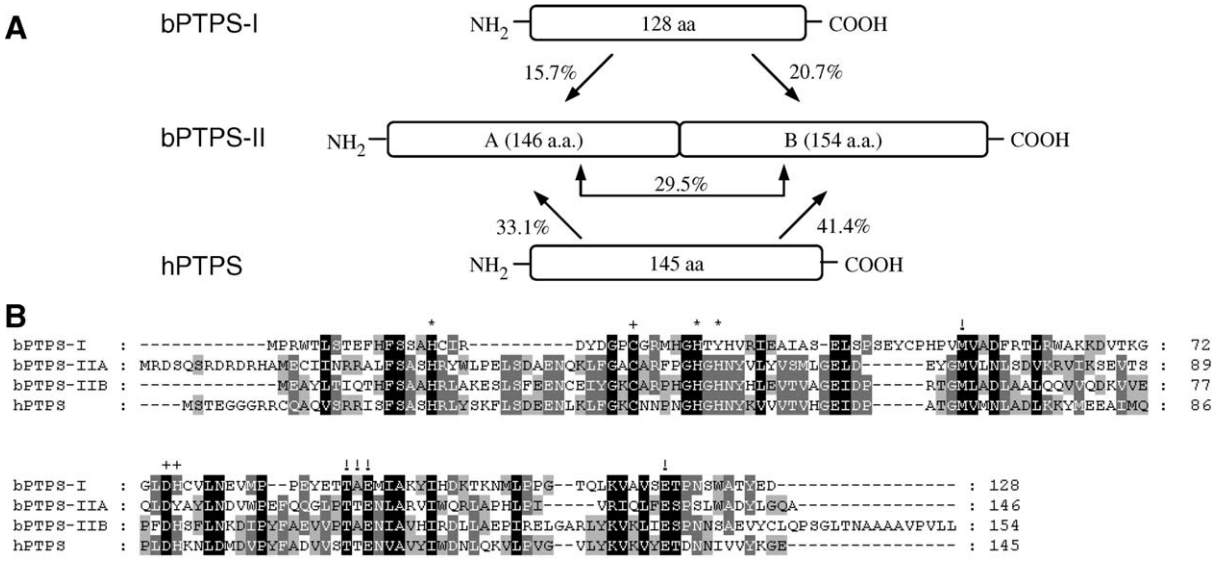

Fig. 2. Primary structures of the deduced PTPS polypeptides. (A) Schematic drawing of PTPS polypeptides to show sequence identities among bPTPS-I (*Synechococcus* sp. PCC 7942), bPTPS-II (*Synechococcus* sp. PCC 7942), and hPTPS (Accession No. Q03393). (B) Amino acid sequence alignment of PTPS homologs. Conserved sequences are shaded at four levels using GeneDoc software and the residues suggested for catalysis (+), positioning of the pterin ring (!), and coordinating with zinc ion (*) are indicated.

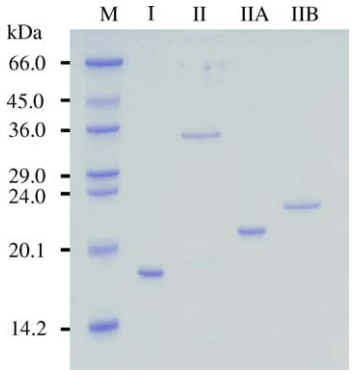

Fig. 3. SDS–PAGE of the purified recombinant PTPS proteins. Purified His-tag proteins were analyzed on a 12.5% SDS–polyarylamide gel. The deduced molecular masses containing His-tag were 16.6 kDa (I, bPTPS-I), 35.1 kDa (II, bPTPS-II), 19.2 kDa (IIA, bPTPS-IIA), and 19.2 kDa (IIB, bPTPS-IIB). Protein markers (M) are depicted to the left.

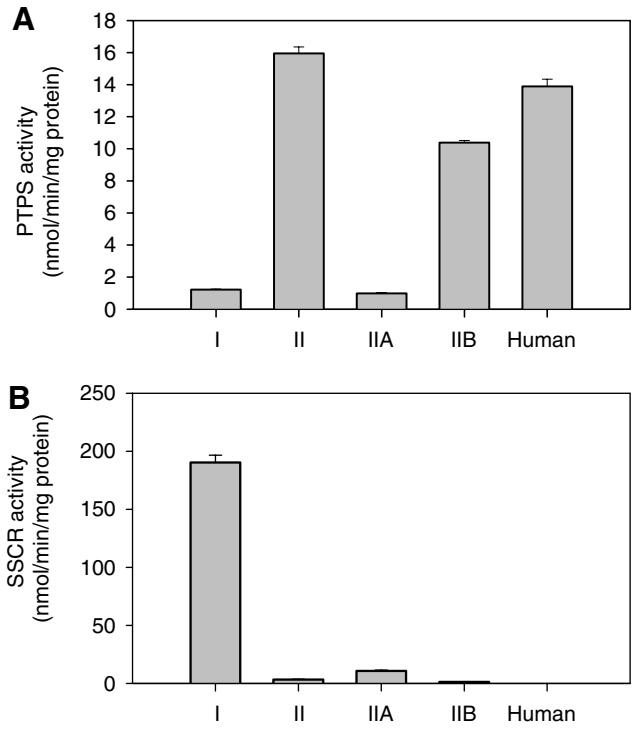

Fig. 4. Enzymatic activities of the recombinant PTPS proteins. The purified recombinant proteins of bPTPS-I, bPTPS-II, bPTPS-IIA, bPTPS-IIB, and hPTPS were assayed for the activities of PTPS (A) and SSCR (B) as described in Section 2. The mean ± SD values were determined from three independent experiments.

(190.17 ± 6.48 nmol/min/mg) (Fig. 4B). It therefore seemed undeniable that bPTPS-II has genuine PTPS activity to involve in BH4 synthesis in *Synechococcus* sp. PCC 7942.

On the other hand, bPTPS-IIB fragment exhibited significant PTPS activity (10.38 ± 0.14 nmol/min/mg of PTPS activity), amounting to 65% of the whole protein, while bPTPS-IIA was only 0.99 ± 0.04 nmol/min/mg. SSCR activity was trivial in both of them, although bPTPS-IIA fragment exerted higher activity (10.74 ± 0.64 nmol/min/mg) than bPTPS-IIB (1.23 ± 0.26 nmol/min/mg) and the whole protein.

3.4. bPTPS-II orthologs in bacterial genomes

So far cyanobacteria and thermophilic green sulfur bacteria *Chlorobium limicola* and *C. tepidum* have been known to produce BH4-glycosides [6,7]. In order to see if bimodular bPTPS-II orthologs are common in them, a BLAST search was performed against the genome sequences in NCBI (http://www.ncbi.nlm.nih.gov). Among 24 cyanobacterial genome sequences, 22 were found to have bimodular bPTPS-II orthologs sharing more than 65% identities (data not shown). However, no ortholog was found in a cyanobacterium *Synechocystis* sp. PCC 6803, which produces 6-hydroxylmethyltetrahydropterin glycoside and therefore does not require PTPS activity for the synthesis [14], further supporting the in vivo function of bPTPS-II orthologs. On the other hand, *C. limicola* and *C. tepidum* genome sequences revealed two PTPS homologs of monomodular structures: one was more similar to bPTPS-I, while the other was to bPTPS-IIB. The bPTPS-IIB homologs consisted of 149 amino acid residues, whose sequence identities with bPTPS-I, bPTPS-IIB, and hPTPS were more than 26%, 35%, and 39%, respectively. In order to see more clearly the sequence differences between the *Chlorobium* bPTPS-I (CL- and CT-PTPS-I) and -IIB homologs (CL- and CT-PTPS-II), they were multiple aligned with other PTPS homologs and computed for a neighbor-joining phylogenetic tree (Fig. 5). The homologs grouped into two divergent groups, which also coincided with the functional grouping depending on the activity of PTPS or SSCR, supporting that the monomodular *Chlorobium* bPTPS-IIs belong to proteins of PTPS activity.

A further homology search of PTPS-II orthologs against the currently available microbial genomes revealed mono and bimodular polypeptides exclusively in green sulfur bacteria and cyanobacteria, respectively, although bimodular ones were also found in a thermophilic *Bacillus*-related species *Geobacillus kaustophilus* HTA426 [15] and anaerobic green non-sulfur bacteria *Chloroflexus aurantiacus J-10-fl* (Accession number NZ_AAAH02000012). The occurrence of bPTPS-II orthologs most exclusively in BH4-glycoside producing bacteria, while bPTPS-I orthologs are widespread, further augmented that bPTPS-II orthologs are truly responsible for BH4 synthesis in bacteria.

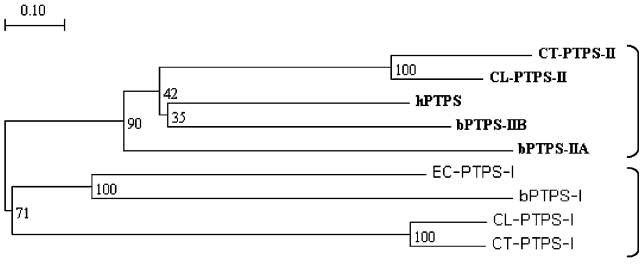

Fig. 5. Phylogenetic tree for PTPS homologs. The multiple alignment was prepared by ClustalW and further analyzed by a distance-based method to build the unrooted neighbor-joining phylogram. The scale bar indicates a distance corresponding to 0.1% difference between sequences. The protein sequences of bPTPS-I, -IIA, -IIB, and hPTPS are described in Fig. 2. The *Escherichia coli* PTPS (EC-PTPS-I) is encoded by *ygc*M [10]. The *Chlorobium* species PTPS homologs were retrieved from the genome sequence of *C. limicola* DSM 245 and *C. tepidum* TLS: CL-PTPS-I (143 amino acid residues encoded by the nucleotide sequence 43554-43126), CL-PTPS-II (149 a.a., the nt sequence 51765-52211), CT-PTPS-I (142 a.a., the nt sequence 1348469-1348894), and CT-PTPS-II (149 a.a., the nt sequence 754397-754131).

3.5. Conclusions

Our results conclude that a new paralogous group of bacterial PTPSs (bPTPS-IIs) consisting of either mono or bimodular polypeptides, not the previously identified ones (bPTPS-Is), are responsible for BH4 synthesis in bacteria. The results thereby clarify the ambiguous occurrence of the homologous PTPS sequences in bacterial genomes, although the physiological function remains unknown for bPTPS-I orthologs.

The bPTPS homologs may worth of intensive structural investigation. As shown in Fig. 3, the *Synechococcus* bPTPS-I and -II exhibited different dominant activities: compared to each other, higher SSCR and lower PTPS activities by bPTPS-I and vice versa by bPTPS-II. Considered the high sequence similarities between them, the protein structures probably differ in the substrate binding sites, which are known to be constituted by monomers in hexameric animal PTPSs [13]. X-ray crystallography of the proteins may be useful for understanding the functional divergence and the putative physiological function of SSCR activity. It may be also interesting to investigate the structural and functional differences between the bPTPS-II orthologs of single and dual domain structures. Based on the results that *Synechococcus* bPTPS-II showed higher PTPS activity than human enzyme (Fig. 3) and bPTPS-II orthologs are ubiquitous mostly in cyanobacteria, which are well known for the high production of BH4-glycosides, we speculate that bimodular structure in bPTPS-II provided a chance to evolve to a protein of higher machinery efficiency.

Acknowledgements: This work was supported by a grant from the Inje Academic Research Fund, 2004, and Environmental Biotechnology National Core Research Center Grant R15-2003-002-01001-0 (to K.H.L.).

References

[1] Thöny, B., Auerbach, G. and Blau, N. (2000) Tetrahydrobiopterin biosynthesis, regeneration, and functions. Biochem. J. 347, 1–16.

[2] Forrest, H.S. and Van Baalen, C. (1970) Microbiology of unconjugated pteridines. Ann. Rev. Microbiol. 24, 91–108.

[3] Wachi, Y., Burgess, J.G., Iwamoto, K., Yamada, N., Nakamura, N. and Matsunaga, T. (1995) Effect of ultraviolet-A (UV-A) light on growth, photosynthetic activity and production of biopterin glucoside by the marine UV-A resistant cyanobacterium *Oscillatoria* sp.. Biochim. Biophys. Acta 1244, 165–168.

[4] Noguchi, Y., Ishii, A., Matsushima, A., Haishi, D., Yasumuro, K., Moriguchi, T., Wada, T., Kodera, Y., Hiroto, M., Nishimura, H., Sekine, M. and Inada, Y. (1999) Isolation of biopterin-α-glucoside from *Spirulina* (*Arthrospira*) *platensis* and its physiologic function. Mar. Biotech. 1, 207–210.

[5] Chung, H.J., Kim, Y., Kim, Y.J., Choi, Y.K., Hwang, Y.K. and Park, Y.S. (2000) Purification and characterization of UDP-glucose: tetrahydrobiopterin glucosyltransferase from *Synechococcus* sp. PCC 7942. Biochim. Biophys. Acta 1524, 183–188.

[6] Cha, K.W., Pfleiderer, W. and Yim, J. (1995) Isolation and characterization of limipterin (1-*O*-(L-erythro-biopterin-2′-yl)-β-*N*-acetylglucosamine) and its 5,6,7,8-tetrahydro derivative from green sulfur bacterium *Chlorobium limicola f. thiosulfatophilum* NCIB 8327. Helv. Chim. Acta 78, 600–614.

[7] Cho, S.H., Na, J.U., Youn, H., Hwang, C.S., Lee, C.H. and Kang, S.O. (1998) Tepidopterin, 1-O-(L-threo-biopterin-2′-yl)-*N*-acetylglucosamine from *Chlorobium tepidum*. Biochim. Biophys. Acta 1379, 53–60.

[8] Kang, D., Kim, S. and Yim, J. (1998) Biosynthetic enzymes of tetrahydrolimipterin from green sulfur bacterium *Chlorobium limicola*. Pteridines 9, 69–84.

[9] Choi, Y.K., Hwang, Y.K. and Park, Y.S. (2001) Molecular cloning and disruption of a novel gene encoding UDP-glucose: tetrahydrobiopterin α-glucosyltransferase gene in *Synechococcus* sp. PCC 7942. FEBS Lett. 502, 73–78.

[10] Woo, H.J., Hwang, Y.K., Kim, Y.J., Kang, J.Y., Choi, Y.K., Kim, C.G. and Park, Y.S. (2002) *Escherichia coli* 6-pyruvoyltetrahydropterin synthase ortholog has a new catalytic activity to convert sepiapterin to dihydropterin. FEBS Lett. 523, 234–238.

[11] Choi, Y.K., Hwang, Y.K., Kang, Y.H. and Park, Y.S. (2001) Chemical structure of 1-*O*-(L-erythro-biopterin-2'-yl)- α-glucose isolated from a cyanobacterium *Synechococcus* sp. PCC 7942. Pteridines 12 (3), 121–125.

[12] Li, R. and Golden, S.S. (1993) Enhancer activity of light-responsive regulatory elements in the untranslated leader regions of cyanobacterial *psbA* genes. Proc. Natl. Acad. Sci. USA 90, 11678–11682.

[13] Ploom, T., Thöny, B., Yim, J., Lee, S., Nar, H., Leimbacher, W., Richardson, J., Huber, R. and Auerbach, G. (1999) Crystallographic and kinetic investigations on the mechanism of 6-pyruvoyl tetrahydropterin synthase. J. Mol. Biol. 286, 851–860.

[14] Lee, H.W., Oh, C.H., Geyer, A., Pfleiderer, W. and Park, Y.S. (1999) Characterization of a novel unconjugated pteridine glycoside, cyanopterin, in *Synechocystis* sp. PCC 6803. Biochim. Biophys. Acta 1410, 61–70.

[15] Takami, H., Takaki, Y., Chee, GJ., Nishi, S., Shimamura, S., Suzuki, H., Matsui, S. and Uchiyama, I. (2004) Thermoadaptation trait revealed by the genome sequence of thermophilic *Geobacillus kaustophilus*. Nucleic Acids Res. 32, 6292–6303.