A Novel Tryptophan Synthase β-Subunit from the Hyperthermophile Thermotoga maritima

QUATERNARY STRUCTURE, STEADY-STATE KINETICS, AND PUTATIVE PHYSIOLOGICAL ROLE*

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Tryptophan synthase catalyzes the last two steps in the biosynthesis of the amino acid tryptophan. The enzyme is an αβα complex in mesophilic microorganisms. The α-subunit (TrpA) catalyzes the cleavage of indoleglycerol phosphate to glyceraldehyde 3-phosphate and indole, which is channeled to the active site of the associated β-subunit (TrpB1), where it reacts with serine to yield tryptophan. The TrpA and TrpB1 proteins are encoded by the adjacent trpA and trpB1 genes in the trp operon. The genomes of many hyperthermophilic microorganisms, however, contain an additional trpB2 gene located outside of the trp operon. To reveal the properties and potential physiological role of TrpB2, the trpA, trpB1, and trpB2 genes of Thermotoga maritima were expressed heterologously in Escherichia coli, and the resulting proteins were purified and characterized. TrpA and TrpB1 form the familiar αβα complex, in which the two different subunits strongly activate each other. In contrast, TrpB2 forms a β-homodimer that has a high catalytic efficiency $k_{cat}/K_m^\text{indole}$ because of a very low $K_m^\text{indole}$ but does not bind to TrpA. These results suggest that TrpB2 acts as an indole rescue protein, which prevents the escape of this costly hydrophobic metabolite from the cell at the high growth temperatures of hyperthermophiles.

Hyperthermophilic microorganisms grow optimally close to the boiling point of water (1). It is interesting to identify those molecular adaptations that allow proper function of metabolism under these extreme conditions (2). In particular, enzymes from hyperthermophiles must be extremely thermostable, and labile metabolites must be protected from spontaneous degradation (3–5).

The pathway of tryptophan biosynthesis from chorismate comprises seven catalytic functions (6). In most organisms, the trp genes are organized in operons, which guarantees their coordinated expression in response to the amount of tryptophan available in the growth medium (7). The order of the trp operon from the hyperthermophile Thermotoga maritima trpE(GD)CFBA (8) resembles the organization of the trp operon from Escherichia coli (9). The last four steps of tryptophan biosynthesis are catalyzed by phosphoribosyl anthranilate isomerase (TrpF), indoleglycerol phosphate synthase (TrpC), and the α- and β-subunits of tryptophan synthase (TrpA and TrpB1, respectively). It was shown that TrpF and TrpC from T. maritima are far more thermostable than their homologs from mesophiles (10, 11), probably because of an increased association state in the case of TrpF (12, 13) and an increased number of salt bridges in the case of TrpC (11, 14). Moreover, both TrpF and TrpC from T. maritima are catalytically more active at 80 °C than the orthologous enzymes from E. coli at 37 °C, thus outrunning the unproductive hydrolysis of their thermolabile substrates under physiological conditions (10, 11, 15).

Less is known about the specific structural and functional adaptations of the tryptophan synthase from T. maritima, which catalyzes the conversion of indoleglycerol phosphate (IGP) and serine to tryptophan (16). The tryptophan synthases characterized so far consist of two TrpA (α) and two TrpB1 (β) structural entities, which are organized either as four monofunctional subunits or as two bifunctional αβ-subunits (17). The x-ray structure at 2.8 Å resolution of the tryptophan synthase from Salmonella typhimurium revealed an αβα quaternary structure (18). The structure of isolated TrpA from Pyrococcus furiosus confirmed that this enzyme has a βαβα-barrel fold (19), which is the most frequently encountered topology among single domain proteins and is also adopted by TrpF and TrpC (20, 21). TrpB1 consists of two domains, which both comprise a central open β-sheet that is surrounded by α-helices (18). TrpA catalyzes the aldol cleavage of IGP to glyceraldehyde 3-phosphate (GA3P) and indole, which condenses with serine at the active site of TrpB1 to yield tryptophan (Fig. 1). The hydrophobic intermediate indole passes directly from the α-site to the β-site via a long tunnel, which prevents its loss from the cell by diffusion across the cytoplasmic membrane (22, 23). There is pronounced allosteric communication between the TrpA and TrpB1 subunits from E. coli, which is reflected in a mutual activation of their catalytic activities which keeps the two reactions in phase and prevents accumulation of indole (24). It appears that the αββα complex is in an equilibrium between a low activity “open” and a high activity “closed” state, which is shifted by allosteric ligands and monovalent cations (25). The basis of the corresponding conformational transitions has been characterized by x-ray structure analysis of a number of enzyme-ligand complexes (26–30).

Recently, the genome sequencing of T. maritima (31) and of other hyperthermophiles has identified a trpB2 gene outside of the trp operon. To reveal the roles in tryptophan biosynthesis of the two different TrpB variants, tmTrpA, tmTrpB1, and tmTrpB2, respectively, ec TrpA, ec TrpB1, respectively. It was shown that TrpF and TrpC from T. maritima are far more thermostable than their homologs from mesophiles (10, 11), probably because of an increased association state in the case of TrpF (12, 13) and an increased number of salt bridges in the case of TrpC (11, 14). Moreover, both TrpF and TrpC from T. maritima are catalytically more active at 80 °C than the orthologous enzymes from E. coli at 37 °C, thus outrunning the unproductive hydrolysis of their thermolabile substrates under physiological conditions (10, 11, 15).

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tmtmtrpB2 from T. maritima were expressed heterologously in E. coli, and the corresponding protein products were purified and characterized by hydrodynamic measurements and steady-state enzyme kinetics. The results show that tmtmTrpB1 associates with tmTrpA to an αβα complex, in which the two different subunits strongly activate each other. tmTrpB2, which does not bind to tmTrpA but is catalytically highly active, has an extremely low $K_m$ value for indole. It appears that tmTrpB1 has the same role in tryptophan biosynthesis as the known TrpB1 enzymes from mesophiles, whereas tmTrpB2 acts as a salvage protein that prevents the loss of indole at the physiological growth temperatures of hyperthermophiles.

EXPERIMENTAL PROCEDURES

DNA Manipulation and Sequence Analysis—Preparation of DNA, amplification, extraction, digestion with restriction endonucleases, ligation, and sequencing were performed as described (32).

Subcloning of tmtmtrpA, tmtmtrpB1, and tmtmtrpB2 Genes from T. maritima—The genes tmtmtrpA and tmtmtrpB1 were amplified by PCR using the plasmid pDS3tmtrpA (16) as template. For amplification of tmtmtrpA, the oligonucleotides 5′-GGTTCATATGAAAGGCCGTTGATACATC-3′ with a NdeI site (in boldface type) and 5′-CGATGAATTCCTCTTTCGAGGAGTCTTTT-3′ with an EcoRI site (in boldface type) were used as 5′- and 3′-primers, respectively. The tmtmtrpB1 gene was amplified with the primers 5′-GGATCATATGAAAGGCTTTATTGCATACATC-3′ and 5′-CCGGTGAATTCCTCATCTTATCCGTCCCTA-3′, again introducing NdeI and EcoRI sites (in boldface type). The two newly introduced restriction sites, the amplified DNA fragments were cloned into different PET vectors (Stratagene), yielding the plasmids pET21a-tmtmtrpA and pET24a-tmtmtrpB1. The gene tmtmtrpB2 was amplified by PCR using genomic DNA of T. maritima as the template. The primers 5′-ACCCTATGAGATTGGTGAA-3′ and 5′-CGAGGATTCAAGCTTTCACACGCTTAGGT-3′ were used, introducing NdeI and HindIII sites, respectively (in boldface type). The amplified DNA fragment was cloned into the vector pET21a to yield the plasmid pET21a-tmtmtrpB2. All inserts were sequenced entirely to exclude inadvertent PCR mutations.

Production of tmtmTrpA, tmtmTrpB1, tmtmTrpB2, the [tmtmTrpA+tmtmTrpB1] Complex, and of Glyceraldehyde-3-phosphate Dehydrogenase from T. maritima (tmGAPDH)—Heterologous expression of tmtmTrpA was conducted in E. coli BL21(DE3) cells containing the plasmid pET21a-tmtmtrpA. For reasons unknown so far, tmtmtrpA (and tmtmtrpB1 and tmtmtrpB2) are being expressed in the absence of isopropyl-1-thio-β-D-galactopyranoside. Therefore, the cells were grown overnight at 37 °C in 1 liter of LB medium supplemented with 150 μg/ml ampicillin but without the addition of isopropyl-1-thio-β-D-galactopyranoside. The cell pellet resulting from centrifugation was resuspended in 10 ml potassium phosphate buffer at pH 7.5 and lysed by sonication (Branson Sonifier W-250, 3 × 3 min, 50% pulse, 0 °C). According to SDS-PAGE, 90% of tmTrpA was found in the soluble fraction of the cell extract. 100 units of benzozene (Merck) was added to this fraction, which was then incubated for 1 h at 37 °C to degrade nucleic acids and subsequently for 20 min at 75 °C to denature the benzozene. The resulting suspension was centrifuged (Sorvall SS34, 13,000 rpm, 30 min, 4 °C), and the pellet, which contained heat-labile host proteins, was discarded. The supernatant was loaded on an anion exchange column (Mono Q, 2 × 11 cm, Amersham Biosciences, Inc.) that was equilibrated with 10 mM potassium phosphate buffer, pH 7.5, at room temperature, and sequencing were performed as described (32).

Heterologous expression of tmtmTrpA was conducted in E. coli BL21(DE3) cells containing the plasmids pET24a-tmtmtrpB1 and pET21a-tmtmtrpB2. Cells were grown as described for tmtmtrpA, with the exceptions that kanamycin instead of ampicillin was added for maintenance of PET24a-tmtmtrpB1 and 20 °C instead of 37 °C was used, which increased the fraction of soluble protein to about 40% for tmtmTrpB1 and about 10% for tmtmTrpB2 (33). Harvesting of cells, cell lysis, incubation with benzozene, heat precipitation of host proteins, and anion exchange chromatography were performed as described for tmTrpA, with the exception that the buffer solutions were supplemented with 40 μM pyridoxal 5′-phosphate (PLP). Both tmTrpB2 proteins eluted from the Mono Q column at about 150–200 mM potassium chloride. Fractions containing either tmTrpB protein were pooled, concentrated using Centriclon-10 devices (Millipore), and dialyzed against 10 mM potassium phosphate buffer, pH 7.5, containing 50 mM potassium chloride. The protein was shock frozen in liquid nitrogen at a concentration of 10 mg/ml. The purification yielded 50 mg of tmTrpA out of 1 liter of cell culture with a purity of about 99% as judged by SDS-PAGE.

For expression of tmtmtrpB1 and tmtmtrpB2, E. coli BL21(DE3) cells containing the plasmids pET24a-tmtmtrpB1 and pET21a-tmtmtrpB2 were used. Cells were grown as described for tmtmtrpA, with the exceptions that kanamycin instead of ampicillin was added for maintenance of PET24a-tmtmtrpB1 and 20 °C instead of 37 °C was used, which increased the fraction of soluble protein to about 40% for tmtmTrpB1 and about 10% for tmtmTrpB2 (33). Harvesting of cells, cell lysis, incubation with benzozene, heat precipitation of host proteins, and anion exchange chromatography were performed as described for tmTrpA, with the exception that the buffer solutions were supplemented with 40 μM pyridoxal 5′-phosphate (PLP). Both tmtmTrpB proteins eluted from the Mono Q column at about 150–200 mM potassium chloride. Fractions containing either tmtmTrpB protein were pooled, concentrated using Centriclon-10 devices, and loaded on a gel filtration column (Superdex 75, HiLoad 26/60, Amersham Biosciences, Inc.) equilibrated with 50 mM potassium phosphate at pH 7.5, containing 300 mM potassium chloride and 40 μM PLP. The tmtmTrpB proteins, which eluted with a purity above 95% as judged from SDS-PAGE, were shock frozen in liquid nitrogen at concentrations of 4 mg/ml (tmtmTrpB1) and 1.5 mg/ml (tmtmTrpB2). From 1 liter of cell culture, 16 mg of tmtmTrpB1 and 6 mg of tmtmTrpB2 were obtained.

The tmgapdh gene cloned into the plasmid pKM1 was expressed using E. coli BL21(DE3) cells (34). The cells were grown at 37 °C in 1 liter of LB medium supplemented with 150 μg/ml ampicillin, inducted with 1 mM isopropyl-1-thio-β-D-galactopyranoside at $A_600 = 0.6$, and incubated overnight. Harvesting of cells, cell lysis, and incubation with benzozene were performed in a way similar to that described for tmTrpA, but a 50 mM EPPS buffer at pH 7.5 was used instead of 10 mM potassium phosphate. After heat precipitation of host proteins at 75 °C for 30 min, tmgapdh was pure to 90%. The protein was dialyzed against 10 mM EPPS buffer at pH 7.5, containing 10 mM potassium chloride, concentrated to 5.2 mg/ml using Centriclon-10 devices, and shock frozen in liquid nitrogen. From 1 liter of cell culture, 14 mg of tmgapdh was obtained.

![Fig. 1. Reactions catalyzed by the [TrpA-TrpB]$_2$ complex and its TrpA and TrpB subunits.](image)
**RESULTS**

**The Genomes of Many Hyperthermophiles Contain Two Different trpB Genes**

The genes trpA and trpB1 of the hyperthermophilic bacterium *T. maritima* are adjacent in the *trp* operon (8). The sequencing of the whole genome of *T. maritima* (31) identified a gene outside of the *trp* operon, which has significant sequence similarity to trpB1 and was designated trpB2. It is likely that the trpB2 gene is expressed in *T. maritima* because the upstream region on the genome contains consensus sequences that are typical of bacterial promoters and ribosome binding sites (data not shown). A database search revealed that trpB2 genes are also present in the genomes of most of the other investigated hyperthermophilic Bacteria and Archaea but are generally absent from the genomes of mesophiles. A phylogenetic tree based on amino acid sequence comparisons shows that TrpB1 and TrpB2 proteins form two separate groups (Fig. 2). Within the two groups, proteins display sequence identities of about 60%, whereas between members from different groups the identities are only about 30%. Most hyperthermophiles contain one TrpB1 and one TrpB2 protein; others, for example *Sulfolobus solfataricus*, possess two different TrpB2 variants but lack TrpB1. Fig. 3a presents the amino acid sequence alignment of the two TrpB variants from *T. maritima*, tmTrpB1 and tmTrpB2, which show an overall identity of 38%. It is evident that those amino acids, which are conserved both
in the TrpB1 and TrpB2 sequences, cluster at the putative active sites. In contrast, amino acids that are conserved in only one of the two TrpB groups are distributed along the sequences. The major differences between the two proteins are a long N-terminal extension and two shorter insertions in tmTrpB2, which are located in regions where tmTrpB1 interacts with tmTrpA, as deduced from the structure of Thermotoga maritima tryptophan synthase (Fig. 3b).

Production and Purification of tmTrpA, tmTrpB1, and tmTrpB2

The tmtrpA, tmtrpB1, and tmtrpB2 genes were cloned into different pET vectors and expressed heterologously in E. coli BL21(DE3) cells (44). tmTrpA could be produced in soluble form at 37 °C, but tmTrpB1 and tmTrpB2 had to be expressed at 20 °C to suppress in part the formation of insoluble aggregates (33). The resulting thermostable tmTrpA, tmTrpB1, and tmTrpB2 proteins were purified from the soluble fraction of the cell extract, using a heat step to remove thermolabile host proteins followed by ion exchange chromatography. The three proteins are homogeneous species, yielding sedimentation equilibrium runs confirms that separated tmTrpA exists mainly as an a-subunit and shows that both tmTrpB1 and tmTrpB2 are b2-dimers. Runs that were performed with a mixture of tmTrpA and tmTrpB1 show that they form an aββ complex, as observed for other investigated tryptophan synthases (6, 45). In accordance with analytical gel filtration, analytical ultracentrifugation detected no complex formation between tmTrpA and tmTrpB2.

Association States and Complex formation of tmTrpA, tmTrpB1, and tmTrpB2

Analytical gel filtration on a calibrated Superdex 75 HR column was used to test whether tmTrpB1 and tmTrpB2 form a complex with tmTrpA at 25 °C. The results are summarized in Fig. 4. Separately, tmTrpA, tmTrpB1, and tmTrpB2 elute as well defined peaks. When tmTrpB1 is mixed with a molar excess of tmTrpA, the tmTrpB1 peak is replaced by a new and faster elution peak, which represents a complex of tmTrpA and tmTrpB1 (Fig. 4a). In contrast, the elution profile of a mixture of tmTrpA and tmTrpB2 excludes any significant complex formation between these proteins (Fig. 4b). The elution time of separated tmTrpA corresponds to a molecular mass of 26.8 kDa, comparing well with the calculated molecular mass for the monomer (26.7 kDa). The elution times of tmTrpB1 and tmTrpB2, however, correspond to molecular masses of 49.4 and 61.7 kDa, respectively, which are between the calculated molecular masses for the respective monomers (42.9 and 46.4 kDa) and homodimers (85.8 and 92.8 kDa). Analytical ultracentrifugation was therefore performed to clarify the association states of tmTrpB1 and tmTrpB2 and to assess the stoichiometry of the complex between tmTrpA and tmTrpB1 (Table I). Sedimentation velocity runs showed that the separated proteins are homogeneous species, yielding sedimentation coefficients values of 2.8 for a subunit of tmTrpA and 5.4 for both tmTrpB1 and tmTrpB2. The analysis of sedimentation equilibrium runs confirms that separated tmTrpA exists mainly as an a-monomer and shows that both tmTrpB1 and tmTrpB2 are b2-dimers. Runs that were performed with a mixture of tmTrpA and tmTrpB1 show that they form an aββ complex, as observed for other investigated tryptophan synthases (6, 45).
drawn after different time intervals were chilled on ice, and their residual catalytic activities were measured at 60 °C. For all proteins, a time-dependent monoeexponential decay of the catalytic activity was observed. The measured half-lives were 125 min for tmTrpA, 320 min for both tmTrpB1 and tmTrpB2, and 400 min for the αββα complex. These results suggest that all proteins are very thermostable and that complex formation further stabilizes both tmTrpA and tmTrpB1.

Steady-state Enzyme Kinetics

The catalytic activities at 80 °C of separated tmTrpA (A-reaction), separated tmTrpB1 and tmTrpB2 (B-reaction), and of the αββα complex of tmTrpA and tmTrpB1 (A-reaction, B-reaction, and AB-reaction, Fig. 1) were determined under steady-state conditions.

A-reaction—Table II shows that the catalytic efficiency kcat/Km of tmTrpA is increased about 270-fold by complex formation with tmTrpB1 because of an increase of kcat and a decrease of Km. The strength of the activation of tmTrpA by tmTrpB1 increases with temperature from 30 to 60 °C, yielding a linear relationship in the Arrhenius plot (data not shown). As a consequence, tmTrpA is activated at 80 °C by tmTrpB1 to a similar extent as ecTrpA is activated by ecTrpB at 25 °C (Table II).

Analytical gel filtration and analytical ultracentrifugation performed at room temperature suggest that tmTrpA and tmTrpB2 do not form a complex (Fig. 4b and Table I). To test whether such a complex might be formed at higher temperatures, the catalytic activity of tmTrpA was measured at 60 °C in the presence and absence of an equimolar concentration of tmTrpB2. Because the presence of tmTrpB2 did not affect the tmTrpA activity, the formation of a functional complex between tmTrpA and tmTrpB2 can be excluded both at 25 and at 60 °C.

B-reaction—The B-activities of separated tmTrpB1(β2) and of tmTrpB1 in the [tmTrpA tmTrpB1]α complex are compared in Table III, which shows that tmTrpB1 is activated by tmTrpA. Whereas kcat and Km are only slightly improved by complex formation, Kcat/Km is significantly decreased, and, as a result, kcat/Km is increased 65-fold. Remarkably, by complex formation with the corresponding TrpA subunits, tmTrpB1 at 80 °C and ecTrpB at 25 °C are activated to a similar extent. This result means that at the corresponding physiological temperatures, the mutual activation of the α- and the β-subunits in the αββα complex is comparably strong in the enzymes from Thermotoga maritima and E. coli. This finding supports the concept of “corresponding states,” which postulates that mesophilic and
hypertermophilic enzymes are comparably flexible, stable, and active at their respective physiological temperatures (3, 46–48).

Activity measurements with tmTrpB2 (β₂) showed the absence of significant activation by tmTrpA, as expected (data not shown). The \( k_{cat}/K_m^{IND} \) of separated tmTrpB2 is similar to that of complexed tmTrpB1 (Table IV) because both \( k_{cat} \) and \( K_m^{IND} \) of tmTrpB2 are decreased by about 1 order of magnitude compared with tmTrpB1. This result suggests that tmTrpB2 binds indole much more tightly than tmTrpB1 but converts it more slowly to tryptophan. In contrast, the \( k_{cat}/K_m^{scc} \) of separated tmTrpB2 is much lower compared with complexed tmTrpB1 because \( k_{cat} \) is decreased, and \( K_m^{scc} \) is increased by about 1 order of magnitude.

**AB-reaction**—In the AB-reaction (Fig. 1), the \( \alphaβββ \) complex catalyzes the conversion of IGP and serine to tryptophan. Table V summarizes the steady-state enzyme kinetic constants \( k_{cat}, K_m^{GP}, K_m^{GP}, K_m^{scc} \) for both the [tmTrpAttmTrpB1] complex at 80 °C and the *E. coli* [ecTrpAecTrpB] complex at 25 °C. For comparison, the values of the A- and B-reaction of the complex (see Tables II and III) are also listed. The \( k_{cat} \) value of the AB-reaction in the *E. coli* complex is much higher than the \( k_{cat} \) of the A-reaction, but about 10-fold lower than the \( k_{cat} \) of the B-reaction. The rate of the AB-reaction of the tryptophan synthase complex from *E. coli* thus appears to be limited by the rate of the A-reaction. In contrast, in the *T. maritima* tryptophan synthase complex the \( k_{cat} \) values of the \( A, \) the \( B, \) and the \( AB \)-reactions are similar, suggesting that both partial reactions influence the rate of the overall reaction. Moreover, in the *T. maritima* tryptophan synthase both \( K_m^{GP} \) and \( K_m^{scc} \) are much lower in the AB-reaction compared with the A- and B-reactions, respectively. In contrast, in the *E. coli* enzyme both \( K_m^{GP} \) and \( K_m^{scc} \) are similar in the overall and in the individual reactions. The mechanistic basis of these differences between the *T. maritima* and *E. coli* tryptophan synthases has yet to be elucidated.

### DISCUSSION

The phylogenetic tree depicted in Fig. 2 shows that TrpB1 and TrpB2 proteins form two separate groups, which presumably evolved independently from each other after an early gene duplication event. In separated form both tmTrpB1 and tmTrpB2 are homodimers (Table I) and have similar amino acid sequences at their putative active sites containing all residues that are catalytically essential for the TrpB reaction (Fig. 3a and Ref. 17). In accordance with these observations, both tmTrpB variants catalyze the B-reaction with high efficiency at 80 °C (Table IV). Moreover, the tmTrpB1 gene is part of the trp operon of *T. maritima* (8), and the upstream sequence of the tmtrpB2 gene contains a putative promoter and a ribosome binding site (31). It therefore has to be assumed that both tmTrpB1 and tmTrpB2 are produced in *T. maritima* and play a functional role in tryptophan biosynthesis.

The most significant difference between the two tmTrpB proteins is that tmTrpA forms a functional complex only with tmTrpB1 (Tables I–III and Fig. 4). Sequence alignment shows that tmTrpB2 contains additional amino acids compared with tmTrpB1 (Fig. 3a), which are inserted at sites where TrpA and TrpB interact with each other (Fig. 3b). It appears therefore that the binding of tmTrpA to tmTrpB2 is prevented by steric hindrance caused by these insertions. Remarkably, maize contains two enzymes of secondary metabolism which show significant sequence similarities to TrpA and catalyze the production of indole from IGP efficiently in the absence of TrpB (49). Sequence deviations of these TrpA homologs have been identified in the intermolecular interaction domain, which might prevent their complex formation with TrpB.

Whereas tmTrpB1 is likely to receive indole from tmTrpA by intermolecular channeing (22, 23), the cellular source of indole to be used by tmTrpB2 is not evident. In *E. coli*, the cleavage of tryptophan as the sole carbon source to pyruvate and indole is the main source for indole (50). This reaction is catalyzed by the enzyme tryptophanase, but no gene with significant sequence similarity to known tryptophanase genes appears to be present on the genome of *T. maritima* (31). Alternatively, at 80 °C IGP might be spontaneously degraded with a significant rate into GA3P and indole, which could then be used by tmTrpB2 for tryptophan biosynthesis. However, at 80 °C no conversion of IGP into tryptophan could be detected in the presence of tmTrpB2 (or tmTrpB1) and serine (data not shown). It has been shown for the *E. coli* tryptophan synthase that less than 1% of the indole produced by ecTrpA is released into the solvent at 25 °C instead of being channeled to ecTrpB (51, 52). It is possible, however, that a larger fraction of indole leaks from the channel connecting tmTrpA with tmTrpB1 at the high physiological temperature of *T. maritima*. Consistent with this hypothesis, tmTrpB2, because of its extremely low \( k_{cat} \) (Table IV), would be ideally suited to scavenge the liberated indole and to prevent it from penetrating through the cytoplasmic membrane (Fig. 5). The putative function of tmTrpB2 as an indole salvage protein is supported by the observation that trpB2 genes are only found in hyperthermophiles (Fig. 2), the high physiological temperatures of which promote passive diffusion of metabolites significantly. Some hyperthermophiles such as *S. solfataricus* possess two trpB2 genes but lack a trpB1 gene (Fig. 2), and it remains to be tested whether one of the two TrpB2 proteins forms an \( \alphaβββ \) tryptophan synthase complex with TrpA. In mesophilic organisms the trpB2 gene might have been lost in the course of evolution because
of the lack of selective pressure, provided that life began in boiling water (53).

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Table IV

| Protein* | $k_{cat}$ | $K_{M}^{IND}$ | $k_{cat}/K_{M}^{IND}$ | $k_{cat}$ | $K_{cat}^{SER}$ | $k_{cat}/K_{cat}^{SER}$ |
|----------|-----------|---------------|----------------------|-----------|----------------|-----------------------|
| [tmTrpB2]$_2$ | 0.46 | <0.77 | >0.6 | 0.44 | 50.2 | 8.7 \times 10^{-3} |
| [tmTrpA*tmTrpB1]$_2$ | 10 | 25 | 0.4 | 8 | 3.7 | 2.2 |

* 100 mM potassium phosphate buffer, pH 7.5, 180 mM KCl, 40 μM PLP, 0.2–0.4 μM tmTrpB2 or 0.23 μM [tmTrpA*tmTrpB1]$_2$ was used. The given values for $K_{M}^{IND}$ and $k_{cat}/K_{M}^{IND}$ are upper and lower limits, respectively.

Table V

| $k_{cat}$ | $k_{cat}^{GP}$ | $k_{cat}/K_{cat}^{GP}$ | $k_{cat}$ | $K_{cat}^{SER}$ | $k_{cat}/K_{cat}^{SER}$ |
|-----------|---------------|----------------------|-----------|----------------|-----------------------|
| T. maritima (80 °C) | | | | | |
| A-reaction | 5.6 | 0.19 | 29.5 | 8 | 3.7 | 2.2 |
| B-reaction | 5.6 | 0.19 | 29.5 | 8 | 3.7 | 2.2 |
| AB-reaction | 0.076 | 0.14 | 0.48 | 5.3 | 0.43 | 12 |

| E. coli (25 °C) | | | | | |
| A-reaction | 1.4 | 0.069 | 20 | 1.4 | 0.34 | 4.1 |

* 100 mM EPPS, pH 7.5, 180 mM KCl, 40 μM PLP, 20 mM arsenate, 6 mM NAD$^+$, 1 mM [tmTrpA*tmTrpB1]$_2$, molar excess of tmGAPDH.

** 100 mM potassium phosphate, pH 7.5, 180 mM KCl, 40 μM PLP, 0.23 μM [tmTrpA*tmTrpB1]$_2$, molar excess of tmGAPDH.

† 100 mM potassium phosphate, pH 7.5, 180 mM KCl, 40 μM PLP, 20 mM arsenate, 6 mM NAD$^+$, 0.4–0.5 μM [tmTrpA*tmTrpB1]$_2$, molar excess of tmGAPDH.

‡ 100 mM potassium phosphate, pH 7.6, 40 μM PLP, 5 mM EDTA, 0.2 mM DTT; concentrations of [ecTrpA*ecTrpB1]$_2$: 16.7 μM for the A-reaction, 0.16 μM for the B-reaction, 8.35 μM for the AB-reaction. Data were taken from Ref. 43.

Fig. 5. Putative functions of TrpB1 and TrpB2 enzymes in hyperthermophiles. TrpB1 forms a functional complex with TrpA, in which IGP and Ser are converted to Trp (circled 1). TrpB2 could bind and convert free IND (circled 2), which might either leak out from the channel between TrpA and TrpB1 (circled 2a) or be produced by the spontaneous degradation of IGP (circled 2b).

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