The *glnA* gene of the extremely thermophilic eubacterium *Thermotoga maritima*: cloning, primary structure, and expression in *Escherichia coli*

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The structural gene (*glnA*) encoding the glutamine synthetase (GS) of the extremely thermophilic eubacterium *Thermotoga maritima* has been cloned on a 6.0 kb *HindIII* DNA fragment. Sequencing of the region containing the *glnA* gene (1444 bp) showed an ORF encoding a polypeptide (439 residues) with an estimated mass of 50088 Da, which shared significant homology with the GSI sequences of other Bacteria (*Escherichia coli*, *Bacillus subtilis*) and Archaea (*Pyrococcus woesei*, *Sulfolobus solfataricus*). The *T. maritima* *glnA* gene was expressed in *E. coli*, as shown by the ability to complement a *glnA* lesion in the glutamine-auxotrophic strain ET8051. The recombinant GS has been partially characterized with respect to the temperature dependence of enzyme activity, molecular mass and mode of regulation. The molecular mass of the *Thermotoga* GS (590000 Da), estimated by gel filtration, was compatible with a dodecameric composition for the holoenzyme, as expected for a glutamine synthetase of the GSI type. Comparison of the amino acid sequence of *T. maritima* GS with those from thermophilic and mesophilic micro-organisms failed to detect any obvious features directly related to thermal stability.

### Introduction

With the discovery of extremely thermophilic and hyperthermophilic Archaea and Bacteria, the upper limit of temperature compatible with life has been raised above 100 °C (Huber *et al.*, 1986; Zillig *et al.*, 1990; Pledger & Baross, 1991). The strategies whereby proteins from these micro-organisms remain stable at temperatures equal to or higher than the boiling point of water are still largely undefined. It is also unclear how thermophilic enzymes succeed in combining the structural rigidity required for stabilization with the flexibility required for catalytic function. Because proteins from thermophilic micro-organisms retain their heat-resistance upon expression in mesophilic hosts (Love & Streiff, 1987; Love *et al.*, 1988; Tiboni *et al.*, 1989), their ability to withstand high temperatures appears to be determined only by features of primary structure. Therefore, sequence comparison of homologous proteins from mesophilic and thermophilic organisms may enable sequence traits critical for thermal stabilization to be identified (Fabry *et al.*, 1989; Schultes *et al.*, 1990).

In this work on the extremely thermophilic eubacterium *Thermotoga maritima*, attention has been focused on the key enzyme for ammonia assimilation glutamine synthetase (GS). This protein has been extensively investigated at the biochemical and molecular levels in a wide range of phylogenetically diverse bacterial/archaeal and eukaryotic species (Streicher & Tyler, 1980; Bhatnagar *et al.*, 1986; Cullimore & Bennet, 1988). In both Bacteria and Archaea, GS is an oligomeric enzyme, termed GSI, consisting of twelve identical subunits each with a molecular mass of 50–55 kDa. The only known exception is the anaerobic bacterium *Bacteroides fragilis*, whose GS is composed of six subunits each of 83 kDa (Hill *et al.*, 1989). The eukaryotic GS, termed GSII, differs from the bacterial and archaeal enzymes in comprising eight subunits, each of 45–48 kDa, whose primary sequence has little similarity to those of the

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Abbreviations: EF, elongation factor; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GS, glutamine synthase; SVP, snake venom phosphodiesterase.

The nucleotide sequence data reported in this paper have been submitted to GenBank and have been assigned the accession number X60160.

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bacterial and archael enzymes. Interestingly, members of *Rhizobium* and *Streptomyces* groups harbour both GSI (bacterial) and GSII (eukaryotic) enzymes (Carlson & Chelm, 1986; Shatters & Kahn, 1989; Behrmann et al., 1990; Kumada et al., 1990). Although bacterial GSs differ substantially from their eukaryotic homologues, it is possible to identify five highly conserved regions, all of which are associated with putative active sites (Almassy et al., 1986; Rawlings et al., 1987).

Glutamine synthetases from different organisms also differ in their mode of regulation. The enzyme synthesizes glutamine from ammonia and glutamate, which in turn is the nitrogen source for the biosynthesis of a range of metabolites, some of which act as feedback inhibitors to GS turn off. The enzyme synthesis in *E. coli* (enteric bacteria, *Streptomyces*) and *A. clypeatus* (large and small scale) was isolated and purified according to Maniatis et al., 1982. DNA sequencing.

**Methods**

**Bacterial strains and plasmids.** *T. maritima* MSB8 (DSM 3109) cells, grown as described by Huber et al. (1986), were kindly supplied by R. Huber. Plasmid pSpC2 (Riccardi et al., 1985), containing the glnA gene of *Spirulina platensis*, was used to prepare the heterologous probe and was also used as the control in complementation experiments. Plasmids pBR322 (Bolivar et al., 1977) and pUC19 (Vieira & Messing, 1982) were used as the vectors and *E. coli* HB101 as the host. *E. coli* ET8051 [ΔargA-glnA] harbours rbs nai-l] kindly provided by D. MacNeil, Merck, Sharp & Dohme Research Laboratories, Rahway, USA, was used in complementation experiments.

**Recombinant DNA techniques.** Chromosomal DNA from *T. maritima* was prepared as described by Blin & Stafford (1976). Plasmid DNA (large and small scale) was isolated and purified according to Maniatis et al. (1982). DNA fragments were recovered from low-melting agarose gels as reported by Higuchi et al. (1981). Transformation experiments were carried out as described by Dagert & Ehrlich (1979). Hybridization experiments (Southern, 1975) were performed at 37 °C in the presence of 35% formamide.

**DNA sequencing.** The inserts of plasmids pTM15 and pTM16 were digested with different restriction enzymes, and fragments ranging from 100 to 650 bp were subcloned in the polylinker cloning sites of pUC19. Sequencing of both DNA strands was done by the dideoxy chain-termination method of Sanger et al. (1977) with overlapping templates. The DNA was radiolabelled with [35S]dATP (deoxy-adenosine 5'-α-[35S]chitriphosphate) (>1000 Ci mmol⁻¹) (>3.7 x 10^3 Bq mmol⁻¹) and primed as specified by the manufacturer using a T7 Sequencing kit (Pharmacia).

Analysis of the DNA sequence was performed using a Macintosh IIcx computer and the DNA Strider 1.0 program (Marcck, 1988). Alignment of protein sequences was performed using an IBM PC and the PRTALN and DM subroutines of the NIH-MBUG program (Wilbur & Lipman, 1983).

**Expression studies.** Plasmids pTM16, pSpC2 and pBR322 were used to transform the *E. coli* strain ET8051. Transformants, selected on LB plates supplemented with 50 μg ml⁻¹ ampicillin and 0.2% glucose, were replicated on plates of M9 minimal medium and M9 minimal medium with 0.2% glucose and incubated at 37 °C for 2-4 d. For biochemical analysis, cells were grown at 37 °C in M9 minimal medium with constant shaking to OD₆₅₀ ≈ 0.7. The cells were harvested by centrifugation and washed once with extraction buffer (100 mM-Tris/HCl, pH 7.4; 5 mM-β-mercaptoethanol).

**Enzyme assays.** Cell-free extracts of *E. coli* and *T. maritima* were prepared at 4 °C by grinding (in a mortar) 1 g of cells with 2 g of alumina powder (Sigma). The paste was resuspended in 5 ml of extraction buffer and centrifuged at 20000 g for 30 min. The supernatant (crude extract) was then desalted through a column (1.6 x 10 cm) of Bio-Gel P30G (Biorad) equilibrated with the extraction buffer. For GS transferase activity assays, crude extracts were buffer-exchanged by using a column equilibrated with 50 mM-imidazole/HCl buffer (pH 7.4) containing 1 mM-β-mercaptoethanol. Glutamine synthetase was assayed by both hemoisynthetic and transferase activities as described by Haizer & Moses (1978) and Stadman et al. (1979). The synthetic assay was used routinely, because it provides a better estimate of the catalytic rate of the enzyme under physiological conditions; appropriate controls in the presence of 100 mM-proline were performed in order to avoid interference by γ-glutamyl kinase activity. One unit (U) of GS activity is defined as the amount of enzyme catalysing the production of 1 nmol s⁻¹ of γ-glutamylhydroxamate (nmk). The pH dependence of the transferase activity was determined in the presence of 200 mM-imidazole buffer over the pH range 6.0-8.5. The occurrence of enzyme deamination was tested in imidazole buffer assay mixture (Stadman et al., 1979) by monitoring the shift in metal ion specificity after incubation of the enzyme with snake venom phosphodiesterase (type I from *Crotalus adamanteus* (Sigma) (Shapiro et al. 1967). Protein concentrations were determined as described by Bradford (1976) using bovine serum albumin as the standard.

**Relative molecular mass determination.** Native molecular mass was estimated by gel filtration using a Sephacryl S300 HR (Pharmacia) column (1 x 6 x 82 cm) calibrated with the appropriate molecular mass standards. The column was equilibrated and eluted with ice-cold extraction buffer. Samples (2 ml) were applied, and elution was carried out at a flow-rate of 12 ml h⁻¹, while collecting 1 ml fractions.

**Results**

**Cloning of the *T. maritima* glnA gene**

To identify the *T. maritima* glnA gene, genomic DNA digested with different restriction enzymes was probed with the glnA gene from *S. platensis*. Among the digests exhibiting a single hybridization signal with
the cyanobacterial probe, a 3-5 kb EcoRI fragment was selected for study.

A size-selected EcoRI genomic library of *T. maritima* DNA (3.0-4.3 kb EcoRI fragments) was constructed in pBR322 using *E. coli* HB101 as the host. Since the signal produced by the *T. maritima glnA* gene was as weak as that generated by the *glnA* gene of the host, it was impossible to carry out colony hybridizations. Thus, recombinant plasmids harbouring sequences homologous to the probe were selected by analysing the inserts. Plasmid DNA, prepared from 30 pools of 18 recombinant clones each, was digested with EcoRI. The resultant fragments were separated by gel electrophoresis, and hybridized with the *S. platensis glnA* probe. The individual transformants from two positive pools were analysed for plasmid content, and a recombinant plasmid, containing a 3.5 kb EcoRI fragment (designated pTM15), was isolated. The restriction map of pTM15 was determined and the gene region was localized to a 1.0 kb HaeIII/EcoRI fragment that included the unique EcoRV site (Fig. 1). The HaeIII/EcoRI fragment from pTM15 was subcloned into pUC19 and sequenced, following the strategy outlined in Fig. 1.

Sequence analysis revealed the presence of an incomplete ORF encoding a polypeptide of 328 amino acid residues highly homologous to the N-terminal portion of eubacterial GSs. Thus, in order to localize the entire *glnA* gene, genomic DNA of *T. maritima* was digested with a range of restriction enzymes, and probed with the HaeIII/EcoRI fragment of pTM15 and the HindIII digestion, which showed hybridization with a 6.0 kb fragment, was selected.

Colony hybridization of the partial HindIII genomic library allowed the isolation of two recombinant plasmids with an insert homologous to the probe. The restriction map of one of these plasmids, designated pTM16, demonstrated the presence, within the cloned fragment, of the entire insert of pTM15 (Fig. 1).

**Nucleotide sequence of the *T. maritima glnA* structural gene**

The complete nucleotide sequence of the 1444 bp *T. maritima* DNA fragment containing the *glnA* gene was determined following the strategy outlined in Fig. 1. Analysis of the sequence (Fig. 2) revealed the presence of
A. M. Sanangelantoni and others

1

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<th>Function</th>
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</tr>
</tbody>
</table>
an ORF of 1320 bp preceded by a ribosome binding site (Shine & Dalgarno, 1976) (GAGGAGG) 7 bp upstream from the putative start codon. The derived polypeptide (439 amino acid residues, with a predicted mass of 50088 Da) is formally similar to GS subunits from Bacillus subtilis (Nakano et al., 1989), Clostridium acetobutylicum (Janssen et al., 1988) and Methanococcus voltae (Possot et al., 1989), but slightly shorter than GS subunits from E. coli (Colombo & Villafranca, 1986) and other bacteria (Toukdarian et al., 1990). The identity of the 1320 bp ORF with the T. maritima glnA gene was unambiguously established by the alignment of the derived polypeptide with the GS proteins of two extremely thermophilic archaeotes (Pyrococcus woesei and Sulfolobus solfataricus) and with those of the mesophilic bacteria B. subtilis and E. coli (Fig. 3). The T. maritima enzyme shares considerable sequence similarity with those of P. woesei (48.3%) and B. subtilis (53.7%) and significant similarity with those of E. coli (32.8%) and S. solfataricus (37.5%). Fig. 3 also shows that the five regions (marked by asterisks) associated with active sites of the enzyme (Rawlings et al., 1987) are rather stringently conserved.

The T. maritima enzyme shares with P. woesei, M. voltae, C. acetobutylicum and B. subtilis a deletion of 26 amino acids from positions 145 to 171. This region has been identified as a large protease-sensitive loop (Almassy et al., 1986). No thermophilic-specific features are detectable that distinguish the T. maritima, S. solfataricus and P. woesei GS sequences from their mesophilic counterparts, except for the presence of a tyrosine residue at position 372, which is replaced by a valine in the mesophilic sequences. The relevance of this substitution with respect to thermal stability, however, is uncertain.

The amino acid sequence around Tyr residue 411 (Fig. 3) is of particular interest, because this residue has been identified in E. coli as the site of GS adenylation (Shapiro & Stadtman, 1968). The region flanking this site (18 amino acids) is highly conserved in all GSs whose activity is regulated by adenylation (Janssen et al., 1988). Although T. maritima GS has a Tyr residue at the correct position, only 4 out of the 18 nearby residues are identical to those present in E. coli, suggesting that the enzyme activity is not modulated by an adenylation-deadenylation mechanism.

Expression of the T. maritima glnA gene in E. coli

The E. coli glutamine auxotroph ET8051 carrying a deletion for the glutamine synthetase gene was transformed with the recombinant plasmids pTM16 and pSpC2 carrying, respectively, the glnA gene of T. maritima and of S. platensis (a mesophilic bacterium) and with the vector pBR322 as control. Transformants selected on LB medium containing ampicillin were replicated on M9 minimal medium containing 0.1% NH4Cl as the sole nitrogen source. After 48 h incubation at 37 °C, only the transformants harbouring pSpC2 were able to grow on minimal medium; transformants harbouring the plasmid pTM16 exhibited appreciable growth only after four days. Under identical conditions, no growth was observed of transformants harbouring the vector pBR322 alone.

These results indicate that the glnA gene of T. maritima is able to complement the glnA lesion present in the E. coli ET8051 mutant, although less efficiently than the glnA gene from S. platensis. The long time period (96 h) required for the growth of cells complemented by pTM16 might reflect low activity of the thermophilic T. maritima GS enzyme at the host growth temperature (37 °C).

To investigate this point, GS activities were determined at increasing temperatures by the hemibiosynthetic or the transferase assays, using cell extracts prepared from recombinant strains grown in the presence or absence of 0.2% glutamine (Table 1). The former assay estimates the rate of glutamate conversion to γ-glutamyl hydroxamate when ammonia is replaced by hydroxylamine, whereas the latter evaluates the reverse reaction from glutamine and ADP. As Table 1 shows, at 37 °C extracts from E. coli transformed with plasmid pTM16 exhibit considerably less GS activity than comparable extracts prepared from E. coli cells transformed with plasmids carrying the S. platensis glnA gene. Conversely, at a temperature (90 °C) close to the physiological optimum for T. maritima growth, only the extracts from E. coli cells carrying the pTM16 exhibit GS activity.

Table 1. GS specific activities in cell-free extracts from recombinant strains

<table>
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<tr>
<th>E. coli strain</th>
<th>glnA allele</th>
<th>Synthetic assay</th>
<th>Transferase assay</th>
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<td></td>
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<td>37°C</td>
<td>70°C</td>
</tr>
<tr>
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<td>ND</td>
</tr>
<tr>
<td>ET8051 pSpC2</td>
<td>S. platensis</td>
<td>2.50</td>
<td>17.67</td>
</tr>
</tbody>
</table>

* Not determined.
ND, not determined.

Table 1. GS specific activities in cell-free extracts from recombinant strains

GS activity is expressed as nkat (mg protein)⁻¹. Results are means of at least three independent determinations.

Fig. 2. Complete nucleotide sequence of the T. maritima glnA gene. The deduced amino acid sequence is shown below the DNA sequence. The Shine-Dalgarno (SD) sequence is underlined.
Fig. 3. Alignment of the *T. maritima* (Tm) GS amino acid sequence with the sequence of GS enzymes from: Pw, *P. woesei* (our laboratory, in preparation); Ss, *S. solfataricus* (Sanangelantoni et al., 1990); Bs, *B. subtilis* (Nakano et al., 1989); and Ec, *E. coli* (Colombo & Villafranca, 1986). The five conserved regions are underlined with asterisks. The Tyr residue adenylated in *E. coli* is indicated by an asterisk. † indicates the Tyr residue present in the thermophiles and substituted in the mesophiles by a Val residue.
The results in Table 1 also show that the GS activity of E. coli ET8051 cells complemented with pTM16 is not significantly affected by the presence or absence of exogenous glutamine during cell growth. Since the host strain also carries a deletion for genes involved in the transcriptional regulation of the gene (Reitzer & Magasanik, 1987), the insensitivity of GS to glutamine levels during cell growth indicates that no genes equivalent to ntr are present on the cloned fragment.

Molecular mass and temperature dependence of T. maritima GS expressed in E. coli

To determine whether the thermophilic GS subunits are correctly assembled during their synthesis in the mesophilic host (E. coli), the molecular mass of the recombinant holoenzyme was compared with that of authentic T. maritima enzyme. To this aim, crude extracts prepared from E. coli cells harbouring the recombinant plasmid (pTM16) and from T. maritima cells were chromatographed on a gel filtration column calibrated previously with appropriate molecular mass standards, and GS was identified by monitoring the synthetic activity of the effluent fractions. Both the recombinant and the authentic enzymes eluted as a single peak, with a retention volume indicating a molecular mass of about 590000 Da (Fig. 4). Since sequence data indicated a subunit of 50088 Da, the molecular mass of the holoenzyme is consistent with a dodecameric composition of the native protein.

Because the recombinant GS is synthesized at a temperature 50 °C lower than the physiological optimum for T. maritima growth, it seemed possible that its folding pattern might differ more or less substantially from that of authentic T. maritima GS synthesized at 87 °C. This has been shown to occur in the case of T. maritima elongation factor Tu (EF-Tu) expressed in E. coli, which requires thermal activation for GDP-binding activity (Tiboni et al., 1989). This possibility is ruled out by the results in Fig. 5, which illustrates the temperature dependence of the recombinant GS activity and the corresponding Arrhenius plot. As the graphs show, the velocity of the forward reaction increases with a Q10 of about 2 over a broad temperature range (Fig. 5a).

Accordingly, the Arrhenius plot does not exhibit deviations from linearity over the range 30–75 °C (Fig. 5b). This is consistent with no activation energy being required to overcome conformational barriers that hinder the achievement of the physiological folding pattern. The results in Fig. 5 also show that the T. maritima enzyme is considerably more stable than the mesophilic enzyme. In fact, no rapid inactivation of the enzyme occurs at temperatures above 90 °C (Fig. 5a). In contrast, the GSs from E. coli and S. platensis (expressed in E. coli) are completely inactivated at 75–80 °C (data not shown). From the Arrhenius plot in Fig. 5(b), the activation energy of the reaction was estimated to be 65.8 kJ mol⁻¹, a value significantly higher than that calculated for the cyanobacterial GS expressed in E. coli (49.8 kJ mol⁻¹, data not shown).

Effect of snake venom phosphodiesterase (SVP) treatment on GS transferase activity

Finally, the possible occurrence of an adenylylation-deadenylylation mechanism modulating the T. maritima GS activity was investigated. In bacterial systems, Mg2⁺ ions are known to inhibit the transferase activity of the adenylylated subunits of the enzyme (Stadtman et al., 1979). Since SVP removes adenylic residues covalently bonded to the GS subunits, pretreatment of an adenylated enzyme with SVP is expected to relieve Mg2⁺ inhibition of GS transferase activity (Shapiro et al., 1967). As Table 2 shows, Mg2⁺ ions did, in fact, exert a strong inhibition on the T. maritima GS activity in extracts of E. coli cells grown in the presence of ammonia, as might have been expected if the T. maritima enzyme was adenylylated. However, treatment of the enzyme with SVP neither increased GS activity in the presence of Mg2⁺ nor caused a significant shift in the pH-activity relationship, which still exhibited a broad
optimum around pH 7.0 (data not presented). These results seem to indicate that the *T. maritima* GS is not regulated by an adenylylation-deadenylylation system. Similar data were obtained for the *S. platensis* GS (Table 2). This conclusion is also supported by the low degree of conservation of the residues surrounding the *T. maritima* tyrosine residue which in *E. coli* acts as the adenylylation site (Fig. 3).

**Discussion**

The expression of genes encoding proteins from extreme thermophilic micro-organisms in a mesophilic host offers two significant advantages. Firstly, it overcomes the problem of low biomass yield usual with thermophilic cultures. Secondly, it allows the partial purification, in a single step, of the heterologous heat-stable proteins by selective thermal denaturation of the mesophilic host cell proteins (Tiboni *et al.*, 1989). However, during synthesis in a mesophilic organism, the thermophilic proteins may adopt a folding pattern different from the physiological one, as shown by the case of *T. maritima* EF-Tu expressed in *E. coli* (Tiboni *et al.*, 1989). On the other hand, many thermophilic proteins are able to complement defective *E. coli* mutants, implying that they are correctly folded at the host growth temperature (Branlant *et al.*, 1989; Cubellis *et al.* 1990).

Here we have shown that an *E. coli* strain auxotrophic for glutamine is able to grow, albeit slowly, on minimal medium following transformation with a plasmid carrying the *glnA* gene from the extreme thermophilic bacterium *T. maritima*. This is in accordance with *in vitro* evidence that the recombinant subunit proteins are

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**Table 2. Effect of Mg²⁺ ions on GS transferase activity before and after SVP treatment**

Cultures were grown in excess nitrogen. GS activity is expressed as nkat (mg protein)⁻¹. Results are means of two independent experiments.

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<td>HB101</td>
<td><em>E. coli</em></td>
<td>6.4</td>
<td>ND</td>
</tr>
<tr>
<td>ET8051 pTM16</td>
<td><em>T. maritima</em></td>
<td>53.2</td>
<td>34%</td>
</tr>
<tr>
<td>ET8051 pSpC2</td>
<td><em>S. platensis</em></td>
<td>222.0</td>
<td>82%</td>
</tr>
</tbody>
</table>

ND, not determined.
correctly folded and assembled into a dodecamer during synthesis in E. coli. The activation energy calculated from the Arrhenius plot of GS activity evaluated over a wide temperature spectrum is consistent with the T. maritima GS being a rather rigid molecule, and this rigidity adversely affecting catalytic rates at temperatures below the physiological optimum for T. maritima growth. At high temperature, however, the T. maritima enzyme could become sufficiently flexible to be fully active, while retaining enough rigidity to withstand heat denaturation. This would explain the low efficiency of the in vivo complementation in the mesophilic host growth conditions.

Although the GSs from different micro-organisms exhibit structural similarities, their activities are regulated by different mechanisms, such as divalent-cation-induced conformational changes, feedback inhibition by various end-products, including glutamine, and covalent modification of the enzyme subunits by an adenylation-deadenylylation system (Tyler, 1978). In general, modification of the enzyme subunits by an adenylated GS, e.g., itylated by different mechanisms, such as divalent-cation-dependent mechanisms, a conclusion also supported by the low degree of sequence conservation (4 out of 18 amino acids) around the tyrosine residue which functions as the adenyllylation site in E. coli.

An intriguing aspect of the results is the evidence that the sequence similarity between the GS of T. maritima (a bacterium) and the GS of P. woesei (an archaeote) is much greater than that between T. maritima GS and E. coli GS (two members of the same domain). The significance of such obvious discrepancy between the similarities in GS sequences and the phylogenetic placement of the organisms inferred from analysis of 16S rRNA sequence (Woese, 1987) is a matter of speculation.

As far as thermostability is concerned, a comparison of thermophilic and mesophilic GS sequences does not reveal any obvious trend towards replacement of small amino acids with bulkier and more hydrophobic ones in the thermophilic protein, as appears to be the case for GAPDHs from thermophilic and mesophilic sources (Fabry et al., 1989; Shultes et al., 1990).

It has been suggested that in thermophilic proteins, cysteine residues (susceptible to oxidation at elevated temperatures) are replaced in non-essential positions (Harris & Perham, 1968). When the thermophilic GSs were analysed for cysteine content, the number of such residues was found to be lower (2, 1, 1 respectively for Thermotoga, Pyrococcus and Sulfolobus) than that of E. coli (4 residues) and B. subtilis (3 residues) GSs. The significance of this finding, however, is questionable if one considers that the GS of Streptomyces coelicolor (a mesophile) contains a single cysteine residue (Wray & Fisher, 1988). The cysteine replacement hypothesis is also contradicted by the evidence that the number of cysteine residues in the T. maritima GAPDH (Shultes et al., 1990) does not differ significantly from that found in corresponding mesophilic enzyme. This suggests that no uniform strategy can be envisaged to explain protein adaptation to high temperatures.

The only possible signature distinguishing the GSs of the three extreme thermophiles from those of mesophiles is the presence of a tyrosine residue in place of a valine residue within the catalytically important region V of the enzyme. The relevance of this valine to tyrosine change to thermal stability will only be elucidated by site-directed mutagenesis experiments.

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References


