Affinity of ribosomal protein S8 from mesophilic and (hyper)thermophilic archaea and bacteria for 16S rRNA correlates with the growth temperatures of the organisms

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Received 9 May 2003; revised 23 June 2003; accepted 28 June 2003

First published online 25 July 2003

Edited by Horst Feldmann

Abstract The ribosomal protein S8 plays a pivotal role in the assembly of the 30S ribosomal subunit. Using filter binding assays, S8 proteins from mesophilic, and (hyper)thermophilic species of the archaeal genus *Methanococcus* and from the bacteria *Escherichia coli* and *Thermus thermophilus* were tested for their affinity to their specific 16S rRNA target site. S8 proteins from hyperthermophiles exhibit a 100-fold and S8 from thermophiles exhibit a 10-fold higher affinity than their mesophilic counterparts. Thus, there is a striking correlation of affinity of S8 proteins for their specific RNA binding site and the optimal growth temperatures of the respective organisms. The stability of individual rRNA-protein complexes might modulate the stability of the ribosome, providing a maximum of thermostability and flexibility at the growth temperature of the organism.

Key words: rRNA-ribosomal protein S8 interaction; Filter binding assay; Archaea; Mesophilic and (hyperthermophilic) *Methanococcus*; Thermostability

1. Introduction

The recent solution of ribosome structures at atomic resolution by X-ray crystallography was a big step forward in the understanding of the mechanisms of protein synthesis. Ribosomes suitable for X-ray crystallographic studies have thus far been obtained exclusively from 'extremophiles' [1], the 50S subunits of the halophilic archaeon *Haloarcula marismortui* [2] and of the extremely robust bacterium *Deinococcus radiodurans* [3], the 30S subunit alone [4] and as part of the 70S ribosome [5] from the thermophilic bacterium *Thermus thermophilus*.

To date, still very little is known about the structural features regulating the inherent thermal stability of ribosomes of thermophilic organisms. The mechanisms involved in the thermal stabilization of the ribosome have been investigated in some detail only for the hyperthermophilic archaeon *Sulfolobus solfataricus*. The individual ribosomal components (rRNAs and ribosomal proteins) are less thermally stable than the ribosome as a whole. In addition to the intrinsic stabilization of the helical rRNA domains by a higher number of G+C base pairs, a more extensive interaction of ribosomal proteins and rRNA, compared to mesophilic organisms, seems to play an essential role [6–9]. In fact, we could demonstrate that the affinity of ribosomal protein L1 from (hyper)thermophilic bacteria and archaea to the specific binding site on 23S rRNA is more than one order of magnitude higher than that of their mesophilic counterparts [10,11].

To study the difference in affinity of ribosomal proteins from mesophilic and (hyper)thermophilic organisms for their specific rRNA target sites, ribosomal protein S8 is a suitable candidate. It is a primary 16S rRNA binding protein and plays an essential role in the assembly of the central domain of 16S rRNA. The rRNA binding site of S8 is confined to a short region of helix H21 (nucleotides 588–602/636–651; *Escherichia coli* nomenclature), which contains the structural elements determining S8 binding specificity [12–15]. The comparison of the recently published structures of the bacterial TthS8-16S rRNA complex (as part of the 30S subunit of *T. thermophilus* [4]) and of the MjaS8-16S rRNA complex from the hyperthermophilic archaeon *Methanococcus jannaschii* [16] reveals that the structures of both, S8 and the target site on 16S rRNA, as well as their specific interactions are highly conserved in bacteria and archaea.

The archaeal genus *Methanococcus* comprises mesophilic species (such as *M. vannielii*,), thermophilic (*M. thermolithotrophicus*) and hyperthermophilic species (*M. jannaschii*, *M. igneus*) that grow over an unusually broad temperature range (Fig. 1). This broad range of optimal growth temperatures among phylogenetically closely related organisms has made the genus *Methanococcus* an ideal model system not only to study thermal adaptation [17], but also to investigate the strategies by which RNA binding proteins are fine-tuning the affinity for their RNA targets [10,11].

We have used filter binding assays to examine the interaction of ribosomal proteins S8 from the *Methanococcus* species mentioned above with their specific binding sites on the 16S rRNA. We discovered a significant correlation of S8 binding affinity with the optimal growth temperature of the organisms: S8 from the mesophilic *M. vannielii* (MvaS8) exhibits a rather low affinity in the micromolar range, S8 from the thermophilic *M. thermolithotrophicus* (MthS8) binds with an about 10-fold higher affinity and the S8 proteins from both hyperthermophilic organisms *M. igneus* (MigS8) and *M. jannaschii* (MjaS8) show an about 100-fold higher affinity for 16S

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Abbreviations: PMSF, phenylmethylsulfonyl fluoride; BSA, bovine serum albumin

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doi:10.1016/S0014-5793(03)00760-9

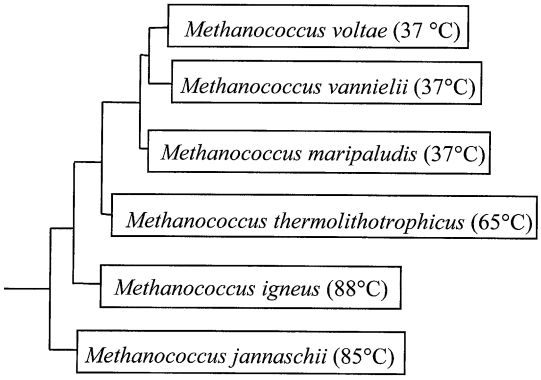

Fig. 1. Phylogeny of the genus *Methanococcus* (based on release 7.1 of the Ribosomal Data Base Project [38]). Only those species are shown for which the S8 gene sequence is available in a database. The optimal growth temperature of each species is given in parentheses.

rRNA as compared to MvaS8. Furthermore, we have re-examined the interaction of S8 from *E. coli* (EcoS8) and from the thermophilic bacterium *T. thermophilus* (TthS8) with 16S rRNA. The interaction of EcoS8 with its 16S rRNA binding site has been investigated in several studies, but the published binding constants are not consistent [12,18–21]. We could reproduce the very low K_d of 2×10^{-8} M for the TthS8-16S rRNA complex [22], but we find a lower affinity of EcoS8, similar to that published by the Zimmermann group [19–21].

2. Materials and methods

2.1. Amplification by polymerase chain reaction (PCR) and sequence determination of S8 genes from M. igneus, M. thermolithotrophicus and M. voltae

Genomic DNA of the *Methanococcus* species *M. igneus*, *M. thermolithotrophicus* and *M. voltae* was prepared as described [23] and used as template to amplify by PCR the genes encoding ribosomal protein S8. Heterologous oligonucleotides [5′-TGCAGACA(AG)-TGCTT(CT)AG(AG)GAA(AT)TAGC-3′ (S14.2fwd) and 5′-AC-(AG)TTT(CT)CAGG(AG)ATTT-3′ (L6.1rev) for the amplification of the MigS8 gene; S14.2fwd and 5′-TTT(AT)ACAACAACTTC-3′ (L6.2rev) for the MthS8 gene; 5′-ATGACAAAAGAACCATTTAA-GACGAAATATGGCCAAGG-3′ (S14.0fwd) and 5′-TTCCCTTAT-TAAAGCTGCAACTGGCAT-3′ (L6.0rev) for the MvoS8 gene] were derived from the highly conserved regions of the MvaS14 and MvaL6 genes from *M. vannielii* [24] which are located upstream and downstream, respectively, of the MvaS8 gene. For the PCR reactions, the Advantage KlenTaq Polymerase Mix (Clontech, Palo Alto, CA, USA), which provides an antibody-mediated 'hot start' and a proofreading activity, was used. PCR amplification was carried out at an annealing temperature of 45°C (MigS8 and MvoS8 gene) and 55°C (MthS8 gene), respectively. The nucleotide sequence of the resulting PCR products was determined by GATC Biotech AG (Konstanz, Germany). The sequence data are available from GenBank under accession numbers AF404828 (MigS8 gene), AF404829 (MvoS8 gene), and AF404830 (MthS8 gene).

2.2. Plasmid constructions

To clone the S8 genes from *E. coli*, *M. igneus*, *M. jannaschii*, *M. thermolithotrophicus*, and *M. vannielii* into the high-level expression vector pET11a [25], two restriction sites were created by PCR upstream and downstream of the coding sequence using genomic DNA as a template. The primer at the 5′ end of the gene contained an *Nde*I site (including the ATG start codon); the primer at the 3′ end contained a *Bam*HI site downstream of the stop codon. *Nde*I–*Bam*HI fragments carrying the respective S8 genes were inserted in the corresponding sites of the vector to give plasmids pEcoS8.4, pMigS8.4 pMjaS8.4 and pMvaS8.4. The MthS8 gene contains an internal *Nde*I site. Therefore, the 315-bp *Nde*I fragment of the PCR product was cloned into *Nde*I-digested pET11a first, then, in a second step, the 75-bp *Nde*I–*Bam*HI fragment of the PCR product was cloned into the vector containing the *Nde*I fragment in the correct orientation to give plasmid pMthS8.4. The correct sequences of the cloned PCR products were confirmed by double-stranded sequencing.

An 85-bp DNA fragment containing the S8 binding site on 16S rRNA of *E. coli* was amplified from genomic DNA generating a 5′-*Sac*I and a 3′-*Hin*dIII restriction site. The *Sac*I–*Hin*dIII fragment was cloned into vector pBluescriptKS [26] giving plasmid pEco16S-S8BS.3. Accordingly, a 220-bp DNA fragment containing the binding site for MvaS8 on 16S rRNA of *M. vannielii* was amplified using chromosomal DNA as a template and cloned into pBluescriptKS, giving plasmid pMva16S-S8BS.3. The binding site for MjaS8 on 16S rRNA of *M. jannaschii* was amplified as a 180-bp *Sac*I–*Bam*HI fragment using genomic DNA as a template. Cloning of the fragment into pBluescriptKS resulted in plasmid pMja16S-S8BS.3.

2.3. Overproduction and purification of S8 proteins

In general, S8 proteins used in this study were overproduced in *E. coli* BL21(DE3) [27] transformed with the respective plasmid. In *Methanococcus*, the codons mainly used for arginine are AGG and AGA which are the less frequently used codons in *E. coli*. To obtain maximal yields and to avoid a potential misincorporation of amino acids [28], the archaeal S8 proteins were overproduced in *E. coli* BL21(DE3) cotransformed with pUBS520, a plasmid that carries the gene for the rare $tRNA^{Arg}_{AGA/AGG}$ [29].

Overproduction of ribosomal proteins MigS8, MjaS8, MthS8, and MvaS8 was performed as described for MvaL1 by Mayer et al. [30]. Briefly, the *E. coli* host cells were harvested by centrifugation 3 h after induction with IPTG (isopropyl-β-thiogalactopyranoside), washed and resuspended in 6 ml/g (wet mass) of 50 mM Tris–HCl, pH7.8, 5 mM $MgCl_2$, 0.8 M NaCl, 18 mM β-mercaptoethanol, 0.1 mM phenylmethylsulfonyl fluoride (PMSF). Cells were passed twice through a chilled French pressure cell at 10 000 psi, *E. coli* cell debris and ribosomes were removed by two consecutive centrifugation steps (10 000 × *g* for 10 min and 100 000 × *g* for 3 h).

For the purification of MvaS8 the resulting supernatant was diluted with 13 volumes of 50 mM Na_2HPO_4, pH7, 0.2 M NaCl, 18 mM β-mercaptoethanol, 0.1 mM PMSF and heated for 40 min at 40°C. The precipitate was removed by centrifugation (10 000 × *g* for 10 min), and the supernatant containing MvaS8 was loaded onto a SP Sepharose Fast Flow (Pharmacia) column equilibrated with the dilution buffer. The protein was eluted at 4°C with a linear gradient of 0.2–1.0 M NaCl.

The purification of MjaS8 and MigS8 was performed with heat treatment of the post-ribosomal fraction. The main contaminating proteins were denatured by heating for 20 min at 70°C (MjaS8), and 75°C (MigS8) and removed by centrifugation (10 000 × *g* for 30 min). The supernatant containing MjaS8 and MigS8, respectively, was diluted into 50 mM Na_2HPO_4, pH 8, 0.2 M NaCl, 18 mM β-mercaptoethanol, 0.1 mM PMSF and purified by IEC (ion-exchange chromatography) as described for MvaS8 at pH8. For purification of MthS8, the optimal temperature for the heat treatment was 50°C. After centrifugation (10 000 × *g* for 30 min), the supernatant was applied to a Hi-Trap Butyl-Sepharose (Pharmacia) column, equilibrated with 50 mM Na_2HPO_4, 1.3 M $(NH_4)_2SO_4$, pH 8, 0.4 M NaCl, 18 mM β-mercaptoehanol. MthS8 was eluted with a linear gradient of 1.3–0 M $(NH_4)_2SO_4$. EcoS8 accumulated as insoluble inclusion bodies. For its purification, we followed the large-scale preparation protocol detailed in [20].

Fractions containing pure S8 proteins were collected and pooled. After precipitation with ammonium sulfate, the proteins were dissolved in the respective TMK buffer (described in Section 2.5.) and stored in small fractions at −20°C. To remove precipitated protein, the stock solution was centrifuged at 16 000 × *g* for 10 min before the concentration of proteins was determined by a Bio-Rad protein assay using bovine serum albumin (BSA) as a standard.

2.4. Preparation of RNA transcripts

Uniformly labelled 16S rRNA fragments of *E. coli*, *M. vannielii* and *M. jannaschii* containing the S8 binding sites were synthesized in vitro in the presence of [α-^{32}P]UTP (800 Ci/mmol; New England Nuclear Corp.) using the MAXIscript T7 Kit (Ambion Inc., Austin, TX, USA). Plasmids pMva16S-S8BS.3 and pMja16S-S8BS.3 linearized

with *Sty*I and plasmid pEco16S-S8BS.3 linearized with *Hin*dIII, respectively, were used as templates to produce run-off transcripts. The purity and integrity of the transcripts were verified by electrophoresis on 5% polyacrylamide gels containing 7 M urea.

2.5. Filter binding assays

The affinity of S8 proteins to their binding site on 16S rRNA was measured by a nitrocellulose filter binding assay as described in detail by Köhrer et al. [10]. TMK-Cl binding buffer contained 50 mM Tris–HCl, pH 7.6, 20 mM $MgCl_2$, 350 or 500 mM KCl, 5 mM β-mercaptoethanol, 0.04% BSA, TMK-Ac binding buffer contained 50 mM Tris–HCl, pH7.6, 20 mM $Mg(OAc)_2$, 350 or 500 mM KOAc, 5 mM β-mercaptoethanol, 0.04% BSA, and CMK binding buffer contained sodium cacodylate instead of Tris. S8 proteins and RNA were incubated separately for 45 min at 40°C in binding buffer to allow complete refolding of both binding partners. A fixed amount of RNA (10 000–20 000 cpm) was mixed with increasing amount of S8 proteins varying from 10 pM to 10 μM in a total volume of 50 μl and incubated for a further 15 min at 40°C, followed by 15 min at 0°C. Protein-RNA complexes were retained by filtration at room temperature on nitrocellulose membranes. After filtration, filters were washed with 300 μl binding buffer and dried. Radioactivity was determined in a liquid scintillation counter (Beckman LS 1801).

Background retention of RNA in the absence of protein, which was normally in the range of 1.5–7% total RNA, was subtracted from the data before plotting. Each binding curve was calculated from at least two experiments by non-linear regression using the computer package 'SPSS for Windows' (SPSS Inc., Release 7.5, 1996). The fundamental equation characterizing a single binding curve was formulated by Mougel et al. [18].

3. Results

3.1. Interaction of ribosomal protein S8 from mesophilic, thermophilic and hyperthermophilic Methanococcus species with their specific 16S rRNA target site

To test in vitro the binding capacity of purified MvaS8, MthS8, MigS8 and MjaS8 proteins to the 16S rRNA target site, the MvaS8 binding site was synthesized as part of a 145-nucleotide transcript comprising nucleotides 493–638 of the 16S rRNA of *M. vannielii* and the MjaS8 binding site was synthesized as part of a 145-nucleotide fragment of the 16S rRNA from *M. jannaschii* (corresponding to nucleotides 504–649). Both 16S rRNA fragments are highly similar, the area determining S8 binding specificity is identical in both organisms (Fig. 2A).

In a first set of filter binding experiments, the affinity of MvaS8, MthS8, MjaS8 and MigS8 to the same 16S rRNA fragment from *M. vannielii* was measured in TMK-Cl_{350} buffer (containing 350 mM KCl), which is very similar to the ribosome reconstitution buffer. As shown in Fig. 3, MvaS8 binds the Mva 16S rRNA with a rather low affinity (average dissociation constant K_d of $1.3 \pm 0.2 \times 10^{-6}$ M), MthS8 binds with a higher affinity (K_d of $2.3 \pm 0.15 \times 10^{-7}$ M), and MjaS8 and MigS8 exhibit an even higher affinity for the 16S rRNA (K_d of $2.6 \pm 0.7 \times 10^{-8}$ M and K_d of $4.5 \pm 0.4 \times 10^{-8}$ M, respectively). To assess the level of non-specific binding of the *Methanococcus* S8 proteins, we used a 23S rRNA fragment

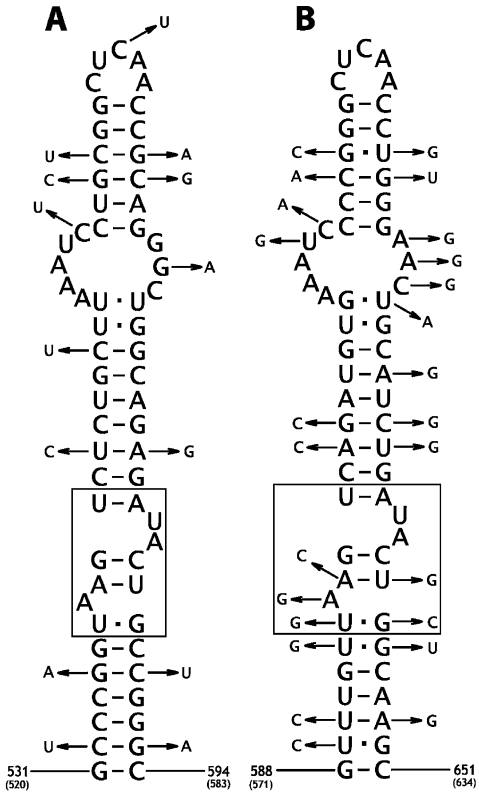

Fig. 2. Secondary structure of H21 of 16S rRNA. The central conserved core representing the essential elements for S8 binding is boxed. A: *M. jannaschii*, nucleotide changes in *M. vannielii* are indicated. B: *E. coli*, nucleotide changes in *T. thermophilus* are indicated.

[11] that was roughly the same size as the 16S rRNA fragment. Only a very weak interaction of all four S8 proteins with the non-cognate RNA was observed (Fig. 3).

The drastic difference in affinity for the Mva 16S rRNA of mesophilic, thermophilic and hyperthermophilic S8 proteins was confirmed by further filter binding assays using buffers TMK-Cl_{500}, TMK-Ac_{350} and TMK-Ac_{500}. The average dissociation constants obtained in the four different buffers are summarized in Table 1. The saturation level, defined as percentage of total rRNA bound to the nitrocellulose membrane, ranged between 45 and 94%. In TMK buffer containing 500 mM KCl, the affinity of the S8 proteins to the 16S rRNA target site is similar to that measured in TMK-Cl_{350}. In buffers where the anion Cl^- was replaced by CH_3COO^- (TMK-Ac_{350} and TMK-Ac_{500}), the dissociation constants were found to decrease by a factor of 10. This anion effect on K_d was described for EcoS8 before [18].

The K_d values characterizing the 16S rRNA-S8 complexes were found to be almost identical for the two hyperthermophilic proteins MigS8 and MjaS8, about an order of magni-

Table 1
Dissociation constants K_d ($\times 10^{-8}$ M) of S8-16S rRNA complexes from mesophilic and (hyper)thermophilic *Methanococcus* species in different buffers as indicated

Complex	TMK-Cl_{350}	TMK-Cl_{500}	TMK-Ac_{350}	TMK-Ac_{500}
MigS8-16S rRNA	4.53 ± 0.40	6.4 ± 0.5	0.49 ± 0.20	0.28 ± 0.10
MjaS8-16S rRNA	2.60 ± 0.70	6.2 ± 2.2	0.16 ± 0.03	0.26 ± 0.06
MthS8-16S rRNA	23.5 ± 1.5	19.7 ± 6.9	2.06 ± 0.50	4.29 ± 1.4
MvaS8-16S rRNA	130 ± 20	210 ± 40	22 ± 4	23 ± 6

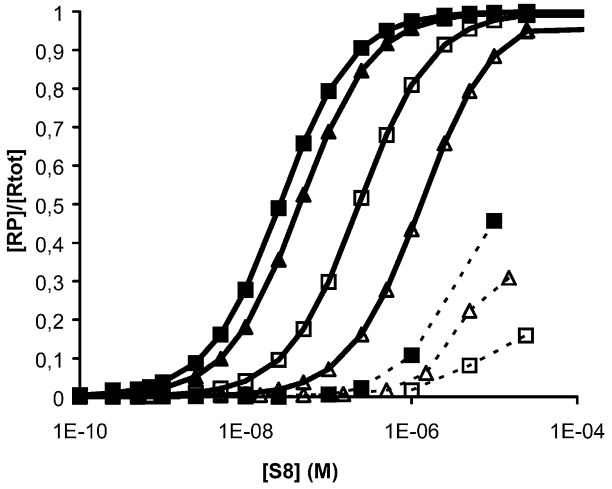

Fig. 3. Binding curves for the interaction of methanococcal S8 proteins to Mva 16S rRNA (solid lines) and, as a control for unspecific binding, to 23S rRNA (broken lines) in TMK-Cl_{350} buffer. MjaS8 (■), MigS8 (▲), MthS8 (□), MvaS8 (△).

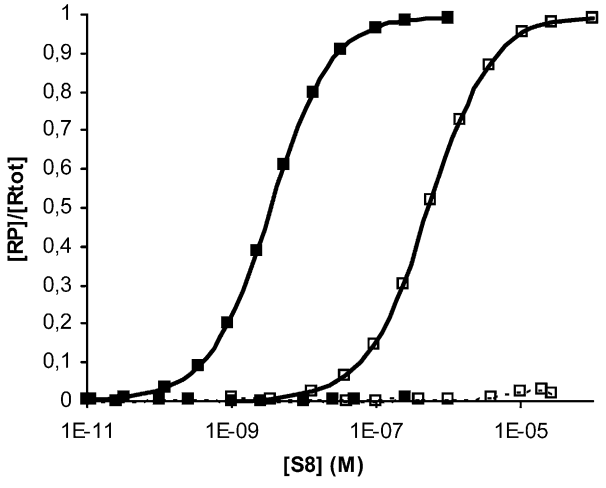

Fig. 4. Binding curves for the interaction of bacterial TthS8 (■) and EcoS8 (□) proteins to Eco 16S rRNA (solid lines) and, as a control for unspecific binding, to 23S rRNA (broken lines) in TMK-Cl_{350} buffer.

tude higher for the thermophilic MthS8, and again 10-fold higher for the mesophilic MvaS8. The four S8 proteins bind the 16S rRNA from *M. jannaschii* (Fig. 2A) with virtually the same affinity as Mva 16S rRNA in all four buffers tested (data not shown).

3.2. Interaction of S8 proteins from mesophilic and thermophilic bacteria with their specific 16S rRNA target site

As the archaeal S8 proteins exhibit such a drastic difference in affinity for their 16S rRNA target site, depending on their mesophilic or (hyper)thermophilic origin, we decided to re-examine the binding of S8 proteins from the mesophilic bacterium *E. coli* and from the thermophilic species *T. thermophilus* to their 16S rRNA target sites. Previous studies showed an identical K_d of 2×10^{-8} M for the EcoS8-16S rRNA complex [18] and for the TthS8-16S rRNA complex [22].

The EcoS8 binding site was transcribed as part of an 89-nucleotide fragment comprising positions 565–653 of the 16S rRNA of *E. coli* (Fig. 2B). Filter binding assays with EcoS8 and TthS8 were carried out in buffers TMK-Cl_{350} and CMK-Cl_{500} where Tris was replaced by sodium cacodylate, a buffer substance that had been used in the previous study of the TthS8-16S rRNA complex [22]. In the presence of 350 mM KCl, EcoS8 binds the Eco 16S rRNA fragment with a K_d of $5.4\pm0.7\times10^{-7}$ M, TthS8 with a K_d of $3.5\pm0.6\times10^{-9}$ (Fig. 4). A similar difference in affinity was observed when using buffer CMK-Cl_{500}, where EcoS8 and TthS8 bind with a K_d of $6.7\pm1.0\times10^{-7}$ M and $1.5\pm0.3\times10^{-8}$ M, respectively. No interaction of both bacterial S8 proteins with the 23S rRNA control fragment could be detected.

4. Discussion

4.1. Correlation between affinity for 16S rRNA, thermostability of S8 proteins and optimal growth temperature

The interaction of the bacterial protein EcoS8 with its 16S rRNA target site has been studied extensively. Interestingly, the apparent K_ds reported vary considerably. Mougel et al. [18] determined a K_d as low as 2×10^{-8} M in a binding buffer containing 350 mM KCl, whereas Wu et al. [20,21] reported a K_d of 1.2×10^{-7} M under the same buffer conditions. The apparent dissociation constant we determined for the thermophilic TthS8-rRNA complex (1.5×10^{-8} M in CMK-Cl_{500}) is virtually identical to that published previously (2×10^{-8} M) [22]. We find that the affinity of EcoS8 for its 16S binding site is an order of magnitude lower, thus only slightly lower than the values published previously by Wu et al. [20,21]. The fact, that primary rRNA binding proteins from thermophiles exhibit a much higher affinity for their specific rRNA binding site compared to their mesophilic counterparts has also been shown for ribosomal proteins L1 [10] and S4 [31] and S7 [32].

The most surprising result of this study was the drastic difference in affinity of the closely related *Methanococcus* S8 proteins for the 16S rRNA binding site. In all four buffers tested, the hyperthermophilic MjaS8 and MigS8 bind 16S

Table 2
Correlation of the dissociation constants of S8-16S rRNA complexes with thermostability of the S8 proteins and optimal growth temperature of the organisms

Protein-RNA complex	K_d ($\times10^{-8}$ M)[a]	Temperature of the heat step used in protein purification (°C)	Optimal growth temperature of the organism (°C)
MigS8-16S rRNA	4.53	75	88 (*M. igneus*)
MjaS8-16S rRNA	2.60	70	85 (*M. jannaschii*)
MthS8-16S rRNA	23.5	50	65 (*M. thermolithotrophicus*)
MvaS8-16S rRNA	130	–	37 (*M. vannielii*)
TthS8-16S rRNA	0.35	70	75 (*T. thermophilus*)
EcoS8-16S rRNA	54	–	37 (*E. coli*)

[a] K_d was measured in TMK-Cl_{350} buffer.

rRNA with a very high affinity, an order of magnitude stronger than the thermophilic MthS8 and two orders of magnitude stronger than the mesophilic MvaS8 (Table 1). As the source of the 16S rRNA fragment – *M. vannielii* or *M. jannaschii* – does not influence the binding constants, the differences of the K_d values are determined by the protein partner. There is a good correlation between the S8 binding affinity for 16S rRNA, the thermal stability of the protein (given as the temperature of the heat step, at which the protein does not denature yet) and the optimal growth temperature of each species (Table 2). The amino acid alignment of six methanococcal S8 proteins (Fig. 5) underlines their close relationship, thus the differences in binding affinity probably do not simply reflect a phylogenetic distance. The ribosome is not a static particle but undergoes large-scale conformational changes during the different steps of protein synthesis [33]. The very strong protein-RNA interaction observed for the hyperthermophilic species might make an important contribution to the thermal stability of their ribosomes. The lower affinity of the thermophilic MthS8 and the much lower affinity of the mesophilic MvaS8 might result in a gain of flexibility of the ribosome. A higher flexibility might be a prerequisite for full 'activity' of the ribosome at lower temperatures. It is tempting to speculate that the stability of the individual rRNA-protein complexes within the ribosome might modulate the stability of the ribosome and provide a maximum of thermostability and flexibility at the growth temperature of the organism. In thermophiles, the rRNA, and thus the whole ribosome, is stabilized by a higher G+C content of helical rRNA structures, and a striking correlation between G+C content of the stems and the optimal growth temperature has been reported [34,35]. Furthermore, the exceptionally high base modification levels of 16S and 23S rRNA in (hyper)thermophiles might contribute to the thermal stabilization of the ribosome [36].

4.2. *Structural elements modulating the affinity of S8 proteins for their specific 16S rRNA binding site*

The analysis of the RNA-protein interactions within the crystal structure of the MjaS8-rRNA complex revealed that nine amino acid residues (marked in red in Fig. 5) interact directly with 16S rRNA [16]. The comparison of all known *Methanococcus* S8 sequences (Fig. 5) shows that MigS8-16S rRNA, the second complex characterized by very tight binding, also contains nine amino acids potentially interacting with RNA. Eight of them are identical to those in the Mja complex, Thr107 is replaced by Ser, but in the TthS8-16S rRNA complex, Ser107 forms an identical interaction with the respective nucleotide [16]. In the MthS8-rRNA complex, which is characterized by an intermediate affinity, one of the direct contacts (position 107) is missing, and in the weak MvaS8-rRNA complex, the number of amino acids potentially contacting RNA seems to be reduced to seven, as Lys83 and Thr107 are replaced by Gln or Tyr and Pro, respectively. It is a reasonable assumption that the number of

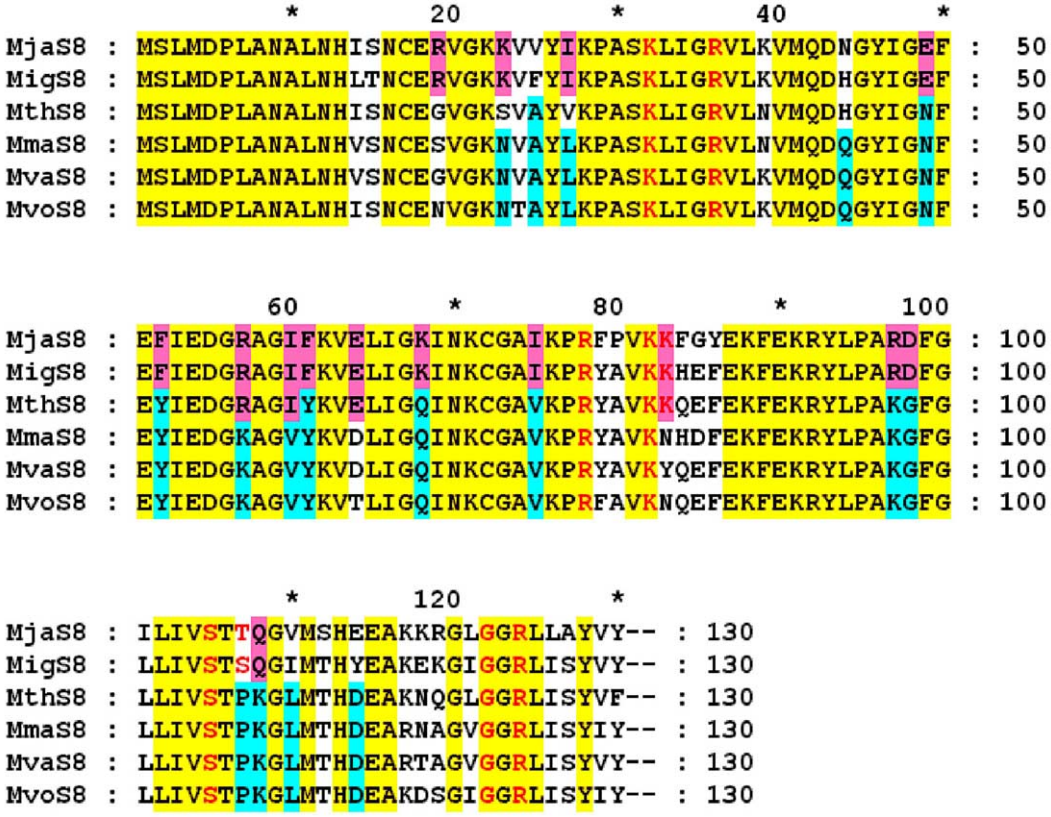

Fig. 5. Alignment of the DNA-deduced amino acid sequences of S8 proteins from mesophilic, thermophilic and hyperthermophilic *Methanococcus* species. Residues identical or highly conserved (V/I/L, T/S, D/E, N/Q, R/K) are shown in a yellow background. Residues identical in the mesophilic MvoS8, MvaS8, MmaS8 (and the thermophilic MthS8) are shown in a blue background, residues identical in the hyperthermophilic MigS8 and MjaS8 (and the thermophilic MthS8) are shown in a magenta background. Amino acid residues which are in direct contact with RNA in the MjaS8-rRNA complex [16] are given in red letters. Mja, *M. jannaschii*; Mig, *M. igneus*; Mth, *M. thermolithotrophicus*; Mma, *M. maripaludis*; Mva, *M. vannielii*; Mvo, *M. voltae*.

amino acid residues directly contacting RNA determines the stability of the complex. The analysis of the 3D structure of *Thermus* 30S subunits revealed special features of proteins, which are probably required for the stability of the ribosome at high temperatures. The extra C-terminal helix of TthS17 which increases the protein-RNA contacts and zinc binding modules in TthS4 and TthS14 are examples of structural features that are supposed to provide additional thermal stabilization to the ribosome [4,37].

Acknowledgements: We thank M. Garber (Pushchino) for the gift of TthS8, A. Klein (Marburg) for the gift of *M. voltae* cells, M. Thomm (München) for providing *M. igneus* cells, and M. Humpel for excellent technical assistance. This work was supported by the Austrian Science Fund (FWF) grant P-14550-B04 to W.P.

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