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Structural analysis of the elongation factor G protein from the low-temperature-adapted bacterium *Arthrobacter globiformis* SI55

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Abstract The first structural analysis of elongation factor G (EF-G) from a cold-adapted bacterium is presented. EF-G is an essential protein involved in the elongation process during protein synthesis and is therefore thought to play a crucial role in the low-temperature adaptation of cold-adapted microorganisms. To define its importance, the EF-G gene (*fus*) from the psychrotolerant bacterium *Arthrobacter globiformis* SI55 was cloned and sequenced. The deduced primary structure of the elongation factor is composed of 700 amino acids with a predicted molecular mass of 77.4 kDa. A three-dimensional model of the protein was constructed based on the known crystal structures of structurally homologous proteins. Structural features that might potentially be important for activity and flexibility at low temperature were deduced by comparisons with models of the EF-G proteins from the closely related mesophiles *Micrococcus luteus* and *Mycobacterium tuberculosis*. These features include a loss in the number of salt bridges in intradomain and interdomain positions, increased solvent interactions mediated by greater charge and polarity on domain surfaces, loop insertions, loss of proline residues in loop structures, and an increase of hydrophobicity in core regions. Specific changes have also been identified in the catalytic domain (G domain) and sites of potential ribosome interaction, which may directly affect guanosine triphosphate (GTP) hydrolysis and elongation rates at low temperature.

Key words Elongation factor G · Psychrotolerant · Protein structure · *Arthrobacter* · Cold adaptation

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Introduction

Of all the physical factors affecting the microbial environment, temperature is one of the most important. Because much of the planet is dominated by low-temperature environments (i.e., below 5°C), it is not surprising that these habitats have been colonized by cold-adapted microorganisms. Cold-adapted microorganisms are referred to as psychrophilic or psychrotolerant (psychrotrophic). Psychrophiles have optimum growth temperatures below 15°C and upper temperature limits below 20°C, whereas psychrotolerant microorganisms are capable of growth at or close to 0°C, have optimum growth above 15°C, and a maximum temperature for growth above 20°C (Morita 1975).

Cellular changes associated with growth at low temperatures include modified membrane lipids, patterns of protein synthesis, and protein structures (Russell 1998). In comparison to proteins from mesophiles and thermophiles, cold-adapted proteins have higher specific activity and catalytic efficiency at low temperatures as a consequence of their greater structural flexibility. Specific structural features implicated in cold adaptation of proteins have been predicted from amino acid sequence and three-dimensional structure comparisons (Davail et al. 1992; Feller et al. 1991, 1994; Rentier-Delrue et al. 1993; Thomas and Cavicchioli 1998). As a result of their unique structural and functional properties, cold-adapted proteins are important models for fundamental studies on protein stablility, folding, and activity and are significant resources for biotechnological application (Russell 1998).

In mesophilic bacteria, the primary effect of a cold shock is a transient inhibition of most protein synthesis, resulting in a growth lag called the acclimation phase and an accumulation of 70*S* monosomes with a concomitant decrease in the number of polysomes (Das and Goldstein 1968; Friedman et al. 1969; Broeze et al. 1978). During this acclimation

phase, several proteins called the cold-shock proteins are induced up to 20 fold, some of which (e.g., RbfA, CsdA, and IF2) might associate with the ribosomal subunits and 70*S* monomers to give polysomes that are able to translate efficiently at the new lower temperature (Thieringer et al. 1998). One of the most significant differences between mesophilic and cold-adapted bacteria is that ribosomes retain their ability to form polysomes after a shift down to 5°C (Szer 1970; Broeze et al. 1978) and the relative rate of synthesis of most cellular proteins is maintained after the cold shock (Araki 1991; Roberts and Inniss 1992; Whyte and Inniss 1992; Berger et al. 1996; Michel et al. 1997). As a consequence, ribosomes and associated factors (e.g., initiation and elongation factors) from cold-adapted bacteria must be adapted to low temperatures (Russell 1990), and their inhibition in mesophiles might be responsible for the incapacity of these bacteria to grow at low temperatures (Gounot and Russell 1999).

EF-G (and its archaeal homolog elongation factor 2, EF-2) is an attractive model for the investigation of cold adaptation as it plays an essential role during protein biosynthesis and is present and active in every organism. Comparative structural studies of EF-2 from psychrophilic, mesophilic, and thermophilic Archaea have indeed identified amino acid residues that may be important for low-temperature activity and increased flexibility (Thomas and Cavicchioli 1998).

In this study, we report the cloning and sequencing of the EF-G encoding gene, *fus*, from *Arthrobacter globiformis* SI55, a psychrotolerant bacterium capable of growth between −5°C and +32°C that was isolated from a Lapland glacier region (Gounot 1976). A model of the *A. globiformis* EF-G has been constructed on the basis of the crystal structures of *Thermus thermophilus* and *Thermus aquaticus* EF-G and EF-Tu. Comparison of the EF-G models with those from closely related mesophiles enabled the identification of residues and domains that may be functionally important in the molecular adaptation of the cold-adapted EF-G for activity at low temperature. This is the first structural analysis of the three-dimensional structure of an elongation factor from a cold-adapted bacterium.

Materials and methods

Bacterial strains and growth conditions

Arthrobacter globiformis SI55 was grown aerobically at 20°C in a liquid synthetic medium (Shaw and Stead 1971) as described previously (Potier et al. 1985). *E. coli* strains DH5α (F^-, ϕ80d *lacZ*ΔM15, Δ[*lacZYA-argF*]U169, *deoR*, *recA*1, *endA*1, *hsdR*17[r_k^-, m_k^-], *supE*44, λ^-, *thi*-1, *gyrA*96, *relA*1) and DH10B (F^-, *mcrA*, Δ[*mrr-hsdRMS-mcrBC*], ϕ80d*lacZ*ΔM15, Δ*lacX*74, *deoR, recA*1, *endA*1, *araD*139, Δ[*ara*, *leu*]7697, *galU*, *galK*, λ^-, *rpsL*, *nupG*) (Life Technologies, MD, USA) and the pBluescript SK vector (Stratagene, La Jolla, CA, USA) were used for subcloning. Following transformation, *E. coli* DH5α and DH10B electrocompetent cells were grown at 37°C in LB medium with ampicillin (100 μg/ml). The host strain for bacteriophages was *E. coli* LE392 (F^-, *hsdR*514[r_k^-, m_k^-], *supE*44, *supF*58, *lacY*1 or Δ[*lacIZY*]6, *galK*2, *galT*22, *metB*1, *trpR*55, λ^-) (Promega, Southampton, UK). For λ lysate preparation, the LB medium was supplemented with 10 mM $MgSO_4$.

Isolation of *A. globiformis* SI55 DNA

Arthrobacter globiformis SI55 cells were harvested by centrifugation at 5000 × *g* for 10 min. The resulting pellet was washed twice in a 0.1 molar phosphate buffer (pH 7) and resuspended in 560 μl 50 mM Tris-HCl, pH 7.5, containing 5 mM EDTA. The cells were treated with 100 mg polyvinylpolypyrrolidone, 40 μl 20% SDS, and 10 μg proteinase K at 37°C for 1 h. Lysis was completed by adding 100 μl 5 M NaCl and 80 μl cethyl trimethyl-ammonium bromide (10% w/v in 4% w/v NaCl) at 65°C for 10 min. The cell wall debris, polysaccharides, and proteins were removed by centrifugation for 10 min at 4000 × *g*. The supernatant was recovered and extracted three times with chloroform : isoamyl alcohol (24 : 1, v/v). The DNA was precipitated with 0.6 vol isopropanol, washed with 70% ethanol, and resuspended in 10 mM Tris-HCl, pH 7.5, containing 1 mM EDTA. The DNA was treated with 0.5 mg/ml RNase A for 30 min at 37°C.

Southern blot analysis

Southern blot analysis was performed as described by Sambrook et al. (1989). *A. globiformis* SI55 DNA was digested with a range of restriction enzymes, separated on a 0.8% (w/v) agarose gel and transferred under vacuum to a Genescreen*Plus*® nylon membrane (NEN, Boston, MA, USA). For probe generation, degenerate primers (primer 1, 5′-AGRTGNAGYTCNCCCATNCC-3′; primer 2, 5′-GCNTTCAARAAYAARGGNGT-3′) were designed from two regions that are conserved within EF-G sequences from gram-positive and gram-negative bacteria. Polymerase chain reaction (PCR) amplification was performed using a 50-μl incubation mixture containing 100 ng chromosomal DNA, 1 μM each primer, 200 μM each of the four dNTPs, and 1 U *Taq* DNA polymerase. The PCR was run for 35 cycles in a thermal cycler (Cetus), during which DNA was denatured at 95°C for 45 s, primers were annealed at 50°C for 45 s, and DNA was extended at 72°C for 1 min. The single 590-bp DNA fragment was sequenced by Genome Express S.A. (Grenoble, France). The nucleotide sequence of the PCR product showed a high level of similarity to genes encoding bacterial EF-G. Probes were generated from the PCR product by random hexanucleotide priming using [α-^{32}P]dCTP with a Random Primed DNA Labelling Kit (Boehringer Mannheim, GmbH, Germany). Prehybridation was carried out at 65°C for 4 h and hybridization with the [α-^{32}P]dCTP radiolabeled probe overnight at 65°C. The membrane was washed once in 2× SSC (20× SSC is 3 M NaCl, 0.3 M sodium citrate) for 5 min at room temperature, once in 2× SSC for 15 min at 65°C, once in 2×

SSC, 1% SDS for 15 min at 65°C, and once in 1× SSC, 1% SDS for 15 min at 65°C. Southern hybridization of genomic DNA digested with *Xho*I resulted in the hybridization of a single band of approximately 2.5 kb.

Isolation and DNA sequencing of the *A. globiformis* SI55 *fus* gene

The construction and amplification of the *A. globiformis* SI55 genomic library using a λGEM-12 vector was carried out as described in the supplier's manual (Promega). *A. globiformis* SI55 DNA was partially digested with *Sau*3AI, and DNA fragments between 9 and 14 kb were purified from agarose gels using a Qiaquick gel extraction kit (Qiagen), ligated into the arms of a λ GEM-12 vector, and packaged. Freshly prepared *E. coli* LE392 cells were infected, and after incubation at 37°C overnight, phage particles from plaques were transferred onto nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany), and the λ DNA was fixed by UV cross-linking (Sambrook et al. 1989). For screening the λ library, membranes were hybridized and washed as already described for the Southern blot procedure. Membranes were exposed to photographic film at −80°C for 3 days. Positive plaques were transferred to 500 µl SM buffer (0.01% gelatin, 50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 8 mM $MgSO_4$) and subjected to secondary and tertiary screenings to isolate pure recombinant λ phages. Small-scale phage DNA preparations were performed with the Qiagen lambda kit using a slightly modified procedure in which the phage lysate was incubated for 1 h with RNaseA (60 µg/ml) and DNaseI (18 µg/ml) before overnight precipitation with polyethylene glycol 6000. Positive clones were digested with *Xho*I, separated on 1% agarose gels, and analyzed by Southern hybridization. A 2.5-kb hybridizing fragment was recovered and ligated into the *Xho*I site of pBluescript SK. The recombinant plasmid was designated pVBU3. The sequence of both strands of the 2.5-kb *Xho*I fragment was determined from fragments subcloned in pBluescript SK constructs and by primer walking on pVBU3, using T3, T7, and internal primers (Genome Express S.A). The nucleotide sequence has been assigned the EMBL/GenBank accession number AJ238083.

Three-dimensional modeling

Arthrobacter globiformis SI55, *Micrococcus luteus*, and *Mycobacterium tuberculosis* EF-G proteins were modeled using the SwissModel program (Peitsch 1995, 1996) with the crystal structures of *Thermus thermophilus* EF-G with no nucleotide, *T. thermophilus* EF-G with GDP, *Thermus aquaticus* EF-Tu with *E. coli* Lys-tRNA, *T. aquaticus* EF-Tu with GTP-analog, *T. aquaticus* EF-Tu + GDP, and *E. coli* EF-Tu with GDP as templates (Brookhaven Protein database entries: 11ELO, 11DAR, 11B23, 11EFC, 11TUI, and 11EFT, respectively). Models were obtained for residues 5–695 for *A. globiformis* SI55, 5–696 for *M. luteus*, and 10–696 for *M. tuberculosis* EF-G. Models were further analyzed and validated with PROCHECK (Laskowski et al. 1993). More than 95% of the residues were found in the most favored or allowed regions of the Ramachandran plot. Stereochemistry of main and side chains was within acceptable limits. The overall G-factor in PROCHECK was −0.2. Structures were superimposed and analyzed with SwissPDB-Viewer (Peitsch 1996). An overall spatial deviation of 0.31 Å for 674 selected residues for the pairs *A. globiformis* SI55/*M. luteus* and 0.42 Å for 657 residues for *A. globiformis* SI55/*M. tuberculosis* were obtained. Models were analyzed for structural differences that may confer thermal adaptation using strategies previously adopted (Thomas and Cavicchioli 1998).

Results and discussion

Analysis of the nucleotide and amino acid sequence

Sequence analysis of the 2.5-kb insert revealed the presence of a single open reading frame (ORF) starting with the initiation codon GTG and ending with the termination codon TAG. The ORF is 2103 bp long and encodes a polypeptide of 700 amino acids with a predicted molecular mass of 77.4 kDa and a pI of 4.84. A GTG start codon has also been described for the phylogenetically related bacterium *Micrococcus luteus* (Ohama et al. 1987). The *fus* gene does not have any recognizable Shine–Dalgarno sequence apart from a GGAG sequence, 16 nucleotides upstream of the GTG start codon. This situation is similar to that of chloroplast mRNAs, in which Shine–Dalgarno sequences can be found up to 25 nucleotides upstream from the initiation codon (Ruf and Kössel 1988).

In bacteria, the gene for EF-G often belongs to a single transcriptional unit, called the streptomycin (*str*) operon, which comprises the ribosomal proteins genes *rps12* (for protein S12) and *rps7* (for protein S7), as well as genes *fus* (for EF-G) and *tuf* (for EF-Tu) in the order 5′-*rps12-rps7-fus-tuf*-3′ (Nomura et al. 1984). The order and linkage of the four streptomycin operon genes appear to be conserved over a wide phylogenetic spectrum encompassing *Thermotoga maritima* (Tiboni et al. 1991), the high G + C gram-positive bacterium *Micrococcus luteus* (Ohama et al. 1987), the low G + C gram-positive *Bacillus subtilis* (Cousineau et al. 1992), Proteobacteria (Post and Nomura 1980), and Cyanobacteria (Buttarelli et al. 1989; Meng et al. 1989). In Archaea, most of the archaeal elongation factor and associated ribosomal protein genes are arranged in operons, although the precise genomic organization is not conserved (for examples, see Lechner et al. 1989; Itoh 1989; Pechmann et al. 1991; Schröder and Klink 1991; de Vendittis et al. 1993; Creti et al. 1995). Analysis of the upstream sequence of the *A. globiformis* SI55 *fus* gene revealed the presence of an incomplete ORF, which shared a high degree of sequence similarity with the C-terminal region of *Micrococcus luteus* and *Mycobacterium tuberculosis* ribosomal protein S7 (95% and 88% identities, respectively). As in *E. coli* (Post and Nomura 1980), no sequence resembling either transcriptional promoters or terminators could be

identified in the 83-bp region between the *rps7* and *fus* genes. The 210-bp downstream sequence from *fus* was analyzed but no ORF corresponding to a *tuf* gene and no promoter-like structure or transcription termination motifs could be identified. In *Micrococcus luteus*, the spacer between EF-G and EF-Tu genes is 275 bp long (Ohama et al. 1987), suggesting that in *A. globiformis* SI55 the *tuf* gene, if it is present, would be located further downstream from *fus* than we had sequenced. All these data suggest that, as in other Bacteria, the *A. globiformis* SI55 *fus* gene is contained in a *str* operon and is cotranscribed with the ribosomal protein S7.

The amino acid sequence of the *A. globiformis* SI55 *fus* gene was compared to others in GenBank with the algorithm Blastp (Altschul et al. 1990). The *A. globiformis* SI55 EF-G exhibited the highest identities with EF-G from *M. luteus* (79%) and *M. tuberculosis* (72%) and lower identities with EF-G from *E. coli* (58%), *B. subtilis* (60%), and *Thermus thermophilus* (58%). The similarity of *A. globiformis* SI55 EF-G with EF-G sequences from other bacteria extends throughout the protein, including the five sequence motifs (Fig. 1), which are considered to be essential for GTP binding (Dever et al. 1987; Chirgadze 1996).

Comparison of the primary sequence of cold-adapted proteins with that of other organisms has revealed some structural features that may be important for cold adaptation (reviewed in Feller et al. 1997). In particular, the proline content of mesophilic and thermophilic proteins is often higher compared to their psychrophilic counterparts. Analysis of the amino acid sequence of *T. thermophilus*, *M. luteus*, *M. tuberculosis*, and *A. globiformis* SI55 EF-G showed that the protein from the thermophile contained more proline residues than the proteins from the mesophiles and the psychrotolerant strain, but there was no striking difference between EF-G from the psychrotolerant bacterium and the mesophiles (Table 1). Other changes observed in some cold-adapted enzymes, such as a decrease in the ratio of arginine + aspartate + asparagine/glutamate + lysine + glutamine (R/D/N to E/K/Q) and in the isoleucine content, were not observed. Increases in both these parameters have been associated with increased thermal stability (Menéndez-Arias and Argos 1989; Zuber 1988).

Table 1. Characteristics of EF-G from *Arthrobacter globiformis* SI55 (*A.g.*), *Micrococcus luteus* (*M.l.*), *Mycobacterium tuberculosis* (*M.t.*), and *Thermus thermophilus* (*T.t.*)

	A.g.	*M.l.*	*M.t.*	*T.t.*
MW (kDa)	77.44	77.42	77.19	77.87
pI	4.84	4.87	4.77	5.13
R/D/N to E/K/Q[a]	0.76	0.72	0.68	0.72
Isoleucine (number of residues)	49	39	41	56
Proline (number of residues)	33	31	31	38

[a] R/D/N to E/K/Q corresponds to the ratio Arg + Asp + Asn/Glu + Lys + Gln

Three-dimensional modeling

Because the primary sequence comparison provided limited information about structural properties for thermal adaptation, a three-dimensional model for the EF-G from *Arthrobacter globiformis* SI55 was generated and compared to models from its most closely related, mesophilic counterparts, *Micrococcus luteus* and *Mycobacterium tuberculosis* (see Fig. 1). Phylogenetically related species were chosen to minimize the number of changes that result from nonthermally derived evolution of the protein (Thomas and Cavicchioli 1998). Based on the crystal structure of EF-G from *Thermus thermophilus* (Czworkowski et al. 1994; AEvarsson 1995; AEvarsson et al. 1994), the following domain sizes and organization was attributed to the three-dimensional model generated for the *A. globiformis* SI55 EF-G, namely G domain, residues 1–281 (with G′ subdomain 153–254); domain II, residues 282–403; domain III, residues 404–484; domain IV, residues 485–610 and 679–700; and domain V, residues 611–678 (Fig. 2).

A. globiformis SI55 *versus M. luteus* EF-G

The pairwise comparison of *A. globiformis* SI55 and *M. luteus* EF-G models showed a reduced number of salt bridges in the EF-G from the psychrotolerant bacterium. These structures are distributed throughout the full length of the protein. Salt bridges are absent from the G domain (position 72), domain III (positions 338 and 453), domain IV (positions 493 and 557), and domain V (position 657); numbers in parentheses are for the *A. globiformis* SI55 EF-G. All the missing salt bridges are involved in intradomain interactions in the *M. luteus* EF-G.

An increase in the charge and polarity of the surface of A. *globiformis* EF-G, particularly in domains G, IV, and V, was observed. This change may result in increased solvent interaction in the cold-adapted protein and facilitate greater structural flexibility of the protein. A proline residue (position 399) is absent from a surface-exposed, 10-amino-acid loop in the *A. globiformis* SI55 EF-G. This lack may confer higher flexibility to this region by reducing structural constraints to the carbon backbone.

Hydrophobic core regions are known to be important for protein folding and stability (Jaenicke 1990; Russell et al. 1998), and a number of changes in hydrophobic core regions were identified in the *A. globiformis* SI55 EF-G. The cold-adapted EF-G exhibited an overall slight increase in hydrophobicity and packing density. For example, a glutamate residue in the *M. luteus* EF-G is replaced by an isoleucine residue in one core region in the G domain of the *Arthrobacter* protein. The hydrophobic cluster in domain 4 is also more hydrophobic and densely packed through a phenylalanine residue in position 596 of *A. globiformis* SI55 EF-G. Hydrophobic interactions form endothermically and are weakened by a decrease in temperature. Consequently, these regions are expected to be destabilized in the cold-adapted EF-G at low temperature and stabilized at high

Fig. 1. Structure-based multiple sequence alignment derived from the superimposition of models for the elongation factor G proteins from *Arthrobacter globiformis* SI55 (*A.g.*), *Micrococcus luteus* (*M.l.*), and *Mycobacterium tuberculosis* (*M.t.*). For *M.l.* and *M.t.* sequences, residues are shown that differ from the *A.g.* sequence. Within the sequences, "−" represents gaps in the alignment. Five conserved motifs involved in nucleotide binding (see text) are *underlined* in the *A.g.* sequence

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1    A.g.    5   LSKVRNIGIMAHIDAGKTTTTERILFYTGVNHKIGETHDGASTTDWMEQE
2    M.l.    5   .H.....H.....L.....GA.....
3    M.t.   10   ..R...F.....Y...I.Y....V....A.M.....
4
5    A.g.   55   KERGITITSAAVTCFWENNQINIIDTPGHVDFTVEVERSLRVLDGAVAVF
6    M.l.   55   .....NDH.....N.....
7    M.t.   60   Q.....T.T..KD..L.....N.....
8
9    A.g.  105   DGKEGVEPQSETVWRQADKYNVPRICFVNKMDKLGADFYFTVDTIISRLG
10   M.l.  105   .....D.....VK...
11   M.t.  110   .....Q.....D.....I.....S.R.MGE...
12
13   A.g.  155   VKPLVMQLPIGAENDFIGVVDLLYMRALVWPGD--SKGDVTMGAKYEIQE
14   M.l.  155   AR.....V.....IS.K.F.....--AN.I.....S...EI
15   M.t.  160   ANAVPI...V...A..E.....VE.N.K..R.ETKLGETYD-----TVE-
16
17   A.g.  203   IPADLKEKAEEYRAALVETVAESSEELMEKYLEGEEITEDELKAGIRKMT
18   M.l.  203   -RQLQEKAE....NE...A...T.....L.VE.IQ..V.QL.
19   M.t.  204   .....A.Q.....TK.L.V....D.H.L....G...L.V..I.GA...L.
20
21   A.g.  253   INSELYPVFCGSAFKNRGVQPMLDAVVDYLPNPLDVPP-MIGHDPR--DE
22   M.l.  252   V.A.A.....A.....AG.VKGHAVND--E.
23   M.t.  254   .A..I...L.....K.....S.....-A...A.AKE..
24
25   A.g.  300   EKELTRKPSSEEPFSALAFKIATHPFFGQLTFIRVYSGHVEAGSQVVNST
26   M.l.  300   VVLER-EV.K.A.....T.....RL.S.A..L.A.
27   M.t.  303   .VVRK--ATTD...A.....K..Y.....T..S....I.A.
28
29   A.g.  350   KGKKERIGKLFQMHANKEMPVDGATAGHIYAAIGLKDTTTGDTLCDPANQ
30   M.l.  349   .....N...EVV.....V.....P
31   M.t.  351   .....L.....S...N...R.S.....V.....S..NQ.
32
33   A.g.  400   IVLESMSFPEPVISVAIEPNTKGDQEKLSTAIQKLSAEDPTFQVSLNEDT
34   M.l.  399   .I....T.....K.....V.....R.N...E.
35   M.t.  401   .....T..D...E.....K..S.....LS....AE.....K.H.DSE.
36
37   A.g.  450   GQTIIAGMGELHLDILVDRMRREFKVEANVGKPQVAYRETIKRAVERHDY
38   M.l.  449   ...E.G.....VF....K.....K.DKV..
39   M.t.  451   ...V.G.....K.....L.QNVE.
40
41   A.g.  500   THKKQTGGSGQFAKIQIAIEPLDTSEGELYEFSNKVTGGRIPREYIPSVD
42   M.l.  499   .....V.LSF.....PR.TV...E.AI....V.....
43   M.t.  501   .....VI.NL..FTGE..AT...ES.....
44
45   A.g.  550   AGIQDALNDGVLAGYPVVGIKATLIDGAYHDVDSSEMAFKIAGRMAFKEA
46   M.l.  549   .....MKF.....M.RV...SL.....R...SQ....G
47   M.t.  551   ..A...MQY.....L.NL.V..L.....E.....SQVL.K.
48
49   A.g.  600   ARKANPILLEPLMDVEVRTPEEYMGEVIGDLNSRRGQM--QSMEDAQGVK
50   M.l.  599   V...T..I.....A.....F..D.....IQI.....T...
51   M.t.  601   .AL.Q.VI...I.A...T...D...D.....I--.A..ERA.AR
52
53   A.g.  648   VIRAHVPLSGMFGYIGDLRSKTQGRAVYSMTFHSYAEVPKAFADEIIQ
54   M.l.  649   .VN.L....E.....V....V.
55   M.t.  649   .V.....E....V.....N...V.D..S...ANVSK...A
56
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temperature. This change may play an important role in the overall dynamics and flexibility of the protein when the cell is growing at different temperatures.

During translation, EF-G interacts with the ribosome to facilitate translocation (Spirin 1985), and specific sites of interaction have been identified in the *E. coli* EF-G and

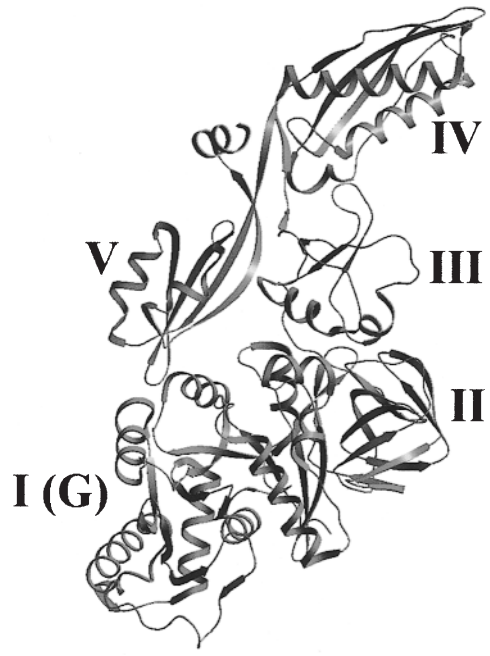

Fig. 2. Three-dimensional model of the elongation factor G protein of *Arthrobacter globiformis* SI55. *Numbers* beside the structure indicate the domains (G/I, II, III, IV, V)

ribosomal RNA (Wilson and Noeller 1998). To determine whether the regions of contact between EF-G and the ribosome may be modified in the cold-active EF-G, the *Arthrobacter* and *Micrococcus luteus* models were superimposed with a model generated for *E. coli* EF-G. Residue 301 in a loop structure in domain II has been shown to directly interact with 16*S* ribosomal RNA in *E. coli*. The corresponding region in the *Arthrobacter* EF-G (residues 285–307) contains four proline residues in comparison to only one in the *M. luteus* sequence. In another part of the protein, a site in domain V involved in interaction with the *E. coli* 23S ribosomal RNA contains a threonine residue in *M. luteus* and a glutamine residue in the *Arthrobacter* protein (position 646). Although the consequences of these alterations are difficult to predict without detailed information concerning the interactions between EF-G and the ribosome in each microorganism, the alterations provide evidence that thermal adaptation might involve modified interactions with the ribosome.

There is evidence for an altered binding or affinity for GTP/GDP in the *A. globiformis* SI55 protein. In the G domain at residue 80, *M. luteus* contains an asparagine residue whereas the *Arthrobacter* protein has a threonine residue. This residue is part of the conserved binding motif G2 (DTPGHV) and is believed to provide an important steric interaction with a conserved aspartate residue within the motif that is involved in complexing magnesium ions necessary for nucleotide binding (Al-Karadaghi et al. 1996). A change in steric conformation mediated by an asparagine to threonine replacement may alter the affinity of the nucleotide binding domain for either GDP or GTP.

A. globiformis SI55 *versus M. tuberculosis* EF-G

A comparison of the EF-G models for *A. globiformis* SI55 and *Mycobacterium tuberculosis* revealed a significant loss of salt bridges in the cold-adapted EF-G. However, in comparison to changes observed for the *Micrococcus luteus* EF-G, the changes occurred mainly in the G domain (positions 72, 147, 171, 178, 185/190, and 217). Through the change in position 171 (*M. tuberculosis* has a salt bridge between glutamate 176 and lysine 271), a salt bridge is missing that is adjacent to the GSAF motif (positions 263–266; see Fig. 1) and may therefore have an effect on nucleotide binding. At position 71, the slat bridge in the *M. tuberculosis* EF-G forms an interdomain link with domain II, but this salt bridge is predicted to be absent in the *Arthrobacter* protein. The anchoring of the G domain and domain II is further weakened by the loss of a hydrogen bond through position 364. Further reductions in stability are predicted by the losses of salt bridges between domains II and III through a change at position 328 and within domain V through changes at position 680.

An electrostatic repulsion in predicted between the surfaces of the *A. globiformis* G domain and domain V because of the positive potential exerted by the presence of a unique arginine residue (position 617), which the arginine at position 119. In addition, there is a loss of two hydrogen bonds at positions 644 and 674. The combined effects may cause a destabilization of the overall architecture of the *A. globiformis* SI55 EF-G. Changes in the general pattern of hydrophobic core regions and surface polarity/charge that were noted when comparing. *A. globiformis* SI55 and *M. luteus* were also observed for comparisons with the *M. tuberculosis* EF-G.

By comparison of the *A. globiformis* SI55 EF-G with *M. tuberculosis* EF-G, an insertion (5 residues) was identified in the G′ subdomain of the *M. tuberculosis* protein. This domain is speculated to function as a nucleotide exchange factor in bacterial EF-G proteins (Al-Karadaghi et al. 1996). An increased flexibility of the region conferred by the addition of a loose extension might increase the dynamics of the domain. The fact that the extension is present in the *M. luteus* protein suggests that alternative strategies are used by this protein to maintain structure and function at mesophilic temperatures. An additional variation in loop length was observed in the G domain of the *M. tuberculosis* EF-G protein. In *E. coli*, the loop has been shown to be involved in interaction with 23*S* ribosomal RNA. In *M. tuberculosis*, the loop is two residues larger. In addition to the small loop insertion, a number of other changes (positions 297, 310, and 580) were identified that may affect interaction with the ribosome.

Alteration in the nucleotide-binding efficiency is indicated by a glutamine residue at position 121 in *M. tuberculosis* that is able to form a hydrogen bond with an arginine residue in position 673 (domain V). This change is in helixC_G of the G domain (Al-Karadaghi et al. 1996), which propagates changes in the P loop (phosphate-binding loop) by a change toward the switch II region during nucleotide binding. The loss of a hydrogen bond observed in *A.*

globiformis SI55 EF-G by the substitution to a threonine may indicate greater dynamics in this region and consequently an increased binding efficiency in the *A. globiformis* SI55 EF-G.

Methionine residues are preferentially buried within the *M. tuberculosis* EF-G protein, whereas they tend to be directed toward the outside of the *Arthrobacter* EF-G. A similar pattern of distribution was observed for EF-2 proteins from psychrophilic, mesophilic, and thermophilic Archaea (Thomas and Cavicchioli 1998). However, their role in thermal adpatation has not been defined.

Conclusion

In this study we present a detailed comparison of the EF-G protein from the psychrotolerant baterium *Arthrobacter globiformis* SI55 with EF-G from the mesophiles *Micrococcus luteus* and *Mycobacterium tuberculosis*. Comparisons of primary amino acid sequences did not reveal differences that could obviously be attributed to adaptations necessary for activity at low temperature. This is not surprising in view of the fact that single amino acid substitutions have been shown to markedly alter the activity of proteins from psychrophilic bacteria (Alvarez et al. 1998) and such differences would be difficult to identify by sequence comparisons. To examine the structural context of sequence differences, three-dimensional protein models were generated and superimposed. From these analyses, modifications were clearly identified that may account for a more flexible structure of EF-G from the psychrotolerant bacterium. These changes included a loss in the number of salt bridges in intradomain and interdomain positions, increased solvent interactions mediated by greater charge and polarity on domain surfaces, loop insertions and loss of proline residues in loop structures, and an increase of hydrophobicity in core regions. Specific changes were also identified in the catalytic domain (G domain) and sites of potential ribosome interaction that may directly affect guanosine triphosphate (GTP) hydrolysis and elongation rates at low temperature.

It is noteworthy that although the types of changes identified between the EF-G model from *A. globiformis* with the models from each of the mesophiles are similar, the specific changes often occur within different molecular contexts. For example, the loss of salt bridges in the model from *A. globiformis* occurs throughout the protein in comparison to the *M. luteus* model, whereas they are largely clustered within the G domain in *M. tuberculosis*. The contextual changes between EF-G from *Arthrobacter* and the two mesophiles may imply unique processes of protein adaptation to similar thermal constraints. The nature of the changes may also have been influenced by specific cytoplasmic environments of the cells (salt concentration/composition or pH) and defined function roles (interactions with the ribosome and accessory factors). The modeling studies presented here provide the basis for comparing the biochemical and biophysical characteristics of wild-type and mutant proteins. Using protein stability and GTPase activity assays, it will be possible to determine the structural features of the *Arthrobacter* EF-G that are necessary for protein function at low temperature.

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