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Molecular cloning, sequencing, and expression of the heat-labile uracil-DNA glycosylase from a marine psychrophilic bacterium, strain BMTU3346

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Abstract The gene encoding a heat-labile uracil-DNA glycosylase (UDG) from a psychrophilic, gram-positive marine strain (BMTU3346) has been cloned, sequenced, and expressed in *Escherichia coli*. The UDG is a cold-active enzyme with an apparent temperature optimum of 35°C and a half-life of 2 min at 40°C. The amino acid sequence shows an identity of 39.1%–46.2% to UDGs from mesophilic bacteria. The primary structure was examined for features that could be related to the thermolability of the enzyme. The amino acid sequence of the heat-labile UDG shows 22 differences with respect to the consensus sequence derived from bacterial UDGs. Features previously recognized in cold-active enzymes such as extended surface loops or a decrease in the number of arginine residues or proline residues in loops were not observed. Because dominant features that could be related to the thermolability of the UDG from BMTU3346 cannot be identified, more subtle modifications of the conformation seem to be responsible for its thermolability.

Key words Uracil-DNA glycosylase · Gene sequence · Expression · Psychrophilic bacterium · Cold-active enzyme

Introduction

The nature of psychrophilic bacteria has stimulated efforts to understand the physiological and biochemical adaption to living at low temperatures. In recent years enzymes from psychrophilic organisms have been studied to investigate the structural basis of enzyme activity at low temperatures and of thermal inactivation and denaturation (Gerday et al. 1997; Danson and Hough 1998). Cold-active enzymes show a high specific activity at low temperatures, a low temperature optimum, and a limited thermal stability, resulting in rapid inactivation at moderate temperatures. With respect to the catalytic activity at low temperatures, it is assumed that cold-active enzymes are more flexible than their mesophilic counterparts, thereby lowering the energy required for conformational changes during catalysis. The thermal lability of psychrophilic enzymes is regarded as a consequence of this increased structural flexibility (Feller and Gerday 1997; Gerday et al. 1997).

UDGs (EC 3.2.2.3) are a highly conserved and extremely specific class of enzymes involved in the base repair pathway for the removal of uracil from DNA. The base uracil is removed from mutagenic U–G mispairs resulting from the deamination of cytosine and from U–A pairs resulting from misincorporation of dUMP during DNA synthesis (Lindahl 1994; Krokan et al. 1997). UDGs hydrolyze the N-glycosidic bond linking the base to the deoxyribose sugar, generating an abasic site, which is subsequently removed by an apurinic/apyrimidinic (AP) endonuclease and a phosphodiesterase. The resultant gap is filled by a DNA polymerase and sealed by a DNA ligase. UDGs have been identified in a variety of prokaryotic and eukaryotic organisms as well as in different viruses (Krokan et al. 1997). They consist of a single polypeptide chain, and the sequences range between 199 and 359 amino acids with a conserved C-terminal component of 199–220 residues and variable N-terminal extensions (Savva et al. 1995). The sequence alignment of several UDGs reveals a high degree of similarity between the genes and several clusters of residues that are highly conserved (Mol et al. 1995). The three-dimensional structures of the UDG from *Escherichia coli*, human, and herpes simplex virus (HSV) type I are very similar and consist of a single α/β domain containing a central four-stranded parallel β-sheet and eight α-helices (Mol et al. 1995; Putnam et al. 1999; Savva

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et al. 1995). Crystallographic and mutational studies have revealed the mechanisms for the selective binding and excision of uracil (Mol et al. 1995; Parikh et al. 1998; Slupphaug et al. 1996).

The first UDG described and characterized was the enzyme from *E. coli* (Lindal 1974; Lindahl et al. 1977). UDGs have also been characterized from such different bacteria as *Bacillus subtilis* (Cone et al. 1977), *Bacillus stearothermophilus* (Kaboev et al. 1981), *Micrococcus luteus* (Leblanc et al. 1982), *Mycoplasma lactucae* (Williams and Pollack 1990), *Thermothrix thiopara* (Kaboev et al. 1985), and *Mycobacterium smegmatis* (Purnapatre and Varshney 1998). UDGs were also isolated from such eukaryotic sources as human (Krokan and Wittwer 1981), and yeast (Crosby et al. 1981). Thermally stable UDGs have been identified in hyperthermophilic microorganisms (Koulis et al. 1996).

We have isolated a heat-labile UDG from a psychrophilic gram-positive marine bacterium, strain BMTU3346. The enzyme is rapidly inactivated at moderate temperatures and is less stable than its counterpart from the mesophilic *E. coli* (Sobek et al. 1996). From the structural comparison of cold-active enzymes from psychrophilic microorganisms to those of homologous mesophilic enzymes, several features have been identified that may be responsible for thermolability, including, for example, a reduced number of intramolecular interactions, reduced number of proline residues, and shorter surface loops (Gerday et al. 1997). To extend the knowledge of the molecular adaption of enzymes from psychrophilic microorganisms, the heat-labile UDG from the psychrophilic strain BMTU3346 was cloned, sequenced, and expressed in *E. coli*.

Materials and methods

Materials

Q-Sepharose fast flow and Ni-Chelating Sepharose fast flow were obtained from Pharmacia (Uppsala, Sweden). The psychrophilic strain BMTU3346 is a marine gram-positive bacterium kindly provided by H. Laatsch (University of Göttingen, Germany). The sequence of its 16*S* rRNA shows a high degree of homology to the 16*S* rRNA sequences of *Arthrobacter globiformis* (96.2%) and *Micrococcus luteus* (96.6%).

E. coli SURE (Stratagene, La Jolla, CA, USA) and *E. coli* NM522 (New England Biolabs, Beverly, MA, USA) were used for cloning and expression. Suicide vector pCAPs was used for the cloning of the *ung* gene and vector pQE30 (Qiagen, Hilden, Germany) was used for the expression of recombinant UDG. All enzymes, kits, and nylon membranes (positively charged) were from Roche (Mannheim, Germany) (unless otherwise indicated). All chemicals were of the highest purity commercially available. Usually, *E. coli* strains were grown in Luria broth, supplemented with ampicillin (100 μg/ml). For expression, the host strains were grown in medium 1 (casamino acids, 30 g/l; yeast extract, 15 g/l; glucose, 5 g/l; KH_2PO_4, 1.5 g/l; K_2HPO_4, 15 g/l; ampicillin, 0.1 g/l).

Polymerase chain reaction

Two highly conserved regions within the sequence of UDG from gram-positive bacteria were chosen to derive degenerate primers H1 (5′-GGS CAG GAC CCS TAC CAC GGS CCS GG-3′) and H4 (5′-GG SSW SGG GTG SGC SSW CTC GAT GAT-3′). Amplification was performed using a 100-μl incubation mixture containing 1 μg chromosomal DNA, 200 pmol of each primer, 1 mM each of dATP, dTTP, dCTP, and dGTP, 2 mM $MgCl_2$, and 5 U *Pwo* DNA polymerase in 1× *Pwo* DNA polymerase buffer. Thirty cycles were applied (3 min at 95°C, 1 min at 60°C, 1 min at 72°C).

Southern blot analysis

Southern blot analysis was performed as described by Ausubel et al. (1990). Chromosomal DNA from strain BMTU3346 was isolated using 500/G Qiagen Genomic Tips and the Genomic DNA Buffer Set according to the instructions for gram-positive bacteria described in the manuals. DIG-labeled hybridization probes were generated using the primers H1 and H4 by PCR according to the manual of the PCR DIG Probe Synthesis Kit. Hybridization was performed at 37°C. Hybridization was detected by color development with phosphatase according to the DIG Systems User's Guide for Filter Hybridization (Roche).

Cloning of the *ung* gene

Chromosomal DNA was partially digested with *Stu*I and cloned into *Mlu*NI-digested suicide vector pCAPs as described in the manufacturer's instruction. Screening for positive clones was performed by direct PCR as described by Güssow and Clackson (1989). Primers used were H1 and H4 for PCR.

Expression of UDG

For expression of UDG, the *ung* gene was cloned into vector pQE30. The *ung* gene was amplified from chromosomal DNA using primers P1 (5′-ACG GAG GGC TGA AGA TCT GAG CTG ATC AGC-3′, *Bgl*II site underlined) and P2 (5′-GGC CGA ACC AAG CTT CAG TCC CGT TCC AC-3′, *Hind*III site underlined). The PCR was set up as described earlier. After restriction of the PCR fragment with *Bgl*II and *Hind*III, the fragment was cloned into *Bgl*II/*Hind*III-digested vector pQE30 to obtain pQE30UNG. *E. coli* NM522 was transformed with pQE30UNG. Overnight cultures of *E. coli* NM522 pQE30UNG were inoculated in

medium 1 at 37°C. When the culture reached OD_{600} = 5.0, it was cooled to 25°C and the expression was induced by adding isopropyl thiogalactoside (IPTG) in a final concentration of 0.5 mM.

Purification of recombinant UDG

During the purification procedure, the temperature was maintained at 4°C. *E. coli* NM522 pQE30UNG cells (80 g) were suspended in 300 ml buffer 1 (10 mM Tris/HCl, pH 8.0, 10 mM 2-mercaptoethanol, 5% glycerol, 1.5 mM phenylmethylsulfonyl fluoride, 3.3 mg/ml lysozyme). After lysis by sonication, Polymin P was added to a final concentration of 0.3%. The lysate was stirred for 30 min and finally centrifuged for 30 min at 13000× *g*. The supernatant was loaded onto a Q-Sepharose fast flow column (2.6 cm × 10 cm) equilibrated with buffer 2 (10 mM Tris/HCl, pH 8.0, 10 mM 2-mercaptoethanol, 10% glycerol). After washing the column was eluted with 600 ml applying a salt gradient (0–1 M NaCl). UDG eluted at about 300 mM NaCl; fractions were pooled and dialyzed against buffer 3 (50 mM sodium phosphate, pH 8.0, 10 mM 2-mercaptoethanol, 10% glycerol, 10 mM imidazole, 300 mM NaCl). The dialyzed fractions were applied to a Ni-Chelating Sepharose fast flow column (2.6 cm × 10 cm) equilibrated with buffer 3. After washing with buffer 3 containing 20 mM imidazole, the elution was performed with a linear gradient from 20 to 500 mM imidazole in buffer 3. Active fractions were pooled, dialyzed against buffer 4 (20 mM Tris/HCl, pH 8.0, 0.1 mM EDTA, 100 mM KCl, 1 mM DTT, 0.5% Tween 20, 0.5% polydocanol), and stored at −20°C.

Analytical methods

UDG activity and protein concentrations were determined as described for the native enzyme (Sobek et al. 1996). Polyacrylamide gel electrophoresis under denaturing conditions was performed using 10% polyacrylamide gels according to the instructions of the supplier (Novex, San Diego, USA). Protein bands were stained with Coomassie brilliant blue R-250.

Thermostability analysis

For studies on the influence of the temperature, additives, and pH on the stability of the purified recombinant enzyme, the experiments were carried out as described for the native enzyme (Sobek et al. 1996). The effect of pH on the stability was tested in 40 mM Britton-Robinson buffer (phosphate, acetate, borate/NaOH).

Computer programs

Protein sequence data were taken from SWISS-PROT Protein Sequence Database. The program package DSSP (Kabsch and Sanders 1983) was used to assign the secondary structure data. Computer analysis of the DNA and protein sequences was performed using the Software package from the Genetics Computer Group of the University of Wisconsin (GCG, version 8).

Results

Cloning of the heat-labile UDG

UDGs contain several regions of highly conserved sequences (Mol et al. 1995). Degenerate primers were chosen from these conserved regions in a multiple alignment of gram-positive UDG sequences to amplify a fragment of the *ung* gene. Chromosomal DNA of BMTU3346 was isolated and used as template in a polymerase chain reaction with primers H1 and H4. The primers bind to the highly conserved regions coding for residues 177–185 and residues 299–307. The 3′-end of primer H4 encodes for the amino acid motif Ile-Ile-Glu-Ser (residues 299–302), which is characteristic for UDGs from gram-positive bacteria (see Fig. 1). A single PCR product of about 400 bp was obtained, cloned into the vector pCAPs, and sequenced. The cloned 400-bp fragment showed overall similarity to UDGs and thus was used as a gene-specific probe. Probing a blot of *Stu*I-digested chromosomal DNA with the DIG-labeled 400-bp fragment resulted in the identification of a single hybridizing band of about 3.8 kb. For the cloning of the entire *ung* gene, chromosomal DNA from BMTU3346 was digested with *Stu*I, cloned into vector pCAPs, and transformed into *E. coli* SURE. Positive clones were identified by direct PCR using primers H1 and H4. The *ung* sequence was obtained by direct sequencing of one of the positive clones. Both strands of the *ung* gene were sequenced.

Analysis of the amino acid sequence

The DNA sequence of the UDG gene from BMTU3346 and the deduced amino acid sequence are shown in Fig. 2. The gene is 684 bp long and codes for a protein of 227 amino acids. The G+C content of the *ung* gene is 71.05%. The psychrophilic UDG has an overall length similar to that of the bacterial UDGs (217–228 amino acid residues) and does not have the extended N-terminus seen in viral HSV type I UDG (334 amino acid residues). The amino acid sequence of the heat-labile UDG was compared to sequences of six other UDGs. They represent sequences of three gram-negative bacteria (*E. coli*, *Pseudomonas denitrificans*, *Haemophilus influenzae*), two gram-positive bacteria (*Streptococcus pneumoniae*, *Bacillus subtilis*), and HSV type I (Fig. 1).

The secondary structure is derived from the high-resolution structure of the UDG of HSV type 1 (Savva et al. 1995). The UDG from BMTU3346 shows the highest identity to the UDG from *P. denitrificans* (46.2%). Lower

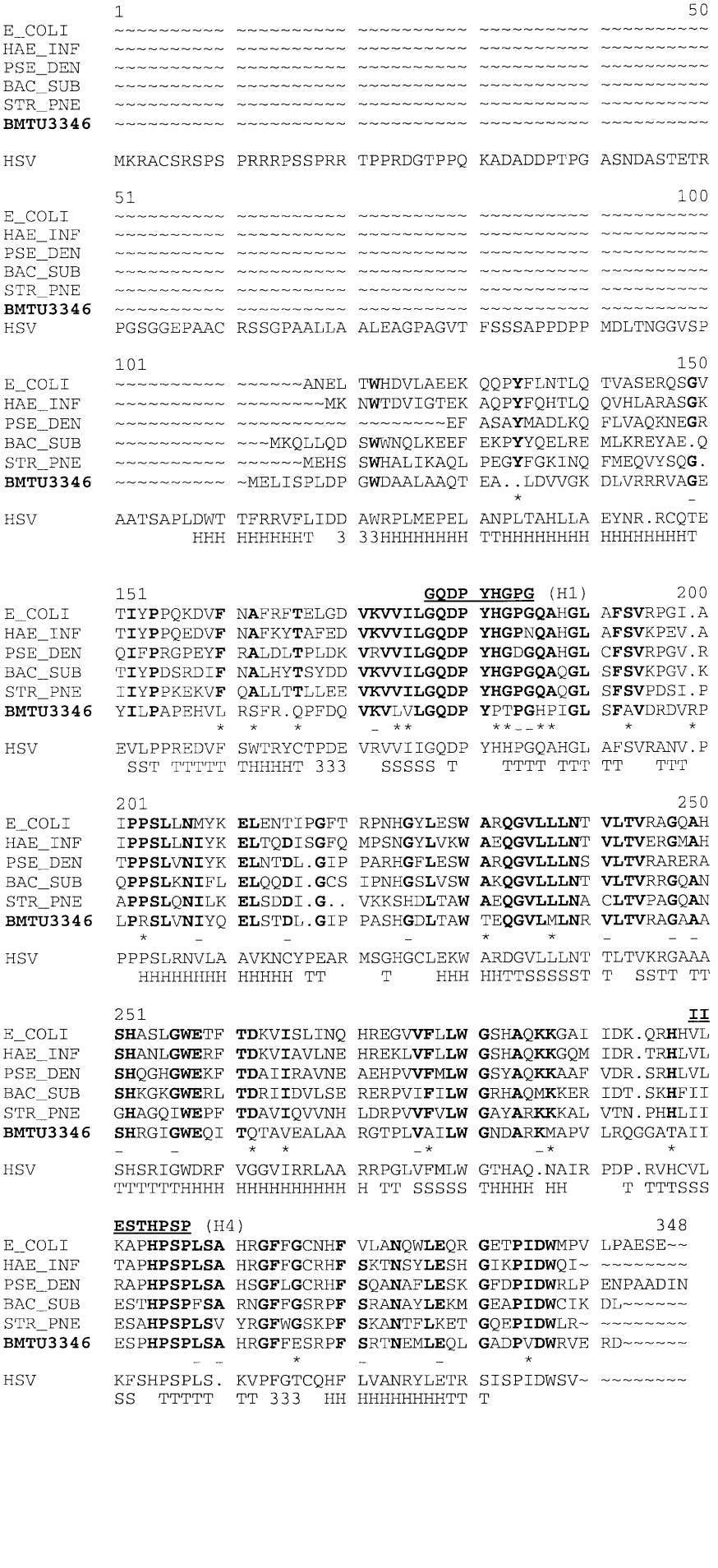

Fig. 1. Amino acid alignment of the UDG from BMTU3346 with the homologous enzymes from *Escherichia coli* (E_COLI), *Haemophilus influenzae* (HAE_INF), *Pseudomonas denitrificans* (PSE_DEN), *Bacillus subtilis* (BAC_SUB), *Streptococcus pneumoniae* (STR_PNE), and herpes simplex virus type I (HSV). The secondary structures shown are α-helices (H), 3_{10}-helices (3), turns (T), and β-strands (S). The secondary structures were derived from the high-resolution structure of the UDG of HSV type 1 (Savva et al. 1995). The regions used for primer design (primer H1 and H4) are indicated. **Bold letters** indicate identical amino acids conserved in at least five of the six bacterial enzymes. Differences in the sequence of the UDG from BMTU3346 (*asterisks*) and in one of the five other bacterial enzymes (*bars*) are indicated

homology is found with UDGs from *S. pneumoniae* (43.7%), *B. subtilis* (42.1%), *H. influenzae* (41.4%), and *E. coli* (39.1%) and the lowest identity to the homologous enzyme from HSV type I (33.5%). The catalytic residues (Asp179, Asn239, His304) have been identified from the high-resolution X-ray structure of the UDG from HSV type I (Savva et al. 1995) and are conserved among all aligned UDGs. Additionally, the amino acid sequence of the heat-labile UDG was compared to the sequences of the five bacterial UDGs to define a bacterial consensus sequence. The heat-labile UDG from BMTU3346 shows 22 differences in positions that are strictly conserved among the other five bacterial UDGs (Fig. 1).

Expression and purification of the psychrophilic UDG

For expression, the *ung* gene was amplified from chromosomal DNA and cloned into expression vector pQE30. The resulting plasmid pQE30UNG was transformed into *E. coli* host strains. The recombinant heat-labile UDG was expressed with a His_6 affinity tag at the N-terminus. In initial experiments to express the enzyme in *E. coli* SURE, it was not produced in a soluble form but was trapped within inclusion bodies. Soluble UDG was obtained in an active form by expression of the *ung* gene in *E. coli* NM522 grown at a reduced temperature of 25°C. Still, the majority of the expressed enzyme was found in inclusion bodies (data not shown). The soluble fraction of the recombinant UDG was purified to homogeneity using the purification procedure described previously. Two chromatographic separations were applied to remove nonspecific *E. coli* nucleases. Because of the high content of nonspecific nucleases, the specific UDG activity could not be determined in crude extracts and in fractions of the first purification steps. The final fraction of the recombinant UDG shows the same specific activity (4×10^5 U/mg) as previously determined for the native enzyme (Sobek et al. 1996). This result confirms that the recombinant enzyme is correctly folded. Purified enzyme preparations showed a single band in SDS-polyacrylamide gel electrophoresis (Fig. 3). The molecular mass of the purified recombinant enzyme was determined to be 26.073 Da by MALDI-TOF mass spectrometry, which is in close agreement with the predicted molecular mass of 26.062 Da for the enzyme fused to the N-terminal His_6 affinity tag (data not shown).

Temperature optimum and thermal stability

The apparent temperature optimum of the recombinant heat-labile UDG was determined; in addition, the temperature optimum for the *E. coli* UDG was measured as a reference mesophilic enzyme. The heat-labile UDG has an apparent temperature optimum of about 35°C, which is significantly lower than the optimum of the UDG from *E. coli*. At 20°C the heat-labile UDG shows about 30% of its

Fig. 2. Nucleotide and deduced amino acid sequence of the UDG from BMTU3346. The nucleotides are numbered consecutively starting from ATG coding for the N-terminal methionine

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1     ATGGAGCTGATCAGCCCGCTGGATCCCGGATGGGACGCGGCCCTGGCCGCGCAGACCGAG     60
      M  E  L  I  S  P  L  D  P  G  W  D  A  A  L  A  A  Q  T  E

61    GCCCTGGACGTCGTCGGGAAGGACCTGGTGCGCCGCCGCGTGGCCGGGGAGTACATCCTG    120
      A  L  D  V  V  G  K  D  L  V  R  R  R  V  A  G  E  Y  I  L

121   CCGGCTCCCGAGCATGTCCTGCGCTCCTTCCGCCAGCCCTTCGACCAGGTCAAGGTCCTG    180
      P  A  P  E  H  V  L  R  S  F  R  Q  P  F  D  Q  V  K  V  L

181   GTGCTGGGACAGGACCCTTACCCCACCCCGGGGCATCCGATCGGCCTGAGCTTCGCCGTG    240
      V  L  G  Q  D  P  Y  P  T  P  G  H  P  I  G  L  S  F  A  V

241   GACCGTGATGTGCGGCCGCTGCCGCGGTCGCTGGTGAACATCTACCAGGAGCTCTCCACT    300
      D  R  D  V  R  P  L  P  R  S  L  V  N  I  Y  Q  E  L  S  T

301   GACCTGGGCATCCCGCCGGCCTCGCACGGAGACCTCACCGCGTGGACGGAACAGGGCGTG    360
      D  L  G  I  P  P  A  S  H  G  D  L  T  A  W  T  E  Q  G  V

361   CTGATGCTGAACCGCGTGCTCACCGTGCGGGCGGGGGCCGCAGCCAGCCACCGCGGGATC    420
      L  M  L  N  R  V  L  T  V  R  A  G  A  A  A  S  H  R  G  I

421   GGGTGGGAACAGATCACCCAGACGGCGGTGGAGGCGTTGGCCGCGCGGGGCACCCCGCTG    480
      G  W  E  Q  I  T  Q  T  A  V  E  A  L  A  A  R  G  T  P  L

481   GTGGCGATCCTGTGGGGCAACGACGCCCGGAAGATGGCTCCGGTGCTGCGGCAGGGCGGT    540
      V  A  I  L  W  G  N  D  A  R  K  M  A  P  V  L  R  Q  G  G

541   GCCACCGCGATCATCGAGTCGCCCCACCCCTCCCCGCTCTCGGCGCATCGGGGGTTCTTC    600
      A  T  A  I  I  E  S  P  H  P  S  P  L  S  A  H  R  G  F  F

601   GAGTCCCGTCCGTTCAGCCGCACCAATGAGATGCTGGAGCAGCTGGGCGCCGACCCCGTG    660
      E  S  R  P  F  S  R  T  N  E  M  L  E  Q  L  G  A  D  P  V

661   GACTGGCGGGTGGAACGGGACTGA      684
      D  W  R  V  E  R  D  *
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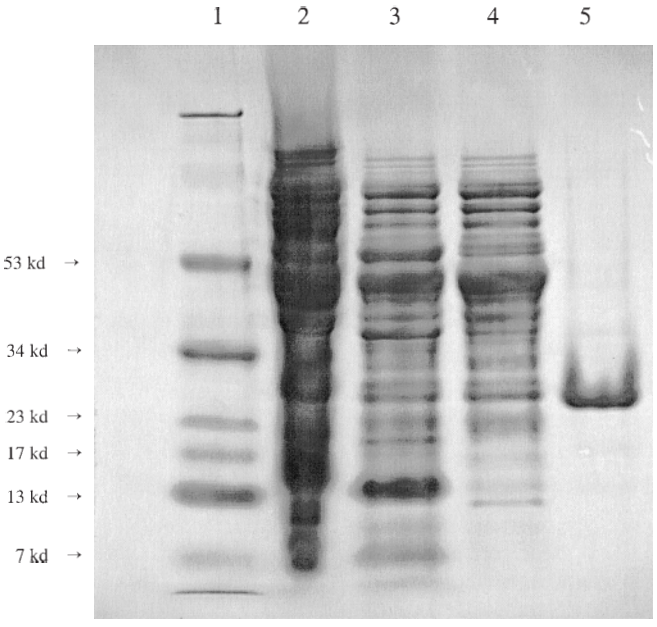

Fig. 3. Expression and purification of recombinant UDG from BMTU 3346. Samples of different purification steps were separated on a 10% polyacrylamide gel under denaturing conditions. Protein bands were stained with Coomassie brilliant blue R-250. *Lane 1*, molecular weight marker; *lane 2*, whole-cell extract of *E. coli* NM522 pQE30UNG; *lane 3*, supernatant of the Polymin P precipitation; *lane 4*, pooled fractions after chromatography on Q-Sepharose; *lane 5*, pooled fractions after chromatography on Ni-Chelating Sepharose. The molecular weights of the marker proteins are indicated

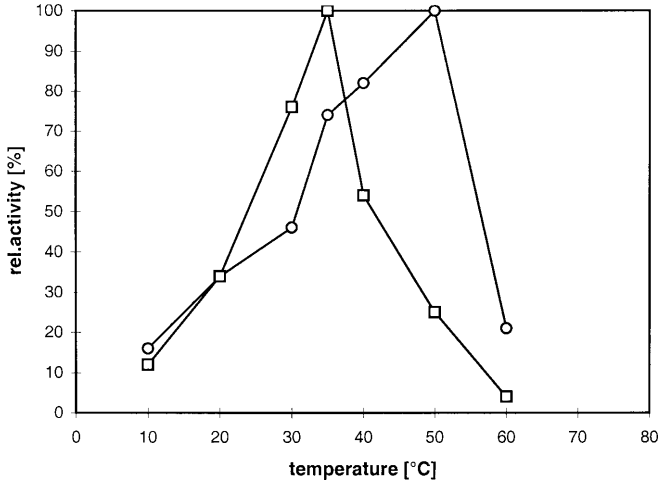

Fig. 4. Temperature optima of the UDG from BMTU3346 (*squares*) and *E. coli* (*circles*). Acitivity was assayed in 60 mM Tris/HCl, pH 8.0, 1 mM EDTA, 1 mM DTT, and 0.1 mg/ml BSA for 10 min at the temperatures indicated. 3H-Uracil-labeled calf thymus DNA was used as substrate as described by Sobek et al. (1996)

apparent maximal activity (Fig. 4). The recombinant heat-labile UDG is stabilized by additives (KCl, glycerol; Fig. 5B). At 40°C the recombinant UDG showed the same half-life time of 2.0 min as described for the native enzyme (Fig. 5A) (Sobek et al. 1996). This result indicates that the stability of the recombinant enzyme is not influenced by the N-terminal His_6 affinity tag.

Discussion

Because of its low apparent temperature optimum and low thermostability, the UDG from BMTU3346 can be classified as a cold-active enzyme (Gerday et al. 1997). To date, only limited thermostability data are available for UDGs (Cone et al. 1977; Koulis et al. 1996; Sobek et al. 1996). Thermostability data and primary sequence data are available for the UDGs of the mesophilic bacteria *E. coli* and *B. subtilis*. The half-time of inactivation of the enzyme from *E. coli* was found to be 27 min at 40°C (Sobek et al. 1996). For the UDG from *B. subtilis*, 57% residual activity was determined after inclubation at 50°C for 10 min (Cone et al. 1977).

To identify features that are related to the thermolability of the heat-labile UDG, its primary structure was compared to the sequences of the enzymes from its mesophilic bacterial counterparts. Extensions of surface loops can be a possible structural characteristic of a cold-active enzyme causing increased flexibility and thus thermolability (Danson and Hough 1998; Feller and Gerday 1997; Gerday et al. 1997). However, the UDG from BMTU3346 has an overall length similar to that of its mesophilic counterparts, and only two unique insertions, each of a single residue, are detected in the amino acid alignment (Arg199, Gly294). Arginine residues have been discussed as playing an important role in the stabilization of proteins by forming salt bridges and ion pairs (Feller and Gerday 1997). However, the UDG from BMTU3346 has a higher content of arginine residues (17 Arg) than the homologous enzymes from *E. coli* (10 Arg) and *B. subtilis* (12 Arg). Proline residues are known to stabilize the folded conformation of a protein by decreasing the entropy of unfolding. The avoidance of proline residues in loops and turns connecting secondary structures has been observed in many psychrophilic enzymes (Feller and Gerday 1997). The UDG from BMTU3346 has a higher content of proline residues (20 Pro) than the enzymes from residues from *E. coli* (16 Pro) and *B. subtilis* (12 Pro). When the positions of proline residues are compared, there are six (two) positions in loops and turns where proline is present in the enzyme from *E. coli* (*B. subtilis*) but absent in the heat-labile UDG. On the other hand, there are ten positions where proline is detected in the sequence of the heat-labile UDG, but not in the enzymes from *E. coli* and *B. subtilis*.

Because dominant features that could be related to the thermolability of the UDG from BMTU3346 cannot be identified, more subtle modifications of the conformation seem to be responsible for its thermolability. The heat-labile UDG shows 22 differences in amino acid positions that are strictly conserved among bacterial UDGs. In 17 of these positions, the differences occur in secondary structure elements and might affect the secondary structure

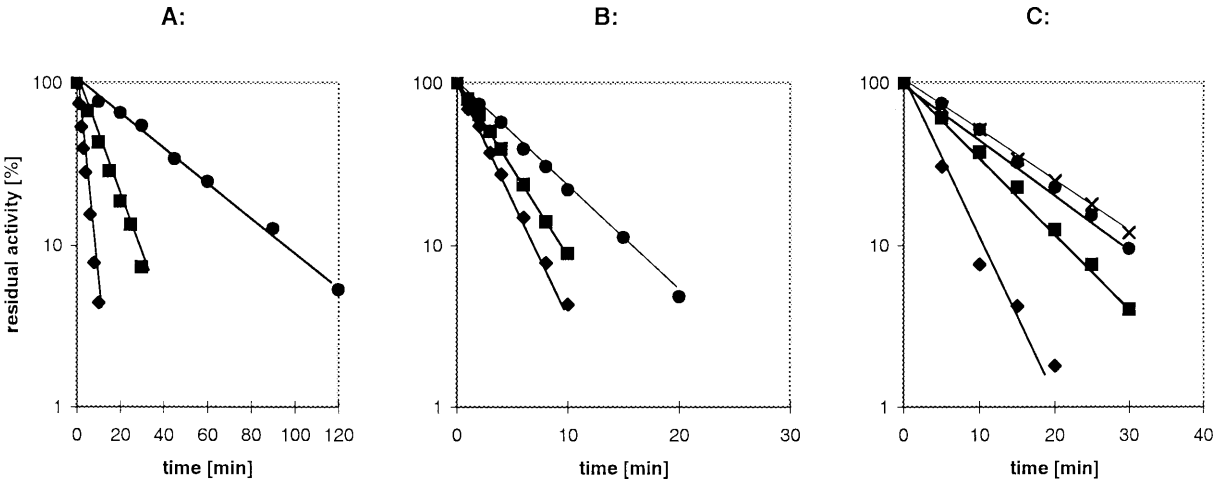

Fig. 5A–C. Stability of recombinant heat-labile UDG. Two units of UDG were incubated under different conditions in 100 µl reaction volume. At different times, aliquots were removed, cooled on ice, and the residual activity determined. **A** Thermostability: UDG was incubated in 10 mM Tris/HCl, pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl at 30°C (●), 35°C (■), and 40°C (◆). **B** Effect of additives: UDG was incubated at 40°C in 10 mM Tris/HCl, pH 8.3, 1.5 mM $MgCl_2$ with 50 mM KCl (◆), 250 mM KCl (■), and 10% glycerol (v/v) (●). **C** Effect of pH. UDG was incubated at 35°C in 40 mM Britton-Robinson buffer at different pH values; pH 6.0 (◆), pH 7.0 (■), pH 8.0 (●), and pH 9.0 (×)

and hence the stability of the enzyme. The deletion of two amino acids (residues 133 and 134) could affect the formation of the third helix in the structure. However, the significance of these changes for the thermolability of the heat-labile UDG is difficult to predict. Only minor alterations in the primary structure of psychrophilic enzymes have been reported (Feller and Gerday 1997). A detailed analysis of the structural features such as surface to volume ratio, hydrogen bonding, and ionic interactions that are related to the thermolability of the enzyme must await the crystal structure of the enzyme. This information would also allow a comparison to the structure of the UDG from *E. coli*, the crystal structure of which has been recently solved (Putnam et al. 1999).

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