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Communication

The pH optimum of native uracil-DNA glycosylase of *Archaeoglobus fulgidus* compared to recombinant enzyme indicates adaption to cytosolic pH

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Uracil-DNA glycosylase of *Archaeoglobus fulgidus* (Afung) in cell extracts exhibited maximal activity around pH 6.2 as compared to pH 4.8 for the purified recombinant enzyme expressed in *Escherichia coli*. Native Afung thus seems to be adapted to the intracellular pH of *A. fulgidus*, determined to be 7.0±0.1. Both recombinant and native Afung exhibited a broad temperature optimum for activity around 80°C, reflecting the *A. fulgidus* optimal growth temperature of 83°C. Adaption to the neutral conditions in the *A. fulgidus* cytoplasm might be due to covalent modifications or accessory factors, or due to a different folding when expressed in the native host.

Key words: DNA repair, uracil-DNA glycosylase, uracil, deamination, hyperthermophiles

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INTRODUCTION

Hydrolytic deamination of cytosine to uracil is, after depurination, the most frequent damaging event to DNA. Such uracils in DNA result in G·C to A·T transition mutations if they are not repaired before replication (Lindahl, 1993). In addition, some dUTP escape hydrolysis by dUTPase causing a certain amount of dUMP introduced into DNA opposite adenine during replication (Kornberg & Baker, 1992). Irrespective of its mode of appearance, uracil is removed from the DNA in virtually all cells by uracil-DNA glycosylase (UDG) enzymes (EC 3.2.2.27) initiating the base excision repair pathway (Lindahl, 1993). The resulting abasic or apurinic/apyrimidinic site is subsequently removed by downstream incising/excising functions and the integrity of DNA is finally restored by DNA polymerase and DNA ligase (Krokan *et al.*, 1997). Since the discovery of the first (family 1) UDG in *Escherichia coli* more than three decades ago (Lindahl, 1974), numerous such enzymes have been characterized as well as at least 4 other families (families 2–5) have been added to the list of UDGs (Chung *et al.*, 2003; Pearl, 2000; Sartori *et al.*, 2002).

The rate of DNA-cytosine deamination is considerably higher at high compared to moderate temperatures (Frederico *et al.*, 1990; Lindahl & Nyberg, 1974), thus adding to the challenge of genomic maintenance in hyperthermophilic organisms (for a review on DNA repair in hyperthermophilic archaeons we refer to: Grogan, 2004). UDG activity in hyperthermophilic microorganisms was first reported in 1996 (Koulis *et al.*, 1996). The hyperthermophilic archaeon *Archaeoglobus fulgidus*, a strict anaerobe growing optimally at 83°C (Stetter, 1992), contains a family 4 type of UDG named Afung, which has been cloned and over-expressed in *E. coli* followed by biochemical characterization (Engstrom *et al.*, 2012; Knævelsrud *et al.*, 2001; Sandigursky & Franklin, 2000). Recently, immunodepletion of UDG activity present in archaeal cell extract showed that Afung is the principal and probably the only UDG in *A. fulgidus* (Knævelsrud *et al.*, 2010).

Here we demonstrate that the UDG activity in cell extracts (i.e., wild type or native enzyme; nAfung) exhibits temperature dependence similar to that of the recombinant enzyme (rAfung) (Knævelsrud *et al.*, 2001), which reflects optimal growth temperature of *A. fulgidus*. This contrasts with the results showing a significantly different pH dependence of the activity of nAfung compared to rAfung, where nAfung has an optimal UDG activity much closer to–and thus exhibits increased adaptation to–the neutral cytosolic pH of *A. fulgidus*. The present report provides the first description of how nAfung activity is dependent on temperature and pH.

MATERIALS AND METHODS

Enzymatic uracil release was analysed using cell extracts prepared from the *A. fulgidus* type strain VC16 (DSMZ 4303 (Stetter, 1992)) grown anaerobically under Ar at 83°C, where the cells were harvested in the early stationary phase essentially as described previously (Knævelsrud *et al.*, 2001). Protein concentration was determined as described (Bradford, 1976) using bovine serum albumin as the standard. To analyse temperature dependence, enzyme was incubated with [^{3}H]uracil-DNA (2000 dpm, 2 pmol DNA-uracils; specific activity, 1110 dpm/pmol) for 10 min in 50 µL 70 mM Mops, pH 7.5, 1 mM EDTA, 1 mM dithiothreitol, 100 mM KCl, 5% (v/v) glycerol (reaction buffer) (Knævelsrud *et al.*, 2001). To analyse pH dependence, exactly the same incubation conditions were used except that in this case the reaction buffer was a modified universal buffer with different pH values (Bjelland & Seeberg, 1987; Johnson & Lindsey, 1939; Knævelsrud *et al.*, 2001). The reactions were carried out at 80°C and 95°C. The amount of radioactivity present in the supernatant was determined

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Abbreviations: Afung, uracil-DNA glycosylase from *Archaeoglobus fulgidus*; nAfung, native Afung; rAfung, recombinant Afung; UDG, uracil-DNA glycosylase

following DNA precipitation with ethanol, and control values from incubations without enzyme were subtracted (Knævelsrud *et al.*, 2001). The intracellular pH of *A. fulgidus* was determined by ^{31}P-nuclear magnetic resonance spectroscopy at SINTEF (The Foundation for Scientific and Industrial Research at the Norwegian Institute of Technology), Trondheim, Norway.

RESULTS AND DISCUSSION

Temperature dependence for the enzymatic uracil release by *A. fulgidus* was determined by incubating cell extracts (nAfung) with [^{3}H]uracil-DNA from 20°C to 100°C. The results show that UDG activity was detected at all temperatures but varied significantly (Fig. 1). Previously we described that the abrupt increase in activity of rAfung at 60°C was accompanied by a conformational conversion to a more open structure (Knævelsrud *et al.*, 2001). The present results show that nAfung follows rAfung by exhibiting an abrupt increase in activity at 60°C to reach its maximum around 80°C (Fig. 1); the latter corresponding neatly with the optimal growth temperature of 83°C for *A. fulgidus*. The activity decreases as the temperature approaches 100°C, although providing a significant repair efficiency several degrees beyond the optimal growth temperature. Since the *A. fulgidus* cell extracts were routinely prepared from cells grown at the optimal temperature, we were also curious as to whether the growth temperature might influence the *in vivo* level of UDG activity. However, extracts prepared from cells grown at 60°C, 83°C and 90°C showed similar levels of UDG activity, as measured at all these three different temperatures (data not shown), arguing against *afung* gene induction or repression as a response to temperature changes.

Then, the pH dependence for the enzymatic release of uracil from [^{3}H]uracil-DNA by cell extracts was determined. The results show a peak of maximal activity at pH 6.2, which is quite different from rAfung which exhibits the highest activity at pH 4.8 (Fig. 2) (Knævelsrud *et al.*, 2001). Importantly, it has been reported that pH optima of proteins measured *in vitro* are dependent on storage pH (Behzadi *et al.*, 1999), which was 6 for rAfung and 7.5 for nAfung (Knævelsrud *et al.*, 2001). Theoretically, this might have influenced our results. However, since the pH optimum changes inversely with storage pH (Behzadi *et al.*, 1999), it would, in our case, imply that the difference in pH optimum between rAfung and nAfung should be even larger if UDG activity was determined after storage of the two Afung preparations at exactly the same pH. In conclusion, nAfung as compared to rAfung exhibits maximal activity at a pH significantly closer to the intracellular pH of *A. fulgidus*, which was determined to be 7.0±0.1 at 61°C (not shown).

In spite of our demonstration of a significant enzymatic difference between Afung in cell extracts (nAfung) and the *E. coli*-produced rAfung protein (Fig. 2), no difference in their molecular weights has been observed (Knævelsrud *et al.*, 2010). Alternative folding of nAfung and rAfung facilitated by different chaperone functions in so distantly related organisms as *A. fulgidus* and *E. coli* (Lund, 2011), where the proteins are produced under extremely different temperature conditions, could in principle explain such a major change in function without an accompanying mass change. This also accords with quite different kinetic parameters determined for nAfung and rAfung (Knævelsrud *et al.*, 2010). However, alternative folding patterns to be stably perpetuated at high

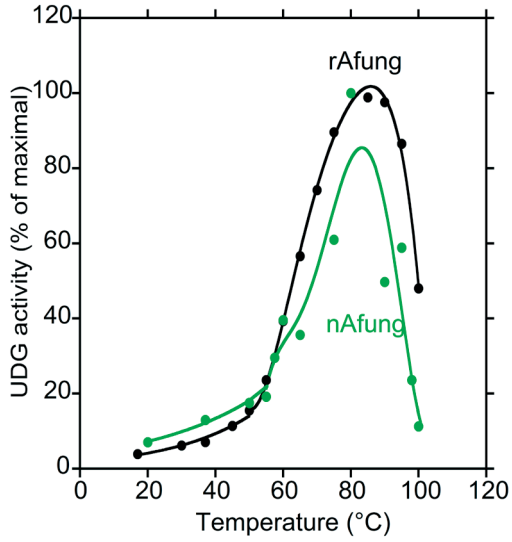

Figure 1. Temperature dependence for the excision of uracil from DNA by *A. fulgidus* cell extracts (nAfung, ●) compared to rAfung (●).
Cell extracts (1–4 µg protein) were incubated with [^{3}H]uracil-DNA as described in Materials and Methods. Each value represents the median of 4–33 (●, maximum = 100%) or 4 [0.9 pmol enzyme; ●, maximum = 100% (Knævelsrud *et al.*, 2001)] independent measurements.

temperature sound unlikely, especially for Afung, which we found, as mentioned above, to convert to a more open structure during transition from low to high temperature (Knævelsrud *et al.*, 2001). Another possibility, although in our opinion also quite unlikely, is that Afung interacts with certain cellular components in a non-covalent manner in order to fulfil the requirements of the *in vivo* conditions. Interestingly, the human family 1 UDG (hUNG2) has recently been shown to be regulated by site-specific phosphorylations of Ser/Thr residues (Hagen *et al.*, 2008). It is tempting to speculate whether

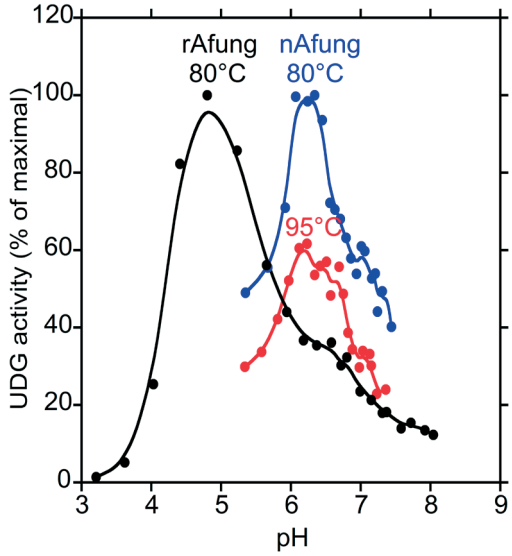

Figure 2. pH dependence for the excision of uracil from DNA by *A. fulgidus* cell extracts (nAfung, ●●) compared to rAfung (●).
Cell extracts (38 µg protein) were incubated with [^{3}H]uracil-DNA at 80°C (●) or 95°C (●) as described in Materials and Methods. Each value represents the median of 4–9 (●, maximum = 100%; ●, % of maximum at 80°C) or 3 [0.2 pmol enzyme; ●, maximum = 100% (Knævelsrud *et al.*, 2001)] independent measurements.

serine-threonine kinases of *A. fulgidus* (LaRonde-LeBlanc *et al.*, 2005a; LaRonde-LeBlanc *et al.*, 2005b; Shi *et al.*, 1998) may serve a similar role.

Afung is one of the most studied family 4 UDGs (Knævelsrud *et al.*, 2010; Knævelsrud *et al.*, 2001; Sandigursky & Franklin, 2000), and is, due to the presence of a $[4Fe–4S]^{2+}$ cofactor, redox-active when bound to DNA. Thus, together with some other DNA glycosylases, Afung has been studied in the context of an interesting theory on the involvement of redox activity and DNA-mediated electron transfer in DNA damage recognition (Boal *et al.*, 2005; Engstrom *et al.*, 2012). Our precise measurement of the optimal pH for Afung in cell extracts (nAfung) compared to purified enzyme (rAfung; Fig. 2) (Knævelsrud *et al.*, 2001), together with the determination of intracellular pH of *A. fulgidus*, may aid the development of *in vitro* conditions that closer mimic *in vivo* electrolyte conditions for damage detection and excision.

We want to conclude the following. Although increase in cell temperature results in an increased DNA damage burden, *in casu* uracil, our results show that the expression of the *afung* gene does not seem to be affected by growth temperature. The catalytic function of nAfung has evolved to neatly adapt to the optimal growth temperature of *A. fulgidus*, because its temperature dependence can be explained by the characteristics of rAfung and thus the primary protein structure. Adaption of Afung to the neutral cytosolic pH can hardly be explained by *afung* gene alterations alone, and is likely provided by certain *A. fulgidus in vivo* reactions or accessory factors.

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REFERENCES

Behzadi A, Hatleskog R, Ruoff P (1999) Hysteretic enzyme adaptation to environmental pH: Change in storage pH of alkaline phosphatase leads to a pH-optimum in the opposite direction to the applied change. *Biophys Chem* **77**: 99–109.

Bjelland S, Seeberg E (1987) Purification and characterization of 3-methyladenine DNA glycosylase I from *Escherichia coli*. *Nucleic Acids Res* **15**: 2787–2801.

Boal AK, Yavin E, Lukianova OA, O'Shea VL, David SS, Barton JK (2005) DNA-bound redox activity of DNA repair glycosylases containing [4Fe-4S] clusters. *Biochemistry* **44**: 8397–8407.

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72**: 248–254.

Chung JH, Im EK, Park H-Y, Kwon JH, Lee S, Oh J, Hwang K-C, Lee JH, Jang Y (2003) A novel uracil-DNA glycosylase family related to the helix–hairpin–helix DNA glycosylase superfamily. *Nucleic Acids Res* **31**: 2045–2055.

Engstrom LM, Partington OA, David SS (2012) An iron-sulfur cluster loop motif in the *Archaeoglobus fulgidus* uracil-DNA glycosylase mediates efficient uracil recognition and removal. *Biochemistry* **51**: 5187–5197.

Frederico LA, Kunkel TA, Shaw BR (1990) A sensitive genetic assay for the detection of cytosine deamination: Determination of rate constants and the activation energy. *Biochemistry* **29**: 2532–2537.

Grogan DW (2004) Stability and repair of DNA in hyperthermophilic archaea. *Curr Issues Mol Biol* **6**: 137–144.

Hagen L, Kavli B, Sousa MML, Torseth K, Liabakk NB, Sundheim O, Peña-Diaz J, Otterlei M, Hørning O, Jensen ON, Krokan HE, Slupphaug G (2008) Cell cycle-specific UNG2 phosphorylations regulate protein turnover, activity and association with RPA. *EMBO J* **27**: 51–61.

Johnson WC, Lindsey AJ (1939) An improved universal buffer. *Analyst (London)* **64**: 490–492.

Knævelsrud I, Moen MN, Grøsvik K, Haugland GT, Birkeland N-K, Klungland A, Leiros I, Bjelland S (2010) The hyperthermophilic euryarchaeon *Archaeoglobus fulgidus* repairs uracil by single-nucleotide replacement. *J Bacteriol* **192**: 5755–5766.

Knævelsrud I, Ruoff P, Ånensen H, Klungland A, Bjelland S, Birkeland N-K (2001) Excision of uracil from DNA by the hyperthermophilic Afung protein is dependent on the opposite base and stimulated by heat-induced transition to a more open structure. *Mutat Res* **487**: 173–190.

Kornberg A, Baker TA (1992) *DNA Replication*, 2nd edition. New York: W.H. Freeman.

Koulis A, Cowan DA, Pearl LH, Savva R (1996) Uracil-DNA glycosylase activities in hyperthermophilic micro-organisms. *FEMS Microbiol Lett* **143**: 267–271.

Krokan HE, Standal R, Slupphaug G (1997) DNA glycosylases in the base excision repair of DNA. *Biochem J* **325**: 1–16.

LaRonde-LeBlanc N, Guszczynski T, Copeland T, Wlodawer A (2005a) Autophosphorylation of *Archaeoglobus fulgidus* Rio2 and crystal structures of its nucleotide-metal ion complexes. *FEBS J* **272**: 2800–2810.

LaRonde-LeBlanc N, Guszczynski T, Copeland T, Wlodawer A (2005b) Structure and activity of the atypical serine kinase Rio1. *FEBS J* **272**: 3698–3713.

Lindahl T (1974) An *N*-glycosidase from *Escherichia coli* that releases free uracil from DNA containing deaminated cytosine residues. *Proc Natl Acad Sci USA* **71**: 3649–3653.

Lindahl T (1993) Instability and decay of the primary structure of DNA. *Nature* **362**: 709–715.

Lindahl T, Nyberg B (1974) Heat-induced deamination of cytosine residues in deoxyribonucleic acid. *Biochemistry* **13**: 3405–3410.

Lund P (2011) Insights into chaperonin function from studies on archaeal thermosomes. *Biochem Soc Trans* **39**: 94–98.

Pearl LH (2000) Structure and function in the uracil-DNA glycosylase superfamily. *Mutat Res* **460**: 165–181.

Sandigursky M, Franklin WA (2000) Uracil-DNA glycosylase in the extreme thermophile *Archaeoglobus fulgidus*. *J Biol Chem* **275**: 19146–19149.

Sartori AA, Fitz-Gibbon S, Yang H, Miller JH, Jiricny J (2002) A novel uracil-DNA glycosylase with broad substrate specificity and an unusual active site. *EMBO J* **21**: 3182–3191.

Shi L, Potts M, Kennelly PJ (1998) The serine, threonine, and/or tyrosine-specific protein kinases and protein phosphatases of prokaryotic organisms: A family portrait. *FEMS Microbiol Rev* **22**: 229–253.