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

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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 in DNA. 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 in DNA. 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 archaea we refer to: Grogan, 2004). UDG activity in hyperthermophilic microorganisms was first reported in 1996 (Koulis et al., 1996). The hyperthermophilic archaeon *Archaebacillus 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 its 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 [3H]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...
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 31P-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 [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 [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 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...
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 (Knaevelsrud et al., 2010; Knaevelsrud 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) (Knaevelsrud 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 *cavi* 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. Adaptation 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 vitro reactions or accessory factors.

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**REFERENCES**


