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The NudA Protein in the Gastric Pathogen *Helicobacter pylori* Is an Ubiquitous and Constitutively Expressed Dinucleoside Polyphosphate Hydrolase*[S]

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The gastric pathogen *Helicobacter pylori* harbors one Nudix hydrolase, NudA, that belongs to the nucleoside polyphosphate hydrolase subgroup. In this work, the enzymatic activity of purified recombinant NudA protein was analyzed on a number of nucleoside polyphosphates. This predicted 18.6-kDa protein preferably hydrolyzes diadenosine tetraphosphate, Ap_4A at a k_{cat} of 0.15 s^{-1} and a K_m of 80 μM, resulting in an asymmetrical cleavage of the molecule into ATP and AMP. To study the biological role of this enzyme in *H. pylori*, an insertion mutant was constructed. There was a 2–7-fold decrease in survival of the mutant as compared with the wild type after hydrogen peroxide exposure but no difference in survival after heat shock or in spontaneous mutation frequency. Western blot analyses revealed that NudA is constitutively expressed in *H. pylori* at different growth stages and during stress, which would indicate that this protein has a housekeeping function. Given that *H. pylori* is a diverse species and that all the *H. pylori* strains tested in this study harbor the *nudA* gene and show protein expression, we consider NudA to be an important enzyme in this bacterium.

The nucleoside diphosphate linked to some other moiety X (Nudix) protein family, formerly called the MutT protein family, consists of about 800 proteins in more than 200 species from all kingdoms (1). This group of proteins may be divided into subfamilies based on the substrate specificity: ADP-ribose, dinucleoside polyphosphates, NADH, nucleotide sugars, or ribo- and deoxyribonucleoside triphosphates (2). The active site of Nudix hydrolases corresponds to the consensus sequence $GX_5EX_7REUXEEXGU$ (where *X* represents any amino acid and U represents Ile, Leu, or Val).

Although enzyme activities of Nudix proteins have been studied extensively, the biological role of these enzymes is still poorly understood (3–6). It has been proposed that Nudix hydrolases are housecleaning enzymes that decrease the intracellular level of toxic substances and cell signaling molecules, *e.g.* dinucleoside polyphosphates (7). The dinucleoside polyphosphate Ap_4A[1] is induced upon oxidative stress and heat shock both in prokaryotes and eukaryotes (8, 9), and the Nudix enzyme subsequently degrades this signaling component to restore the intracellular balance. Experimental data indicate that an increased concentration of Ap_4A is a signal for cell division in *Escherichia coli* (10). Furthermore, it has been suggested that Ap_4A and Ap_3A are physiological antagonists. Ap_4A induces apoptosis, and Ap_3A is a co-inducer of differentiation in both murine and human cells (11).

Nudix enzymes have also been suggested to play a role in bacterial invasion of eukaryotic cells. The *Bartonella bacilliformis* Nudix hydrolase, encoded by the *ialA* gene, was shown to be associated with the ability to invade human erythrocytes (12). An up-regulation of the expression of the *E. coli* K1 orthologue *ygdP* during invasion of human brain microvascular endothelial cells has also been demonstrated (13). Recently, it was shown that transcription of the *Rickettsia prowazekii* orthologue *invA* is temporarily increased during the early stages of infection, similar to the stress-related protein GroEL (14).

Previously, Nudix hydrolases were referred to as MutT proteins since the first studied protein in this group was MutT in *E. coli*. MutT hydrolyzes 8-oxo-dGTP, a compound generating A-T/C-G transversions, and thereby prevents DNA damage caused by oxidative stress (15). The MutT nomenclature used for proteins containing Nudix motifs was misleading since many of these proteins do not seem to hydrolyze toxic compounds such as 8-oxo-dGTP. Recently, the idea of an indirect involvement of *E. coli* MutT in the prevention of DNA damage *in vivo* was questioned (16). In this study, it was shown that the incorporation of 8-oxo-dGTP into the genome by DNA polymerase is rather inefficient *in vitro* at measurable intracellular concentrations. Furthermore, the *mutT* phenotype was still observed at anaerobic conditions when no 8-oxo-dGTP is produced.

About half of the population of the world is colonized with the gastric pathogen *Helicobacter pylori*, and the infection persists lifelong unless treated. This Gram-negative, microaerophilic bacterium causes gastritis, gastric ulcer, and duodenal ulcer

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[S] The on-line version of this article (available at http://www.jbc.org) contains supplementary data showing the sequence alignments of *nudA* from different *H. pylori* strains.

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[1] The abbreviations used are: Ap_4A, adenosine (5′)-tetrahospho-(5′)-adenosine; Ap_3A, adenosine (5′)-triphospho-(5′)-adenosine; Ap_5A, adenosine (5′)-pentaphospho-(5′)-adenosine; Ap_6A, adenosine (5′)-hexaphospho-(5′)-adenosine; Gp_4G, guanosine (5′)-tetraphospho-(5′)-guanosine; Gp_5G, guanosine (5′)-pentaphospho-(5′)-guanosine; HPLC, high performance liquid chromatography.

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and is associated with gastric cancer (17, 18). *H. pylori* is primarily an extracellular bacterium, but it has been observed intracellularly, although it is still unclear whether it is capable of multiplying and persisting within host cells (19, 20). *H. pylori* is transformed from a dividing rod to a nonculturable coccoid form at unfavorable conditions, *e.g.* oxidative stress. The intracellular level of 8-oxo-dGTP accumulates during this transformation (21). The two fully sequenced strains of *H. pylori* harbor one Nudix hydrolase orthologue, *Nud*ix hydrolase *A*, *nudA*, with the gene numbers HP1228 in 26695 (22) and JHP1149 in J99 (23). Sequence analysis of the genome suggests that the NudA protein is the only dinucleoside polyphosphate hydrolase homologue present in this bacterium (2, 22, 23), which makes *H. pylori* a suitable system to study the biological role of Nudix hydrolases.

Because of the functional heterogeneity within this group of proteins, the possible functions of *H. pylori* NudA are involvement in (i) DNA repair through hydrolysis of 8-oxo-dGTP, (ii) oxidative stress and/or heat shock response through degradation of stress signaling molecules, or (iii) bacterial invasion of epithelial cells through degradation of toxic substances induced during invasion. In this work, we describe the enzymatic function of the Nudix hydrolase NudA in *H. pylori* and use a constructed *nudA* insertion mutant to perform functional studies to determine the biological role of this protein.

EXPERIMENTAL PROCEDURES

Bacterial Strains and Culture Conditions—*H. pylori* strains 67:21, CAG7:8, Ca73, Ca52, DU52:2, and Ca34 have been characterized previously in our laboratory (24, 25). Additional *H. pylori* strains used are: 26695 (22), J99 (23), Hp1 (26), and 2808, a mouse-passed strain originating from a German patient diagnosed with ulcer. All *H. pylori* strains were grown on GC agar plates as described previously (24) or in Brucella broth supplemented with 1% IsoVitaleX (BD Biosciences) and 5% fetal bovine serum. Generation of the coccoid form of *H. pylori* was obtained by storing a liquid bacterial culture in phosphate-buffered saline for 40 days at 4 °C (without shaking). *E. coli* strains DH5α and HMS174(DE3) were grown at 37 °C on Luria agar plates containing the appropriate antibiotics for selection of plasmids.

PCR and Sequencing—PCR was performed according to standard protocols using Dynazyme DNA polymerase (Finnzyme, Espoo, Finland). DNA cycle sequencing was performed using the BigDye kit, version 2 (Applied Biosystems, Foster City, CA) and analyzed on an ABI 3100 apparatus.

Cloning, Expression, and Purification of Recombinant NudA—The *H. pylori nudA* gene was amplified with PWO polymerase (Roche Molecular Biochemicals) using genomic DNA from strain 67:21 as template and the forward primer 5′-CAT G**CC ATG G**CA ATG CTA CAT AAA AAA-3′ containing a *Nco*I site (in **bold**) and reverse primer 5′-CCG **CTC GAG** TAA ATA CCC CTC TCT TTT G-3′ containing a *Xho*I site. The resulting PCR fragment was cloned in-frame into the pET32a (+) vector, transformed into DH5α, and subcloned into HMS174(DE3) for protein expression. The HMS174(DE3) strain containing the *nudA* construct was grown at 37 °C in 1.6 liters of LB medium with 50 μg/ml ampicillin to an A_{600} of 0.5. Isopropyl-β-D-thiogalactopyranoside was added to a final concentration of 0.4 mM to induce protein expression. Four h after isopropyl-β-D-thiogalactopyranoside addition, cells were harvested by centrifugation, and the pellet was then resuspended in 15 ml of lysis buffer (50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole, 0.1% *N*-laurylsarcosine, pH 8.0) and disrupted by three passages through a French press at 140 kilopascals. Bacterial cell debris was removed by centrifugation at 39,000 × *g* for 20 min. Protein purification made use of the His tag present on the expressed protein and was performed according the manufacturer's recommendations using a nickel-nitrilotriacetic acid matrix (Qiagen, Hilden, Germany). After purification, the protein was immediately dialyzed twice with 1 liter of DL1 buffer (50 mM Tris-HCl, pH 8.0, 200 mM NaCl, 10 mM $MgCl_2$, 2 mM dithiothreitol, 10% glycerol) and once with 200 ml of DL2 buffer (as DL1 but 50% glycerol). The concentration of the purified protein was determined using the Bio-Rad protein assay (Bio-Rad), and *H. pylori* NudA was stored at −20 °C.

Substrate Specificity Analysis Using HPLC—The substrate specificity was determined by ion-exchange high performance liquid chromatography, HPLC (Äkta Basic, Amersham Biosciences). A 500-μl reaction mixture (50 mM Tris-HCl, pH 9.0, 100 μM substrate (Sigma), 10 mM $MgCl_2$ with or without 1 μg of purified NudA) was incubated for 20 min at 37 °C, snap-frozen, and stored at −20 °C. The samples were thawed and immediately used in HPLC analysis in which 100 μl of sample was applied to a 1-ml Resource Q column (Amersham Biosciences) at a flow rate of 2 ml/min in 35 mM NH_4HCO_3, pH 9.6. The products were eluted and separated by an 18-min gradient elution from 5 to 100% with 0.7 M NH_4HCO_3. The proportion of hydrolyzed substrate was calculated by dividing the peak areas from samples lacking enzyme with the samples containing enzyme. Each value represents the mean of two individual analyses with an experimental difference of 7% or less. The reaction products were determined using the controls AMP, ADP, and ATP to which the retention times of the sample products were compared.

Enzyme Assay for Catalytic Measurements—The enzyme kinetic constant, K_m, for the preferred substrate Ap_4A was determined using an assay based on conversion of a phosphatase-insensitive substrate to phosphatase-sensitive product (4). The reaction mixture consisted of 50 mM Tris-HCl, pH 9.0, 2.5 mM Ap_4A, 10 mM $MgCl_2$, 4 units of calf intestinal phosphatase, and 0.5 μg of purified *H. pylori* NudA in a total volume of 50 μl. The level of free phosphate, P_i, was assayed according to Ames and Dubin (27).

Generation of a nudA Insertion Mutant—The *H. pylori nudA* gene with flanking regions was amplified using forward primer 5′-CTT TAA TGG CAA CC GAT GTT A-3′ and reverse primer 5′-GGG ATG CAT CAT CTC TCG CTT GTT AGG G-3′ with genomic DNA from strain 67:21 as template. The PCR fragment was cloned into the pCR2.1-TOPO vector (Invitrogen) according to the manufacturer's instructions and subcloned into pUC18 vector using the *Eco*RI sites. An *Xho*I site (in **bold**) was generated seven codons upstream of the Nudix box in the *nudA* gene using forward primer 5′-GCG CAT AGA TAT TGC TAG **CTC GAG** GCA GTT CCC CCA AGG-3′ and reverse primer 5′-CCT TGG GGG AAC TGC **CTC GAG** CTA GCA ATA TCT ATG CGC-3′ in the QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA). A kanamycin cassette from pJMK30 vector (28) was ligated into the *Xho*I site to interrupt the *nudA* open reading frame. The insertion mutant, 2808*nudA::Km*, of the strain 2808 was generated through natural transformation by addition of linearized vector construct to the strain cultured in Brucella broth, allowing uptake and homologous recombination. The mutant was confirmed by PCR using primers flanking the construct (InvA2715 5′-TAT GGC TTT AGG CGT TTT AG-3′, InvA3861 5′-TCT ATG CCC ATA CCT TTC TA-3′) and sequencing. The mutant 2808*nudA::Km* and the wild-type *H. pylori* strains were used for the functional analyses.

Mutation Frequency Analysis—The frequency of spontaneous mutations of the 2808*nudA::Km* and the wild type were determined as described previously (29). Briefly, for each strain, 20 independent 1-ml cultures were grown in Brucella broth for 36 h. The total number of bacteria was calculated as the mean of three tubes by plating serial dilutions of the culture on non-selecting media. The number of rifampin-resistant *H. pylori* bacteria was determined by plating onto GC agar plates containing 20 μg/ml (10 × minimal inhibitory concentration) rifampin. The mutation frequency was calculated as the median number of rifampin-resistant bacteria divided by the mean colony-forming units for each culture.

Oxidative and Heat Shock Stress Responses—The mutant and wild-type strains were cultured in Brucella broth to an A_{600} of 0.2, equivalent to $\sim 10^8$ cells/ml, and aliquots of 3 ml were challenged with hydrogen peroxide (H_2O_2) to a final concentration of 100 or 300 μM followed by a 2-h incubation without shaking. Samples were collected in 30-min intervals, and viable counts were performed before and during challenge from two parallel tubes. In the heat shock experiment, 1 ml of each strain was transferred from 37 to 55 °C, and samples were taken at different time points from 0 to 13 min. Viable counts were performed before and after exposure.

Western Blot Analyses—Bacteria were disrupted by sonication on ice, and the cell debris was removed by centrifugation. Total protein concentration was determined using the same method as above, and 2 μg of each sample was separated by 10% SDS-PAGE and transferred to polyvinylidene difluoride membrane (Bio-Rad). Primary polyclonal rabbit antibodies raised against purified NudA (1:8,000, Agrisera, Vännäs, Sweden) and horseradish peroxidase-conjugated secondary goat anti-rabbit IgG antibodies (1:8,000 DAKO, Glostrup, Denmark) were used. The signals were visualized by the ECL detection system (Amersham Biosciences).

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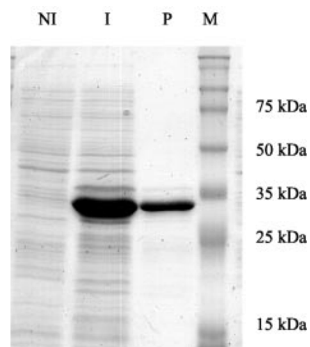

FIG. 1. **Expression and purification of the *H. pylori* Nudix hydrolase.** A 12% polyacrylamide gel containing 1% SDS stained with Coomassie Brilliant Blue included the following samples: crude extract of non-induced (*NI*) and induced (*I*) HMS174(DE3) cells containing pET32a (+) with the inserted *H. pylori nudA* gene, 6 μg of purified protein (*P*), full-length Rainbow protein size marker (*M*) (Amersham Biosciences).

TABLE I
Substrate specificity of H. pylori Nudix hydrolase NudA

The relative enzymatic activity was measured by HPLC analysis as described under "Experimental Procedures." One μg of the *H. pylori* Nudix hydrolase hydrolyzes 0.3 μmol of Ap_4A/min. The proportion of hydrolyzed substrate was calculated by dividing the peak areas in the presence and absence of enzyme. The mean of two assays for each substrate was used. The relative hydrolysis was calculated as the activity on substrate Ap_4A was set to 100%.

Substrate	Relative hydrolysis	Reaction products
	(%)	
Ap_3A	7	AMP, ADP
Ap_4A	100	ATP, ADP, AMP
Ap_5A	34	ATP, ADP, AMP
Ap_6A	45	ATP, ADP, AMP
Gp_4G	77	GTP, GMP
Gp_5G	41	GTP, GDP, GMP
ATP	5	ADP

RESULTS

Sequence Comparison and Protein Prediction—All the 70 *H. pylori* clinical isolates analyzed by PCR or hybridization harbored the *nudA* gene (data not shown). The DNA sequence of *nudA* was determined in four *H. pylori* strains, 67:21, Hp1, CAG7:8, and 2808, and aligned with the two fully sequenced strains 26695 and J99 using the ClustalW program (supplemented data). *H. pylori* strains are known to display high sequence diversity, and in the six strains, 48 of 465 nucleotides were variable, corresponding to variations in 11 of 155 amino acids. Thus, 23% of the substitutions were non-synonymous. Only one amino acid in the Nudix box was variable, indicating conservation in this protein domain as expected.

The NudA protein in strain 26695 has a predicted size of 18.6 kDa and a slightly basic pI of 7.7 (The Institute for Genomic Research). The protein is predicted to be cytoplasmic since no signal sequence is found. Nudix hydrolases are known to be 17–20 kDa globular monomeric proteins (30).

Enzyme Activity and Substrate Specificity—The *H. pylori nudA* gene was cloned in pET32a (+), expressed in *E. coli* strain HMS174(DE3), and purified using the His tag and nickel-nitrilotriacetic acid resin (Fig. 1). Expression from a pET32a (+) vector resulted in a protein fused to an N-terminal thioredoxin (Trx) tag in addition to the His tag, which stabilized the protein and increased the yield so that ~10 mg of pure soluble protein was obtained. Substrate specificity of NudA was determined by HPLC analysis. The relative substrate specificity and reaction products are presented in Table I. The NudA enzyme showed activity on several polyphosphate substrates, and the

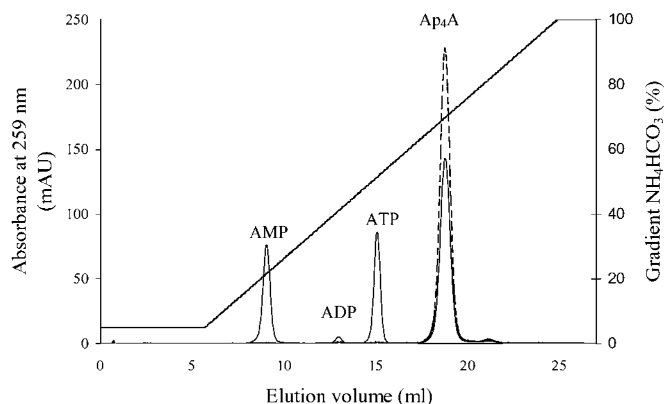

FIG. 2. **NudA hydrolyzes Ap_4A asymmetrically.** The results of HPLC analysis of Ap_4A incubated with the *H. pylori* Nudix hydrolase NudA (*solid line*) and control without the enzyme (*broken line*) are shown. *AU*, arbitrary units.

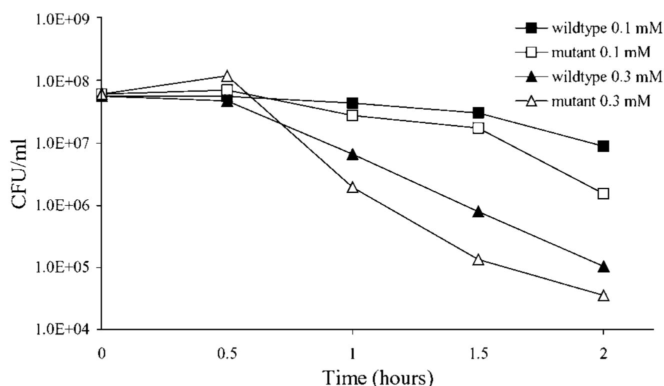

FIG. 3. **Survival of the NudA mutant is lower compared with the wild-type strain after hydrogen peroxide exposure.** *Lines* marked with *filled symbols* represent the wild-type strain 2808, and *lines* marked with *open symbols* represent the 2808*nudA::Km* mutant. *Squares* represent exposure of 100 μM H_2O_2, and *triangles* represent exposure of 300 μM. Each time point is calculated as the mean value of five individual experiments for each strain. *CFU*, colony-forming units.

preferred substrate was diadenosine tetraphosphate, Ap_4A, as for the NudA orthologues in *B. bacilliformis*, *Caenorhabditis elegans*, and *Lupinus angustifolius*. The substrates were all cleaved asymmetrically, and in the case of Ap_4A, the reaction products were AMP and ATP (Fig. 2). In the same reaction, a small amount of the ATP was further hydrolyzed to ADP. NudA was not active on the substrates NADH and ADP-ribose.

Kinetic Properties of NudA—The K_m of NudA was 80 μM on the preferred substrate Ap_4A, calculated as the mean of three independent experiments using a substrate concentration ranging from 10 to 300 μM. NudA hydrolyzes 0.3 μmol of Ap_4A min^{-1} mg^{-1} enzyme corresponding to a k_{cat} value of 0.15 s^{-1}. The enzyme has a broad pH optimum ranging from pH 7.0 to 9.0 and a need of divalent metal ions for activity, *e.g.* Mg^{2+}, as is the case of other Nudix hydrolases (31). To rule out the possibility of reduced activity due to the tags (Trx, S-protein, and His), the enzyme activity of NudA was compared before and after enterokinase digestion that cleaves off the tags. Presence of the tags did not affect the enzyme activity on Ap_4A.

Analyses of NudA Involvement in Stress Responses and in Hydrolysis of DNA Damaging Components—Since *H. pylori* appears to have only one dinucleoside polyphosphate hydrolase, this bacterium provides a useful system to examine the biological role of this enzyme. A mutant of the *H. pylori* Nudix hydrolase was constructed by insertion of a kanamycin resistance cassette into the *nudA* gene. The cassette was inserted upstream of the active site (Nudix box), thereby ensuring that

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all enzymatic activity was lost in this mutant. To test the hypothesis that NudA is a functional orthologue of *E. coli* MutT, we performed a measurement of the mutation rates using the frequency of rifampicin resistance as an overall indicator of the bacterial spontaneous mutation formation (29). Our results showed that there was no difference in the spontaneous mutation frequency of the mutant and wild-type strains. This result suggested that NudA does not hydrolyze oxidized deoxynucleotide triphosphates and that it is not involved in preventing incorporation of mutagenic compounds such as 8-oxo-dGTP that may result in base pair substitutions. An alternative possibility is that the NudA protein is involved in protection against oxidative stress and heat shock. The mutant strain and the corresponding wild-type strain were compared in growth experiments with and without oxidative stress (hydrogen peroxide exposure). The *nudA* mutant was 2–7-fold more sensitive than the wild type after 1–2 h of exposure of 100 and 300 μM H_2O_2 (Fig. 3). In the experiment in which the strains were exposed to heat (55 °C), the mutant as well as the wild type died at the same rate, and starting with 10^8 cells/ml, the colony-forming units decreased with approximately four logs after 13 min.

Constitutive Expression of NudA at Different Growth Phases and during Oxidative Stress—Protein expression of NudA was analyzed by Western blot in 10 different *H. pylori* strains grown on GC plates, including J99 and 26695. All strains produced the protein in similar amounts (Fig. 4*A*). The size of the protein varied slightly, which could not be confirmed by DNA sequencing, suggesting post-translational modifications, as has been seen for many other *H. pylori* proteins (32).

Western blot analyses were also used to determine the expression level of the protein in strain 2808 under different growth phases and during stress (Fig. 4, *B* and *C*). The protein was constitutively expressed throughout growth to stationary phase. In addition, the protein level was similar in the coccoid form of the bacteria. The expression of NudA did not change at any of the tested stress conditions: incubation with 100 and 300 μM H_2O_2 for 2 h, heat shock at 42 °C for 10 min or 30 min and at 55 °C for 2 min or 13 min.

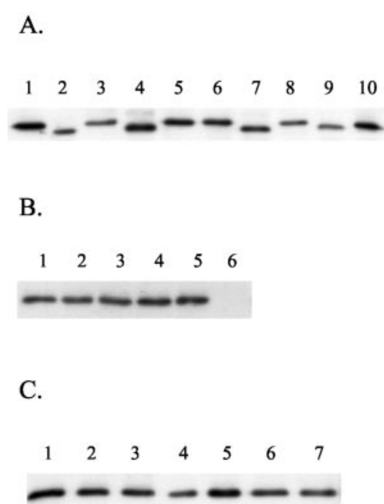

FIG. 4. **Expression of NudA in *H. pylori*.** As shown in *A*, Western blot of the NudA protein from different *H. pylori* strains revealed a difference in protein size. *Lane 1* = 26695, *lane 2* = J99, *lane 3* = 2808, *lane 4* = 67:21, *lane 5* = Hp1, *lane 6* = CAG7:8, *lane 7* = Ca52, *lane 8* = Ca73, *lane 9* = Ca34, and *lane 10* = DU52:2. *B*, Western blot analysis showing constitutive expression of NudA in *H. pylori* strain 2808 at different growth phases measured at A_{600}. *Lane 1* = 0.2, *lane 2* = 0.5, *lane 3* = 0.9, *lane 4* = 1.3, *lane 5* = coccoid from 40-day-old culture stored in phosphate-buffered saline at 4 °C, and *lane 6* = 0.9 of the 2808*nudA::Km* mutant. *C*, *H. pylori* strain 2808 exposed to different stress conditions. *Lane 1* = no stress, *lane 2* = 100 μM H_2O_2 for 2 h, *lane 3* = 300 μM H_2O_2 for 2 h, *lane 4* = 55 °C for 3 min, *lane 5* = 55 °C for 13 min, *lane 6* = 42 °C for 10 min, and *lane 7* = 42 °C for 30 min.

DISCUSSION

Several Nudix enzymes from different organisms, belonging to the dinucleoside polyphosphate hydrolase subgroup, are characterized, and the Nudix box sequences of these enzymes have been compared (2). Interestingly, these enzymes differ in substrate specificity and catalytic capacity (Table II). The preferred substrate for *H. pylori* NudA is Ap_4A, but this Nudix enzyme hydrolyzes a number of nucleoside polyphosphates, as do the orthologues. Compared with the orthologues, the purified *H. pylori* NudA seems to be less efficient in hydrolyzing the dinucleoside polyphosphates. This could be due to incorrect folding of the purified protein since the solubility was low, or the fact that important post-translational modifications are absent when the protein is expressed in *E. coli*. Another explanation could be that the *H. pylori* enzyme is intrinsically less efficient when compared with Nudix enzymes in other species.

Ap_4A binds the heat shock and oxidative stress response proteins DnaK, GroEL, E89, C45, and C40 (33). To determine whether NudA is also involved in oxidative stress, we used H_2O_2 challenge and compared the survival of the mutant and the wild type. A 2–7-fold difference was found, and thus the *H. pylori* NudA seems to be involved in oxidative stress response, possibly through degradation of stress-induced Ap_4A.

Ap_4A has been detected previously *in vitro* as a byproduct of aminoacyl-tRNA synthetases and is suggested to act as a metabolic alarmone (34). The constitutive expression of the protein in *H. pylori* during different growth phases as well as in coccoid forms or after hydrogen peroxide challenge and heat shock indicates that this protein has a housekeeping function rather than being involved in stress responses. Thus, it is not clear whether Ap_nA (n = 3–6) are signaling molecules or toxic byproducts from metabolic processes in *H. pylori*.

The lack of increase in spontaneous mutation frequency in the strain with an inactivated *nudA* gene suggests that NudA is not involved in hydrolyzing mutagenic deoxynucleoside triphosphates. Another indication that NudA and MutT are not functional orthologues is the fact that they use different substrates. In a recent study, it was shown that the *E. coli* MutT protein is active on dGTP and 8-oxo-dGTP but not on Ap_3A, ADP-ribose, or FAD (1).

TABLE II
Comparison of the kinetic parameters of dinucleoside polyphosphate hydrolases from different organisms

Enzyme name, organism	Preferred substrate	K_m	k_{cat}	Reference
		μM	s^{-1}	
NudA, *H. pylori*	Ap_4A	80	0.15	This study
Ia1A, *B. bacilliformis*	Ap_4A		14	4
Ia1A, *B. bacilliformis*	Ap_4A	10	3	3
Y37H9A.6, *C. elegans*	Ap_4A	7	27	38
EC 3.6.1.17, *L. angustifolius*	Ap_4A	2.5	40.8	39
InvA, *R. prowazekii*	Ap_5A	100	1.9	6
Ygdp, *E. coli* K1	Ap_5A	360	1	5

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Recently, it was discovered that a putative *H. pylori* protein NudB (HP0507), containing 5 of the 9 most important amino acids of a Nudix box, is involved in colonization of suckling mice (35). An enzyme activity screen was performed to determine whether this protein had the same substrate specificity as NudA. The purified NudB protein was active on NADH and ADP-ribose but not on any of the dinucleoside polyphosphates tested for NudA.[2] Therefore, we conclude that there is no cross-activity between these two proteins.

To study the possible involvement of NudA in invasion, a gentamicin protection assay was used to compare the invasion frequency of the *NudA* mutant with the wild type in AGS cells (36). No quantifiable differences were found with this method (data not shown). Therefore, it is not clear whether the NudA protein is involved in *H. pylori* invasion of gastric epithelial cells. However, considering the low frequency of invasion of *H. pylori*, the gentamicin protection assay might not be sensitive enough to monitor differences in invasion capacity since complete killing of extracellular bacteria is rarely obtained (20).

H. pylori exhibits high genetic diversity, as shown by a whole genome microarray analysis of 15 strains, in which 22% of the genes were considered dispensable in one or more strains (37). In addition, about 7% of the genes differ between the two fully sequenced strains 26695 and J99 (23). Taking into account that all the 70 clinical isolates of *H. pylori* tested harbor this gene, as well as the fact that 10 out of 10 of the examined strains showed protein expression, we consider NudA to be important but not essential for this bacterium. In conclusion, this study demonstrates that the NudA protein in *H. pylori* is an ubiquitous and constitutively expressed dinucleoside polyphosphate hydrolase preferably hydrolyzing Ap_4A.

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