Crystal structure of the N-terminal anticodon-binding domain of the nondiscriminating aspartyl-tRNA synthetase from *Helicobacter pylori*

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The N-terminal anticodon-binding domain of the nondiscriminating aspartyl-tRNA synthetase (ND-AspRS) plays a crucial role in the recognition of both tRNA^{Asp} and tRNA^{Asn}. Here, the first X-ray crystal structure of the N-terminal domain of this enzyme (ND-AspRS_{1–104}) from the human-pathogenic bacterium *Helicobacter pylori* is reported at 2.0 Å resolution. The apo form of *H. pylori* ND-AspRS_{1–104} shares high structural similarity with the N-terminal anticodon-binding domains of the discriminating aspartyl-tRNA synthetase (D-AspRS) from *Escherichia coli* and ND-AspRS from *Pseudomonas aeruginosa*, allowing recognition elements to be proposed for tRNA^{Asp} and tRNA^{Asn}. It is proposed that a long loop (Arg77–Lys90) in this *H. pylori* domain influences its relaxed tRNA specificity, such that it is classified as nondiscriminating. A structural comparison between D-AspRS from *E. coli* and ND-AspRS from *P. aeruginosa* suggests that turns E and F (₇₈GAGL₈₁ and ₈₃NPKL₈₆) in *H. pylori* ND-AspRS play a crucial role in anticodon recognition. Accordingly, the conserved Pro84 in turn F facilitates the recognition of the anticodons of tRNA^{Asp} (³⁴GUC³⁶) and tRNA^{Asn} (³⁴GUU³⁶). The absence of the amide H atom allows both C and U bases to be accommodated in the tRNA-recognition site.

1. Introduction

Aminoacylated tRNAs, which are biochemically synthesized by aminoacyl-tRNA synthetases (aaRSs), are key components for the biosynthesis of proteins (Ibba & Söll, 2000). In higher organisms, 20 aaRSs generate the full set of aminoacylated tRNAs (Ribas de Pouplana & Schimmel, 2000). In all archaea and some bacteria, however, the gene(s) encoding certain aaRS(s) are absent. These organisms rely on indirect aminoacylation pathways to maintain the fidelity of translation (Ribas de Pouplana & Schimmel, 2000; Ibba & Söll, 2000, 2004; Cathopoulis *et al.*, 2007). The glutamyl-tRNA and asparaginyl-tRNA synthetases (GlnRS and AsnRS) are frequently missing in microbes. In the absence of GlnRS and/or AsnRS, these organisms use a nondiscriminating GluRS (ND-GluRS; Wilcox & Nirenberg, 1968; Lapointe *et al.*, 1986; Schön *et al.*, 1988; Curnow *et al.*, 1998; Racznik *et al.*, 2001), or in some cases GluRS2 (Salazar *et al.*, 2003; Skouloubris *et al.*, 2003), and/or a nondiscriminating AspRS (or AspRS2) (Becker *et al.*, 1997; Curnow *et al.*, 1998; Tumbula-Hansen *et al.*, 2002; Charron *et al.*, 2003) to generate the correctly aminoacylated Glu-tRNA^{Glu} and/or Asp-tRNA^{Asp} and the

incorrectly aminoacylated Glu-tRNA^{Gln} and/or Asp-tRNA^{Asn}. These misacylated tRNAs are then converted to the correctly aminoacylated Gln-tRNA^{Gln} and/or Asn-tRNA^{Asn} by the heterodimeric GatDE (in archaea, exclusively for Gln-tRNA^{Gln}; Feng *et al.*, 2005; Schmitt *et al.*, 2005) or the heterotrimeric GatCAB (in bacteria; Curnow *et al.*, 1997; Becker *et al.*, 2000; Raczniak *et al.*, 2001) *via* transamidation of the amino-acid side chains.

The dual tRNA specificity of the bacterial-type ND-AspRS remains poorly understood. The N-terminal anticodon-binding domain of this enzyme is responsible for the recognition of the anticodon bases of both tRNA^{Asp} (³⁴GUC³⁶) and tRNA^{Asn} (³⁴GUU³⁶). Mutations in this domain, H31L and G83K in *Pseudomonas aeruginosa* (Bernard *et al.*, 2006) and L81N and L86M in *Helicobacter pylori*, revealed key amino-acid residues responsible for tRNA^{Asp} recognition (Chuawong & Hendrickson, 2006). These mutations, based on conservation in D-AspRS, increase tRNA^{Asp} specificity for ND-AspRS from both *P. aeruginosa* and *H. pylori*, making them more discriminating. However, a mutation that enhances tRNA^{Asn} specificity in a bacterial-type ND-AspRS has not yet been identified.

Although several crystal structures of ND-AspRS have been reported, most are of archaeal orthologues (Charron *et al.*, 2003; Sato *et al.*, 2007; Schmitt *et al.*, 1998; Blaise *et al.*, 2010). Recently, the crystal structures of two bacterial-type ND-AspRSs from *Mycobacterium smegmatis* (Baugh *et al.*, 2015) and *P. aeruginosa* (Suzuki *et al.*, 2015) have been reported that focused on the utilization of ND-AspRS as a tuberculosis drug target (*M. smegmatis*) and the significance of the GAD domain, a bacterial-specific domain inserted within the catalytic core of the bacterial-type AspRSs, in Asn-transamidosome formation and catalysis (*P. aeruginosa*). The *P. aeruginosa* ND-AspRS structure contains tRNA^{Asn} and reveals key residues (Arg79, Ala83 and Asn85) responsible for the recognition of U36, the third base in the tRNA^{Asn} anticodon (³⁴GUU³⁶). Unfortunately, there is no bacterial-type ND-AspRS:tRNA^{Asp} structure available to date. Therefore, a direct comparison of the anticodon-recognition patterns for both tRNA^{Asp} and tRNA^{Asn} cannot be achieved.

We report here the first X-ray crystal structure of the anticodon-binding domain of ND-AspRS (ND-AspRS_{1–104}) from *H. pylori*, a Gram-negative bacterium found in the stomach of patients with chronic gastritis and gastric ulcers (Marshall & Warren, 1984). Subtle differences in this anticodon-binding domain compared with those of discriminating and other nondiscriminating AspRSs reveal structural diversity in these enzymes and facilitate our understanding of the relaxed tRNA specificity of the bacterial-type ND-AspRS.

2. Materials and methods

2.1. Cloning, expression and purification of ND-AspRS_{1–104}

The production of *H. pylori* ND-AspRS_{1–104} has previously been reported (Fuengfuloy *et al.*, 2013). In brief, the gene encoding ND-AspRS_{1–104} was amplified and cloned into the

KpnI and PstI sites in the pCDF-1b vector (Novagen, Madison, Wisconsin, USA) to produce a plasmid encoding a six-histidine tag appended onto the N-terminus of ND-AspRS_{1–104} (pPC010). *Escherichia coli* BL21(DE3) cells transformed with pPC010 were grown in LB medium supplemented with streptomycin to a final concentration of 50 µg ml^{−1}. Protein expression was induced with IPTG at a final concentration of 1 mM once the OD₆₀₀ reached 0.8. The expression time was limited to only 30 min to minimize the heterologous toxicity of ND-AspRS_{1–104}. Cells were harvested by centrifugation at 4000 rev min^{−1} and lysed using sonication. The protein was purified using Ni-NTA Spin Columns (Qiagen, Valencia, California, USA) as per the manufacturer's instructions. The protein was further purified by gel-filtration chromatography (HiPrep 16/60 Sephacryl S-200 HR column, GE Healthcare Life Sciences, Uppsala, Sweden) and quantified by BCA assays (Pierce, Rockford, Illinois, USA).

2.2. Crystallization

Extensive crystallization screenings were performed to produce single crystals for X-ray diffraction experiments. For initial crystallization screening, the microbatch method was employed using JBScreen Classic HTS I (Jena Bioscience GmbH, Jena, Germany). The plate was incubated at 291 K. Small needle clusters were obtained after 24 h in the E3 [22% (w/v) PEG 4000, 0.2 M ammonium sulfate, 0.1 M sodium acetate], E4 [25% (w/v) PEG 4000, 0.2 M ammonium sulfate, 0.1 M sodium citrate pH 5.6] and E8 [30% (w/v) PEG 4000, 0.1 M ammonium sulfate, 0.1 M sodium citrate pH 5.6] solutions. Conditions E3, E4 and E8 were further optimized by hanging-drop vapour diffusion using EasyXtal 24-well tools (Qiagen, Valencia, California, USA). Crystallization droplets were made from 1 µl of 1.25, 2.5, 5 and 10 mg ml^{−1} protein solution mixed with 1 µl precipitant solution and equilibrated against 1 ml of the respective precipitating reagent. Crystal optimization was performed by varying the concentration of PEG 4000 [5–30% (w/v)] with a discrete range of pH from 5.0 to 8.5 (0.1 M sodium citrate pH 5.0, 0.1 M sodium citrate pH 5.6, 0.1 M sodium citrate pH 6.0, 0.1 M MES pH 6.5, 0.1 M MOPS pH 7.0, 0.1 M HEPES pH 7.5, 0.1 M Tris–HCl buffer pH 8.0 and 0.1 M Tris–HCl buffer pH 8.5). Finally, rectangular rod-shaped crystals appeared from 10 mg ml^{−1} protein solution in 7% (w/v) PEG 4000, 0.1 M ammonium sulfate, 0.1 M sodium citrate pH 5.6 within 6 d of incubation at 277 K.

2.3. Data collection and processing

Optimized crystals were transferred from the equilibrating drops to 30% (v/v) glycerol in mother liquor, briefly soaked for 5 min and further flash-vitrified by dipping them into liquid nitrogen. The crystals were mounted in cryoloops (CrystalCap Copper Magnetic HT, Hampton Research, Aliso Viejo, California, USA) and further arranged in a cryocane chilled with liquid nitrogen in a dry shipper. Preliminary X-ray diffraction experiments were performed using a MICROSTAR rotating-anode X-ray generator (λ = 1.54 Å; Bruker GmbH, Karlsruhe, Germany) with a MAR165 CCD detector system (Rayonix

Table 1

Crystallographic data-collection and refinement statistics.

Values in parentheses are for the highest resolution shell.

Data collection	
Space group	$P4_12_12$
Unit-cell parameters (Å)	$a = b = 61.83$, $c = 141.38$
Resolution range (Å)	30.00–2.00 (2.07–2.00)
No. of unique reflections	19187 (1760)
No. of observed reflections	95935 (7920)
Completeness (%)	98.9 (93.8)
Multiplicity	5.0 (4.5)
$\langle I/\sigma(I) \rangle$	27.2 (3.9)
$R_{\text{merge}}^\dagger$ (%)	5.7 (42.1)
Refinement	
$R_{\text{work}}^\ddagger$ (%)	18.00
$R_{\text{free}}^\S$ (%)	20.70
No. of protein atoms	1612
No. of ligand atoms	23
No. of water atoms	140
Mean atomic B values (Å ²)	
Protein atoms	32.61
Ligand atoms	48.61
Water atoms	40.52
R.m.s. deviations from ideal geometry	
Bond lengths (Å)	0.0070
Bond angles (°)	1.253

$^\dagger R_{\text{merge}} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of the i th measurement of an equivalent reflection with indices hkl . $^\ddagger R_{\text{work}} = \sum_{hkl} ||F_{\text{obs}}| - |F_{\text{calc}}|| / \sum_{hkl} |F_{\text{obs}}|$, where F_{obs} and F_{calc} are the observed and calculated structure factors, respectively. $^\S R_{\text{free}} = \sum_{hkl} ||F_{\text{obs}}| - |F_{\text{calc}}|| / \sum_{hkl} |F_{\text{obs}}|$ calculated using 5% of the reflections that were selected randomly and omitted from the refinement process.

LLC, Evanston, Illinois, USA), located at the Macromolecular Crystallography (MX) End Station at the Synchrotron Light Research Institute (SLRI), Thailand. A complete data set was collected from an ND-AspRS_{1–104} crystal on the BL15A1 beamline at the National Synchrotron Radiation Research Center (NSRRC), Hsinchu, Taiwan. Diffraction images were collected using an X-ray beam with a wavelength of 1.0 Å on a Rayonix MX-300 CCD detector (Rayonix LLC, Evanston, Illinois, USA) mounted on a Bruker MD2 Microdiffractometer (Bruker GmbH, Karlsruhe, Germany). The crystals were maintained at 110 K in a cold stream of nitrogen throughout data collection using a Cryojel (Oxford Instruments, Oxfordshire, England). The crystal-to-detector distance was set to 280 mm. The mounted crystal was translated and rotated using the goniometer head mounted on a Bruker MD2 Microdiffractometer (Bruker GmbH, Karlsruhe, Germany) to centre the crystal in the monochromatic X-ray beam of synchrotron radiation. During X-ray diffraction, data frames were collected over 0.5° oscillations with an exposure time of 10 s per frame for 150° of rotation in 300 diffraction images. The data set was indexed, integrated and scaled with the *HKL-2000* package (Otwinowski & Minor, 1997).

2.4. Structure determination and refinement

The ND-AspRS_{1–104} crystal belonged to the tetragonal space group $P4_12_12$ based on systematic absences and molecular replacement (see below). The refined unit-cell parameters are $a = b = 61.83$, $c = 141.38$ Å, and the crystal contains two molecules per asymmetric unit with an estimated

Matthews coefficient of $2.92 \text{ Å}^3 \text{ Da}^{-1}$ and a solvent content of 57.83% (Matthews, 1968). The statistics for data collection, processing and refinement are summarized in Table 1. The native structure was solved by molecular replacement with the N-terminal anticodon-binding domain of *E. coli* D-AspRS (PDB entry 1c0a; Eiler *et al.*, 1999) as a search model using *MOLREP* (Vagin & Teplyakov, 2010) from the *CCP4* suite of programs (Winn *et al.*, 2011). D-AspRS_{1–121} from *E. coli* has an identity of 38% to ND-AspRS_{1–104} from *H. pylori*. Molecular replacement employed experimental data from 3.5 to 8.0 Å resolution and revealed a solution score of 0.438, a translation-function peak/noise of 40.96 and a contrast of 7.41. The refinement of the ND-AspRS_{1–104} structure started at $R_{\text{work}} = 54.42\%$ and $R_{\text{free}} = 51.88\%$ and reached $R_{\text{work}} = 39.25\%$ and $R_{\text{free}} = 40.65\%$ after 14 cycles of refinement using *REFMAC5* (Murshudov *et al.*, 2011) with restraint refinement. After successive cycles of model building with *WinCoot* (Emsley & Cowtan, 2004; Emsley *et al.*, 2010) and structure refinement with *REFMAC5*, *ARP/wARP* (Perrakis *et al.*, 1999) was used to iteratively search for potential water molecules when the R_{work} and R_{free} values had converged to 25.66% and 29.95%, respectively. At the end of the structural refinement, R_{work} and R_{free} had converged to 18.00% and 20.70%, respectively.

The geometry of the final model was analyzed by *MolProbity* (Chen *et al.*, 2010). Most residues (>99.8%) were in allowed regions and no residues were found in outlier regions. However, the first residue (Met) of each monomer could not be modelled owing to poor electron density.

After the final model had been constructed, our refined apo structure was superposed with the X-ray crystal structures of *E. coli* D-AspRS complexed with tRNA^{Asp} and *P. aeruginosa* ND-AspRS bound to tRNA^{Asn}. The superpositions were executed by *SUPERPOSE* from the *CCP4* suite using only the coordinates of the anticodon-binding domain and the three anticodon bases (³⁴QUC³⁶ for tRNA^{Asp} and ³⁴GUU³⁶ for tRNA^{Asn}) from the *E. coli* D-AspRS and *P. aeruginosa* ND-AspRS structures, respectively. All structures were created and displayed with *PyMOL* (DeLano, 2002).

2.5. Amino-acid sequence alignment

A structure-based sequence alignment of the anticodon-binding domain of ND-AspRS from *H. pylori* with six selected structures from *P. aeruginosa*, *M. smegmatis*, *E. coli*, *Sulfolobus tokodaii*, *Pyrococcus kodakaraensis* and *Thermus thermophilus* was obtained using the *PROMALS3D* alignment program (Pei *et al.*, 2008). The secondary-structure elements of our reported structure (PDB entry 5gro) are displayed above the sequence.

3. Results and discussion

3.1. Overall structure and quality of the model

The monomeric ND-AspRS_{1–104} with an N-terminal six-His tag (~15.5 kDa) proved suitable for crystallization. After trial experiments, ND-AspRS_{1–104} crystals were obtained that exhibited rectangular morphology with approximate

dimensions of $200 \times 20 \times 10 \mu\text{m}$ (Supplementary Fig. S1). The best diffracting crystal gave data to $2.0 \text{ \AA}$ resolution using 30% glycerol in the mother liquor as a cryoprotectant (Supplementary Fig. S2). This N-terminal domain forms a homodimer. The two monomeric structures are similar, with a root-mean-square deviation (r.m.s.d.) of $0.36 \text{ \AA}$ for their C^α atoms. The r.m.s.d.s of the bond lengths and angles were calculated to be $0.007 \text{ \AA}$ and 1.253° , respectively. The crystal structure of the homodimer comprises 206 amino-acid residues with 140 water molecules solvating the dimeric structure. The two N-terminal methionines were not resolved. The final model gave a good

fit to the electron-density map, with an average atomic temperature factor (B factor) of $32.61 \text{ \AA}^2$ for both molecules.

The overall structure of ND-AspRS_{1–104} from the human pathogen *H. pylori* ($\sim 30 \text{ \AA}$ in width and $\sim 20 \text{ \AA}$ in depth for one monomer) exhibits a characteristic OB-fold (Murzin, 1993; Fig. 1) comprising a five-stranded β -barrel ($\beta 1$ – $\beta 5$) capped by an α -helix ($\alpha 1$; residues 56–59) between strands $\beta 3$ and $\beta 4$. The long L1 loop (residues 77–90) connecting strands $\beta 4$ and $\beta 5$ is located on the opposite side of $\alpha 1$. In full-length ND-AspRS residues 100–104 would connect this N-terminal domain to the catalytic domain.

Figure 1
The overall structure of ND-AspRS_{1–104} from *H. pylori* (PDB entry 5gro). (a) A ribbon representation is shown in salmon; the L1 loop is depicted in red, the 3_{10} -helices are in blue and the $\alpha 1$ helix is in purple. (b) Topology diagram of the secondary structure of *H. pylori* ND-AspRS_{1–104}. The β -strands, helices and loops are shown as arrows, rods and lines, respectively. The five β -strands are referred to as $\beta 1$ to $\beta 5$, whereas the single α -helix between $\beta 3$ and $\beta 4$ is referred to as $\alpha 1$.

		3 ₁₀ 1	3 ₁₀ 2	β1	β2	β3	α1	β4	β5	
<i>H. pylori</i>	2	--RSHFCTEISEKDVGKIVKVGWNCNTYRDHGGVVFIDLRDKSGLVQLVCDPS---	SKAYEKALEVRSEFVLVAKGKVRIRGAGLENPKLTKGKIEVLLEELII	100						
<i>P. aeruginosa</i>	2	--MRSHYCGQLNESLDGQEVTLGCGVHRRRDHGGVIFLDVRDREGLAQVVFDDP---	RAETFAKADRVRSFVVKITGKVRIRPEGARNPNMAGSGSIEVLGYEVLVN	104						
<i>M. smegmatis</i>	2	VLRTHAAGSLRPADAGQTVTLAGVARRRDHGGVIFIDLRDASGVSVVFR---	GDVLAAAHRLRAEFVAVTGVEVRPEGNENPEIPTGQIEVNATSLTVLG	103						
<i>E. coli</i>	1	--MRTEYCGQLRLSHVGOQVTLGCGVNRRLDLGSLIFIDMRDREGIVQVFFDDP---	RADALKLASELRNEFCIQVTGTVARDEKNINRDMATGTEVLASSLTIN	103						
<i>S. tokodaii</i>	1	MYRSHFIADVTPEYDGKEVIWAGVHLLRDLGGKKFILLRDKTGLGQVVDKN---	SSAFGISQELTQESVIVQVRGIVKAD---KRA---PRGIELHAEETLLS	96						
<i>P. kodakaraensis</i>	1	MYRTHYSSEITEELNGQKVKVAGWVVEVKDLGGIKELWIRDRDGIVQITAPKKVDPELFKLIPLKLRSEVVAVEGVVNF---	PKAK---LGFEILPEKIVVLN	99						
<i>T. thermophilus</i>	1	--MRVLVRDL-KAHVGQEVELLGFLHWRRLDGRIOQLLLRDRSGVVQVVTG-----	GLKLPLPEALRVRGLVEN--AKAP-----GGLEVQAKEVEVL	86						
			35	46		75	77	81	83	92

Figure 2
Structure-based sequence alignment of the anticodon-binding domains of different aspartyl-tRNA synthetases. The amino-acid sequence of *H. pylori* ND-AspRS_{1–104} was aligned with those from *P. aeruginosa*, *M. smegmatis*, *E. coli*, *S. tokodaii*, *P. kodakaraensis* and *T. thermophilus*. The secondary structure of *H. pylori* ND-AspRS_{1–104} is displayed as a diagram above the alignment. The α -helix and 3_{10} -helices are represented by cylinders, β -strands by arrows and loops by straight lines. Residues belonging to the L1 loop are underlined. Residues are coloured according to predicted secondary structures (red for α -helix and blue for β -strand). Conserved amino acids are highlighted with yellow shading. The diagnostic residues reported to interact with the tRNA anticodon are marked in italics according to the sequence numbering of *H. pylori* ND-AspRS_{1–104}.

both C and U, the third anticodon of tRNA^{Asp} and tRNA^{Asn}, respectively (Sato *et al.*, 2007).

3.2.2. Structural comparison with D-AspRS. The structures of ND-AspRS_{1–104} from *H. pylori* (PDB entry 5gro), D-AspRS from *E. coli* (PDB entry 1c0a; Eiler *et al.*, 1999) and D-AspRS

from *P. kodakaraensis* (PDB entry 3nem; Schmitt *et al.*, 1998) are superposed in Fig. 3(a). The overall structure comparison indicates that the core structures of both the nondiscriminating and discriminating types are similar in shape and size. There are two short 3_{10} -helices (6CTE_8 and 11EKD_{13} ; Fig. 1) tethered to the common OB-fold by a β -turn in our structure. The long L1 loop (Arg77–Lys90) in our structure is similar to that of D-AspRS from *E. coli* (Arg78–Glu91), but is significantly longer than that of D-AspRS from *P. kodakaraensis* (Thr81–Gly87, Fig. 3a). Moreover, the major difference is the presence of the Pro84 residue discussed above, which is absent in D-AspRS from both *E. coli* and *P. kodakaraensis* (Fig. 2). A structural superimposition of ND-AspRS from *S. tokodaii* and the two D-AspRS structures from *Thermococcus kodakaraensis* and *Saccharomyces cerevisiae* (Sato *et al.*, 2007) showed that the amide protons of Lys85 in *T. kodakaraensis* and Lys180 in *S. cerevisiae* specifically recognize C36 in tRNA^{Asp} through hydrogen bonding between N4 of the cytosine base and the H atom of the main-chain amide group. In *S. tokodaii* ND-AspRS, however, the nearest residue to C36 is Pro82, which lacks a main-chain amide H atom and cannot form a

Figure 3

(a) The superposed structures of *H. pylori* ND-AspRS_{1–104} (this work; PDB entry 5gro, salmon) and the D-AspRSs from *E. coli* (PDB entry 1c0a, yellow) and *P. kodakaraensis* (PDB entry 3nem, blue). The tRNA^{Asp} anticodon (34QUC36) modelled in the structure of *H. pylori* ND-AspRS_{1–104} is shown as a stick model with C atoms in yellow, N atoms in blue and O atoms in red. Turns A, C and D are expanded in the insets to show the differences in turn structures. Black dashed lines represent hydrogen bonds formed in the crystal structures; their distances are given in Å. (b) The amino-acid residues of *E. coli* D-AspRS (PDB entry 1c0a) interacting with the tRNA^{Asp} anticodon are depicted as yellow ball-and-stick models showing crucial interactions discussed in the text. The tRNA^{Asp} anticodon (34QUC36) is shown as a stick model with C atoms in yellow, N atoms in blue and O atoms in red. Black dashed lines represent hydrogen bonds formed in the crystal structures; their distances are given in Å. (c) The amino-acid residues of *H. pylori* ND-AspRS_{1–104} (PDB entry 5gro, salmon) are depicted as salmon ball-and-stick models. The tRNA^{Asp} anticodon (34QUC36) modelled in the structure of *H. pylori* ND-AspRS_{1–104} is shown as a stick model with C atoms in yellow, N atoms in blue and O atoms in red. The predicted hydrogen bonds are shown as green dashed lines.

specific hydrogen-bonding interaction with C36. Homologous to Pro82 in *S. tokodaii* ND-AspRS, Pro84 in *H. pylori* ND-AspRS_{1–104} protrudes away from the third nucleotide of the

anticodon (Fig. 4a), presumably allowing the acceptance of a cytosine base (³⁴QUC³⁶ in tRNA^{Asp}) as well as a uracil base (³⁴GUU³⁶ in tRNA^{Asn}), resulting in the relaxed tRNA specificity of this enzyme.

3.2.3. Structural analysis of tRNA^{Asp} anticodon recognition. The superposition of *H. pylori* ND-AspRS_{1–104} with D-AspRS from *E. coli* gave an r.m.s.d. of 1.22 Å between their C^α atoms, indicating a high similarity in the overall fold of the two proteins (Fig. 3a). The ³⁴QUC³⁶ tRNA^{Asp} anticodon from the *E. coli* D-AspRS:tRNA^{Asp} co-crystal structure fitted into the structure of our bacterial ND-AspRS based on the superimposition. As seen in Fig. 3(c), the N atom of the side chain of Asn83 recognizes the O2 atom of the cytosine base (C36) with a hydrogen bond of 3.5 Å. A similar interaction is present in the *E. coli* D-AspRS:tRNA^{Asp} co-crystal structure (Fig. 3b). In addition, the N atom of Arg77 in our structure forms two hydrogen bonds to N3 and O2 of C36 with distances of 2.8 and 2.7 Å, respectively. We also observe an interaction between the N atom of Arg77 and the O4 atom of U35 (Fig. 3c). Once again, a similar interaction (from Arg78 to U35) is present in the *E. coli* structure (Fig. 3b). The carbonyl backbone of Leu81 forms a hydrogen bond to the N4 atom of C36 with a distance of 2.7 Å (Fig. 3c). A similar interaction was observed in the *E. coli* structure, with Asn82 replacing Leu81 (Fig. 3b). The observed participation of Leu81 in the recognition of C36 in our model agrees with the experimentally demonstrated importance of this residue for tRNA^{Asp}

Figure 4

(a) The superposed structures of *H. pylori* ND-AspRS_{1–104} (PDB entry 5gro, salmon) and the ND-AspRSs from *P. aeruginosa* (PDB entry 4wj4, cyan) and *M. smegmatis* (PDB entry 4o2d, orange). The tRNA^{Asn} anticodon (³⁴GUU³⁶) modelled in the structure of *H. pylori* ND-AspRS_{1–104} is shown as a stick model with C atoms in cyan, N atoms in blue and O atoms in red. Turns A, C, D, E and F are expanded in the insets to show the differences in the turn structures. Black dashed lines represent hydrogen bonds formed in the crystal structures; their distances are given in Å. (b) The amino-acid residues of *P. aeruginosa* ND-AspRS (PDB entry 4wj4, cyan) interacting with the tRNA^{Asn} anticodon (³⁴GUU³⁶) is shown as a stick model with C atoms in cyan, N atoms in blue and O atoms in red. Black dashed lines represent hydrogen bonds formed in the crystal structures; their distances are given in Å. (c) The amino-acid residues of ND-AspRS_{1–104} from *H. pylori* (PDB entry 5gro, salmon) are depicted as salmon ball-and-stick models. The tRNA^{Asn} anticodon (³⁴GUU³⁶) modelled in the structure of *H. pylori* ND-AspRS_{1–104} is shown as a stick model with C atoms in cyan, N atoms in blue and O atoms in red. The predicted hydrogen bonds are shown as green dashed lines.

recognition; an L81M mutation increases the tRNA^{Asp} specificity of *H. pylori* ND-AspRS by 2.4-fold (Chuawong & Hendrickson, 2006).

As seen in Fig. 3(a), the overall structure of the bacterial ND-AspRS_{1–104} from *H. pylori* is less similar to that of the N-terminal domain of the archaeal *P. kodakaraensis* D-AspRS (Schmitt *et al.*, 1998), with an r.m.s.d. of 6.19 Å between C α atoms. The major difference is that the corresponding L1 loop (residues Thr81–Gly87) in *P. kodakaraensis* D-AspRS is much shorter than that of *H. pylori* ND-AspRS. It has been proposed that the short loop in D-AspRS from *P. kodakaraensis* constrains its specificity to tRNA^{Asp} only (Schmitt *et al.*, 1998). However, the length of the L1 loop has no correlation to the tRNA specificity of AspRS enzymes since both short and long L1 loops are found in both discriminating and non-discriminating AspRSs (Charron *et al.*, 2003).

3.2.4. Structural comparison with other ND-AspRS structures. The superposition of our ND-AspRS_{1–104} structure with those of the known bacterial-type ND-AspRSs from *P. aeruginosa* (PDB entry 4wj4; Suzuki *et al.*, 2015) and *M. smegmatis* (PDB entry 4o2d; Baugh *et al.*, 2015) revealed a similar core and size of the closed β -barrel (Fig. 4a), but showed less similarity, with high r.m.s.d. values of 6.29 and 6.21 Å when superposed with the anticodon-binding domains from the archaeal-type ND-AspRSs from the *T. thermophilus* transamidosome (PDB entry 3kfu; Blaise *et al.*, 2010) and *S. tokodaii* (PDB entry 1wyd; Sato *et al.*, 2007), respectively (Supplementary Figs. S3 and S4). The *H. pylori* ND-AspRS_{1–104} structure is similar to that of *T. thermophilus* because both structures contain two short 3_{10} -helices connected to the OB-fold. Contrarily, only one was found in *S. tokodaii* ND-AspRS (Sato *et al.*, 2007). In our structure, the Pro84 residue is shifted away from U36 in the tRNA^{Asn} anticodon (Fig. 4a), similar to the positions of the Pro82 residue in the *S. tokodaii* ND-AspRS structure (Sato *et al.*, 2007).

3.2.5. Structural analysis of tRNA^{Asn} anticodon recognition. Structural comparison of ND-AspRS_{1–104} from *H. pylori* with those from *M. smegmatis* (PDB entry 4o2d; Baugh *et al.*, 2015) and *P. aeruginosa* (PDB entry 4wj4; Suzuki *et al.*, 2015) gave r.m.s.d. values of 1.09 and 1.29 Å, respectively. Taking advantage of the tRNA^{Asn} anticodon (³⁴GUU³⁶) bound to the recognition site of the ND-AspRS from *P. aeruginosa*, it is possible to propose analogous interactions in *H. pylori* ND-AspRS_{1–104}. In our structure, we propose that the N atom of the Asn83 side chain forms a hydrogen bond to the O2 atom of U36 (Fig. 4c). A similar interaction was observed in the *P. aeruginosa* ND-AspRS:tRNA^{Asn} co-crystal structure with the corresponding Asn85 residue (Fig. 4b). Moreover, additional U36 recognition proceeds *via* hydrogen-bond formation between the carbonyl backbone of Leu81 and the N3 and O4 atoms of U36 (Fig. 4c). These interactions can be seen with Ala83 of ND-AspRS from *P. aeruginosa* (Fig. 4b).

The conserved residue Gln46 in *H. pylori* ND-AspRS_{1–104} also forms hydrogen bonds to the U35 nucleotide found in both the tRNA^{Asp} (Fig. 3c) and tRNA^{Asn} (Fig. 4c) anticodons. Similar interactions were also reported in the *E. coli* (Fig. 3b)

and *P. aeruginosa* (Fig. 4b) structures. The formation of hydrophobic stacking by the conserved Phe33 against the pyrimidine ring of U35 was proposed in ND-AspRS:tRNA^{Asn} in the *T. thermophilus* transamidosome (Blaise *et al.*, 2010). Interestingly, the analogous residue, Phe35 in *H. pylori* and Phe36 in *P. aeruginosa*, is located relatively far away from U35 and probably does not exhibit hydrophobic stacking interactions (Figs. 4b and 4c).

3.2.6. Differences in the OB-fold. Our structure differs from that of the archaeal-type ND-AspRS from the *T. thermophilus* transamidosome structure because the helix (α 1) located between strands β 3 and β 4 is absent in the transamidosome structure. The bacterial-type *H. pylori* ND-AspRS_{1–104} contains four residues forming helix α 1; in contrast, a loop with ten residues is found at this position in *T. thermophilus* ND-AspRS (Supplementary Fig. S3). The presence of α 1 located opposite the anticodon-recognition site in our structure is similar to that in the bacterial-type ND-AspRS from *P. aeruginosa* (Suzuki *et al.*, 2015) and from *M. smegmatis* (Baugh *et al.*, 2015) and the archaeal-type ND-AspRS from *S. tokodaii* (Sato *et al.*, 2007) (Fig. 4a, Supplementary Fig. S4). However, it has been proposed that a structural change in this loop might not relate to the evolution of the nondiscriminating function of this enzyme (Sato *et al.*, 2007).

There were six turns formed in our structure (turn A, hydrogen bonding between His30 and Val33; turn B, between Asp40 and Gly43; turn C, between Asp50 and Ser53; turn D, between Ala59 and Val62; turn E, between Gly78 and Leu81; turn F, between Asn83 and Leu86). The distances between residues His30 and Val33, Asp50 and Ser53, and Ala59 and Val62 in turns A, C and D of our structure are closer than those in D-AspRS from *E. coli* by approximately 0.2–0.7 Å (Fig. 3a). These close contacts help to stabilize the closed β -sheets (OB-fold) and make the structure more compact, and might increase the stability of helix α 1 and the OB-fold. Turn B, which connects strand β 2 and β 3, has a similar shape in the ND-AspRS structures from *H. pylori* and *P. aeruginosa* (Suzuki *et al.*, 2015) and D-AspRS from *E. coli*. Both turns E and F are parts of the long L1 loop, and are significantly closer to nucleotide 36, thus facilitating the enfolding of the tRNA anticodon-recognition site.

A minor difference found in turn E in *H. pylori* ND-AspRS_{1–104} (₇₈GAGL₈₁) versus *P. aeruginosa* ND-AspRS (₈₀PEGA₈₃) is the location of Leu81 and the homologue Ala83. The Leu81 residue is located in the recognition loop of *H. pylori* ND-AspRS_{1–104}, but the analogous Ala83 residue is in the short 3_{10} -helix in the *P. aeruginosa* ND-AspRS structure (Fig. 4a). Pro84 significantly affects the formation of turn F (₈₃NPKL₈₆) in *H. pylori* ND-AspRS_{1–104} (Fig. 4a) and in the ND-AspRS structures from *P. aeruginosa* (Suzuki *et al.*, 2015) and *M. smegmatis* (Baugh *et al.*, 2015) (Fig. 2).

In conclusion, the structure of the bacterial-type *H. pylori* N-terminal domain, ND-AspRS_{1–104}, reveals a β -sheet-rich domain comprising a five-stranded β -sheet in a compact β -barrel, which is similar to the structures of D-AspRS from *E. coli* (Eiler *et al.*, 1999) and ND-AspRS from *P. aeruginosa*

(Suzuki *et al.*, 2015). *H. pylori* ND-AspRS_{1–104} has a more compact OB-fold, as revealed by the formation of hydrogen bonds within all structural turns. Moreover, turns E and F presumably play a major role in efficiently recognizing the different tRNA anticodons in tRNA^{Asp} (³⁴GUC³⁶) and tRNA^{Asn} (³⁴GUU³⁶). The long loop in our *H. pylori* ND-AspRS_{1–104} structure, which contains turn F and its conserved Pro84 residue, helps to accommodate these divergent anticodons. Interactions between several conserved residues in this N-terminal domain and the three nucleotides of both tRNA anticodons help to explain the relaxed specificity of the enzyme.

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