Crystal structure of histidyl-tRNA synthetase from *Escherichia coli* complexed with histidyl-adenylate

J.G.Arnez, D.C.Harris, A.Mitschler, B.Rees, C.S.Francklyn¹ and D.Moras²

Institut de Génétique et de Biologie Moléculaire et Cellulaire, CNRS/INSERM/ULP BP 163, 67404 Illkirch Cedex, France and ¹Department of Biochemistry, College of Medicine, The University of Vermont, Burlington, VT 05405, USA

²Corresponding author

The crystal structure at 2.6 Å of the histidyl-tRNA synthetase from *Escherichia coli* complexed with histidyl-adenylate has been determined. The enzyme is a homodimer with a molecular weight of 94 kDa and belongs to the class II of aminoacyl-tRNA synthetases (aaRS). The asymmetric unit is composed of two homodimers. Each monomer consists of two domains. The N-terminal catalytic core domain contains a six-stranded antiparallel β -sheet sitting on two α -helices, which can be superposed with the catalytic domains of yeast AspRS, and GlyRS and SerRS from *Thermus thermophilus* with a root-mean-square difference on the C α atoms of 1.7–1.9 Å. The active sites of all four monomers are occupied by histidyl-adenylate, which apparently forms during crystallization. The 100 residue C-terminal α/β domain resembles half of a β -barrel, and provides an independent domain oriented to contact the anticodon stem and part of the anticodon loop of tRNA^{His}. The modular domain organization of histidyl-tRNA synthetase reiterates a repeated theme in aaRS, and its structure should provide insight into the ability of certain aaRS to aminoacylate minihelices and other non-tRNA molecules.

Keywords: class II aminoacyl-tRNA synthetase/crystal structure/histidyl-adenylate/histidyl-tRNA synthetase/prokaryote

Introduction

The joining of amino acids to the 3' ends of their cognate tRNAs by aminoacyl-tRNA synthetases (aaRS) represents a key step in biological information transfer. These enzymes have been partitioned on the basis of sequence comparisons (Eriani *et al.*, 1990) into two classes of 10 members each that differ with respect to the topology of the catalytic fold and the site of esterification on the tRNA. Class I aaRS share a five-stranded parallel β -sheet based on the nucleotide binding (Rossmann) fold, and charge amino acids to the 2' OH of their cognate tRNAs. Class I enzymes of known structure include tyrosyl-tRNA synthetase (TyrRS) from *Bacillus stearothermophilus* (Brick *et al.*, 1989), tryptophanyl-tRNA synthetase (TrpRS) from *B.stearothermophilus* (Doublié *et al.*, 1995), methionyl-tRNA synthetase (MetRS) from *Escherichia coli* (Brunie *et al.*, 1990) and the glutamyl-tRNA

synthetase (GlnRS):tRNA^{Gln} complex from *E.coli* (Rould *et al.*, 1989, 1991). Class II enzymes have a catalytic domain composed of an antiparallel β -sheet and aminoacylate on the 3' OH of their cognate tRNAs. Class II synthetases whose structures have been obtained by X-ray crystallography include seryl-tRNA synthetase (SerRS) from *E.coli* (Cusack *et al.*, 1990) and *Thermus thermophilus* (Belrhali *et al.*, 1994; Biou *et al.*, 1994), aspartyl-tRNA synthetase (AspRS) from *Saccharomyces cerevisiae* (Ruff *et al.*, 1991) and *T.thermophilus* (Poterszman *et al.*, 1994), lysyl-tRNA synthetase (LysRS) from *E.coli* (Onesti *et al.*, 1995) and glycyl-tRNA synthetase from *T.thermophilus* (Logan *et al.*, 1995, accompanying paper). The structure of AspRS from *S.cerevisiae* is of the complex with its cognate tRNA. In both classes, sequence motifs composed of conserved residues make critical contacts with the universal substrate ATP. In class I enzymes, the conserved KMSKS and HIGH sequence motifs are responsible for ATP binding while, in class II enzymes, the arginine- and glutamate-rich motif 2 and motif 3 regions make analogous contacts (Eriani *et al.*, 1990; Delarue and Moras, 1993). Although several aaRS:adenylate complexes have been reported (Brick *et al.*, 1989; Belrhali *et al.*, 1994; Poterszman *et al.*, 1994; Doublié *et al.*, 1995), the basis of amino acid binding specificity is still emerging, and important mechanistic issues remain to be resolved.

The complexes with tRNA that have been reported for the glutamyl- (Rould *et al.*, 1989), aspartyl- (Ruff *et al.*, 1991; Cavarelli *et al.*, 1993) and seryl- (Biou *et al.*, 1994) tRNA synthetases suggest that the accessory domains joined to the catalytic cores function as specific tRNA recognition domains. In class II enzymes, the location of this accessory domain has been used to subdivide the class into a subgroup (class IIa) that possesses a C-terminal tRNA binding domain, and a second group (class IIb) that shares a conserved N-terminal tRNA binding domain (Cusack *et al.*, 1991; Delarue and Moras, 1993). As observed for the AspRS:tRNA^{Asp} complex (Cavarelli *et al.*, 1993), base-specific recognition of the anticodon is likely to be general to class IIb enzymes, while for class IIa enzymes the relationship between synthetase accessory domains and the location of the identity/recognition elements in the tRNA is not yet clear. The recognition of the tRNA variable loop by the N-terminal coiled-coil domain that is observed in the SerRS:tRNA^{Ser} complex is unlikely to be general to class IIa enzymes in *E.coli*, because none of the other class II enzymes aminoacylate type II tRNAs (Giegé *et al.*, 1993) and, based on sequence alignments, it is unlikely that any of the other class II enzymes possess this domain. Moreover, many of the class IIa enzymes apparently share a conserved C-terminal domain absent in SerRS (Cusack *et al.*, 1991; Delarue and Moras, 1993).

We report here the structure of another class IIa enzyme, the *E.coli* histidyl-tRNA synthetase (HisRS) in a complex with the histidyl-adenylate. An obligate dimer of 94 kDa (Freedman *et al.*, 1985), HisRS recognizes acceptor stem base pairs, both in the context of full-length tRNA (Himeno *et al.*, 1989; Francklyn and Schimmel, 1990; Francklyn *et al.*, 1992) and in mini- and microhelices (Francklyn and Schimmel, 1990; Francklyn *et al.*, 1992). Thus, HisRS serves as a model for those aaRS in which the acceptor stem, rather than the anticodon, is the primary location of identity elements. The structure of *E.coli* HisRS provides further evidence that class II aaRS possess a conserved catalytic domain to which have been appended insertion

domains and independent accessory tRNA binding domains, allowing specific recognition of both cognate amino acids and tRNAs.

Results and discussion

The structure of HisRS was solved by multiple isomorphous replacement (MIR) using two derivatives followed by 4-fold non-crystallographic symmetry averaging of electron density maps. It was refined at 2.6 Å resolution to a final R-factor of 24.6% with good stereochemistry (see Materials and methods).

The histidyl-tRNA synthetase monomer (Figure 1A and B)

consists of three domains: a catalytic domain that resembles the canonical class II fold; a C-terminal domain; and an extra domain that inserts into the catalytic core and is distal to the dimer interface. The structures of the four monomers that comprise the asymmetric unit have virtually the same interiors overall, while some surface side chains exhibit different conformations. The dimers (Figure 1C) are very similar to each other, while the monomers within each dimer are slightly different. The root-mean-square (r.m.s.) differences in backbone atom positions between the dimers are 0.12 Å and between the

monomers within a dimer 0.19 Å. The r.m.s. differences in all atomic coordinates are 0.95 and 1.3 Å, respectively.

Catalytic domain

The N-terminal catalytic domain of residues 1–325 is a seven-stranded β -sheet, comprising one parallel and six antiparallel β -strands bordered by three α -helices, that is topologically related to other class II synthetases of known structure, including the SerRS from *E.coli* (Cusack *et al.*, 1990) and *T.thermophilus* (Belrhali *et al.*, 1994), the AspRS from *S.cerevisiae* (Ruff *et al.*, 1991) and *T.thermo-*

Fig. 1. The structure of HisRS from *E.coli*. (A) The monomer; motif 1 is in red, motif 2 in cyan, motif 3 in dark blue and the insertion in purple. The adenylylation site is drawn in white in a stick rendering. The C-terminal domain is at the bottom. (B) Topology diagram of the secondary structure elements of HisRS; the motifs are colour-coded as in (A). (C) The dimer (dimensions 85×60×45 Å) with the histidyl-adenylate shown in a CPK representation in each active site.

Table IA. Structural comparison of the core domain of HisRS with those of other class II aaRS

	GlyRStt	AspRSsc	AspRStt	SerRSec	SerRStt
Overall	1.70 (180)	1.89 (161)	1.75 (165)	1.80 (168)	1.78 (172)
M1,2,2.5,3	1.18 (84)	1.18 (85)	1.20 (86)	1.43 (87)	1.62 (86)
M2,2.5,3	0.94 (56)	0.91 (56)	0.82 (56)	1.06 (56)	1.28 (55)

The numbers are r.m.s. differences in Å between α -carbon positions; the number of α -carbon atoms used in each comparison is given in parentheses. M1, 2 and 3 are the canonical class II motifs, while M2.5 consists of two β -strands immediately adjacent to the strand of motif 3 (AS8 and AS9 in HisRS). The residues constituting the motifs are shown below in Table IB (the suffixes to aaRS abbreviations stand for the species of origin: ec, *E.coli*, tt, *T.thermophilus*, sc, *S.cerevisiae*).

Table IB.

	HisRSec	GlyRStt	AspRSsc	AspRStt	SerRSec	SerRStt
M1	17–47	37–69	246–276	142–172	168–199	164–194
M2	103–114	210–221	315–326	213–224	258–269	246–257
	121–133	231–243	334–346	231–243	283–295	271–283
M2.5	267–272	290–295	468–473	466–471	339–344	329–334
	281–288	304–311	478–485	476–483	355–362	345–352
M3	301–317	356–372	521–537	521–537	386–403	375–392

The references for the structures are GlyRStt (Logan *et al.*, 1995), AspRSsc (Ruff *et al.*, 1991; Cavarelli *et al.*, 1993), AspRStt (Poterszman *et al.*, 1994), SerRSec (Cusack *et al.*, 1990), SerRStt (Belrhali *et al.*, 1994).

Fig. 2. Superpositions on the C α atoms of the conserved class II active site cores of (A) AspRS (Poterszman *et al.*, 1994), (B) SerRS (Belrhali *et al.*, 1994) and (C) GlyRS (Logan *et al.*, 1995), all from *T.thermophilus*, onto HisRS from *E.coli*. HisRS is traced in red, AspRS in blue, SerRS in cyan and GlyRS in green.

philus (Poterszman *et al.*, 1994) and GlyRS from *T.thermophilus* (Logan *et al.*, 1995). As predicted from sequence alignments, HisRS contains structural elements corresponding to motif 1, motif 2 and motif 3. The overall core domains of the class II aaRS listed above superpose with r.m.s. differences on the C α atoms ranging from 1.7 to 1.9 Å over the regions shared by all of them (Table I).

When only the elements of the structure containing the conserved sequence motifs and two additional structurally conserved strands are compared, the r.m.s. differences range from 1.2 to 1.6 Å. If motif 1 is excluded, the similarities are even more profound, with r.m.s. differences from 0.8 to 1.3 Å. Although both SerRS and HisRS are class IIa enzymes, HisRS from *E.coli* is topologically

Fig. 3. Dimer interface. (A) Interactions between the core domains. (B) Interactions between the C-terminal domain and the core domain of the other monomer.

more similar to AspRS from *T.thermophilus* than SerRS from *E.coli* (Figure 2). The seventh β -strand distal to the subunit interface (AS7A in HisRS, Figure 1B), which is absent in GlyRS, is parallel to the nearest neighbour strand in HisRS and AspRS, but antiparallel in the case of SerRS. Both HisRS and AspRS possess significant insertion domains that are located between motif 2 and motif 3, which SerRS clearly lacks. GlyRS also possesses such a domain but between motif 1 and motif 2. However, the N-terminal coiled-coil domain in SerRS is in a topologically similar position with respect to the insertion domain in HisRS (Figure 2B). In other respects, the topology of HisRS is similar to the other class II aaRS of known structures. One structural feature that distinguishes HisRS from the others (with the possible exception of AspRS from *T.thermophilus*) is the conformation of the long loop between AS7A and AS8 that reaches over part of the active site. This loop contains residues corresponding to a hexapeptide sequence (LV/ARGLDYY) that is strictly conserved among all HisRS, but is not found in other class II aaRS. In HisRS, these residues participate in the recognition of histidine. In GlyRS, SerRS and yeast AspRS this loop does not extend over the amino acid binding site and does not make an appreciable contribution to substrate recognition.

Subunit interface

The 2369 Å² of buried surface that comprises the dimeric interface of HisRS includes contributions from motif 1 and from the four-stranded antiparallel β -sheet, strands AS3 and AS4, located in the small insertion between motif 1 and motif 2 in each monomer. This β -sheet forms an arch over the dimeric interface (top of Figures 1C and 3A) and is structurally homologous to the subunit interfaces of AspRS, GlyRS and SerRS. Several residues provide a structural linkage between dimerization and substrate interactions in the active site (Figure 3A). As in the case of AspRS and SerRS, a strictly conserved proline in motif 1 (Pro44 in HisRS) constrains the conformation of the peptide backbone in this crucial region. This conformation permits a hydrogen bond between the main chain amide of Ile45, which follows Pro44, and the ϵ -oxygen of Gln124; the latter residue precedes the class II conserved Phe125 responsible for stacking on the adenine ring of ATP. The major hydrophobic interface between the monomers features the side chains of Ile45, Val46, Phe112, Phe70 and their symmetry-related counterparts. Phe112 precedes Arg113, a class II conserved residue that contacts the α -phosphate of ATP. The importance of this hydrophobic core in allowing communication between the motif 1 interface and the active site is reflected in the

Fig. 4. Multiple alignment of HisRS sequences from: Ec = *Escherichia coli* (Freedman et al., 1985), Se = *Streptococcus equis* (Menguito et al., 1993), Sc = *Saccharomyces cerevisiae* (Natsoulis et al., 1986), Ce = *Caenorhabditis elegans* (Amaar and Baillie, 1993), Hs = *Homo sapiens* (Raben et al., 1992) and of the GCN2 sequence from *S. cerevisiae* (Wek et al., 1989). The alignment was obtained using the LineUp and Pretty routines from the GCG program package (GCG, 1994). Secondary structure was derived by inspection of the structure aided by the program PROCHECK (Laskowski et al., 1993). The three motifs characteristic of class II aaRS are highlighted, as are the histidine-specific regions, Histidine A and B. Strictly conserved residues are indicated in bold letters.

conservation of a number of its constituents over evolution in all HisRS. Ile45 is replaced by the similarly β -branched valine in eukaryotic HisRS, and Phe112 is replaced by tyrosine. At positions 46 and 70 there is a reciprocal switch, so that the β -branched side chain at 46 and

aromatic at 70 in prokaryotes are replaced by a Phe and a Leu, respectively, in eukaryotes (Figure 4).

As in the case of other class II aaRS of known structure, the long helix of motif 1 in HisRS forms part of the dimeric interface with its symmetrically related counterpart. In

AspRS the interhelix distance is 6.5 Å, while in SerRS, GlyRS and HisRS the distance is 11–12 Å. A key element that stabilizes the helix–helix packing in HisRS is the salt bridge between Arg42 and Glu26' (where the prime refers to a residue in the other monomer). The conformation of Arg42 is maintained by an ionic contact with Glu40. Additional intersubunit contacts are contributed by residues projecting from the antiparallel sheet formed by the interaction of N-terminal β -strand, NS1, with the β -strand in motif 1 of the other monomer, AS1'. In GlyRS and SerRS, the N-terminus also makes these interactions, whereas in AspRS the C-terminus makes analogous contacts. Several residues in the N-terminus of HisRS from *E.coli* are strictly conserved among all HisRS, including Gly10, which stabilizes the bend between the N-terminus and NS1, and Asp13, which forms a salt bridge with Arg90' in helix AH3' (Figure 3A). Also participating in the salt bridge to Arg90' is Glu47, a strictly conserved residue in motif 1 that is located three residues away from Pro44. These salt bridges join portions of motif 1 and the N-terminus of the other subunit to constrain helix AH3, whose N-terminus protrudes into the active site. Thus, the motif 1 subunit interface influences the disposition of both active sites by virtue of direct contacts with the motif 2 loop of the other monomer and helix AH3 in its own monomer.

Insertion domain

A feature of HisRS that is largely absent in the model is the insertion domain located between strand AS7 and helix AH5, which includes the residues from 178 to 228 in one monomer and 167 to 229 in the other. This 60 amino acid domain is disordered in the structure, with electron density present but of insufficient definition to permit an unambiguous chain tracing. Based on its location, this domain may be responsible for contacts to the minor groove side of the acceptor stem of tRNA^{His}. This function would be analogous to the role of the Rossmann fold insertion in GlnRS, which is responsible for interactions with the minor groove of the acceptor stem (Rould *et al.*, 1989). In SerRS the N-terminal coiled-coil domain is topologically anchored in the same location as the disordered domain in HisRS; however, in this case it binds the long variable loop of tRNA^{Ser} and not the acceptor stem (Biou *et al.*, 1994). It is oriented away from the catalytic core, while the insertion in HisRS is most probably oriented toward and above the active site.

C-terminal domain

HisRS from *E.coli* possesses a C-terminal domain of 100 amino acids which, by analogy with AspRS (Figure 2A), is situated with respect to the catalytic domain to permit interaction with the tRNA^{His} anticodon stem. Unlike the β -barrel N-terminal domain of AspRS, the C-terminal domain is a mixed α – β domain of one antiparallel and four parallel β -strands and three α -helices that shows high structural homology with the C-terminal domain of GlyRS from *T.thermophilus*. (Logan *et al.*, 1995). The r.m.s. difference on the α -carbon atoms between the HisRS and GlyRS C-terminal domains is 1.56 Å (Figure 5). The HisRS C-terminal domain is connected to the α -helix AH7 of motif 3 by an eight residue peptide segment whose high B-factors suggest a high degree of conformation

Fig. 5. Superposition of the C-terminal domains of HisRS from *E.coli* (red) and GlyRS from *T.thermophilus* (Logan *et al.*, 1995) (green).

flexibility. This connecting peptide provides one way in which allosteric information resulting from a productive protein–RNA interaction could be transmitted to essential catalytic residues in motif 3. With the exception of this peptide, the C-terminal domain of HisRS interacts exclusively with the core domain of the other monomer (Figure 1C). By contrast, the C-terminal domain of GlyRS shares a substantial interface with both monomers, so that it is in a substantially shifted position with respect to that of HisRS.

Alternatively, packing interactions between the C-terminal domain and other elements of the HisRS catalytic domain could serve to affect interactions in the active site (Figure 3B). The interface between these domains, constituting 903 Å² of buried surface, is characterized by numerous charged and polar residues, providing stabilization by electrostatic interactions and hydrogen bonding. Notable in these contacts is strictly conserved Glu143, which is involved in a salt bridge with Arg104 in the core domain and His364' in the C-terminal domain. Interdomain hydrogen bonds are also present between the phenolic oxygen of Tyr36 and Glu349', and between the main chain carbonyl of Ser35 and the backbone amide of Leu360'. Additional hydrophobic interactions with the aliphatic portions of Arg104 and Glu102 and with His364' are provided by Met361' and Trp379' on the C-terminal domain side, and Val132, Leu135 and Ile140 found in the coil and helix immediately after strand AS6 in motif 2. Because Arg104 and Leu135 are located in motif 2 strands AS5 and AS6, respectively, they are connected directly to the active site and may in turn relay information concerning a productive tRNA interaction from the interface between the C-terminal and catalytic domains to the active site.

The analysis of mutations in *hisS* that confer altered tRNA selection *in vivo* is consistent with this proposal (W.Yan and C.S.Francklyn, in preparation). In the *E.coli* ThrRS, the mutation of an arginine found in the C-terminal domain, Arg583, leads to an increase in the K_m for tRNA^{Thr} and the *thrS* translational operator (Springer *et al.*, 1989), suggesting that the C-terminal domain is important in the tRNA selection by other class IIa aaRS.

The active site

In the structure obtained, the histidyl-adenylate shows well defined density in the active sites of all monomers (Figure 6A). A detailed discussion of enzyme–substrate interactions and the implications of subtle conformational differences observed between the different monomers for the catalytic mechanism will be presented in a forthcoming paper (Arnez *et al.*, in preparation). The active site of HisRS has elements that are common to other class II aaRS, as well as features that, based on the sequence alignment, would be predicted to be conserved within the

HisRS family. As expected from alignments with other class II enzymes, contacts to the adenine are provided by several residues which are strictly conserved among class II aaRS: Phe125, which stacks on the adenine ring; Arg113, which contacts the α phosphate; Arg311, which forms a hydrogen bond with the 2' OH of the ribose (but not in all monomers); and Glu115, which contacts the exocyclic amino group of the adenine (Figure 6B). In the HisRS structure, the conformation of the motif 2 loop is well defined, which, along with the overall layout of the interactions, is consistent with what has been observed for other class II enzymes with bound substrates (Belrhali *et al.*, 1994; Cavarelli *et al.*, 1994; Poterszman *et al.*, 1994).

The binding pocket for histidine is formed both from the residues that project from the surface of strand AS6 in motif 2, and from residues that are found in distinct sequence motifs that are highly conserved among members of the HisRS family, in keeping with the general rule for all aaRS systems whereby these sequences are the most highly conserved within each family (Delarue and Moras,

1993). The residues in the L257VRGLDYY264 peptide that are located in the AS7A–AS8 loop comprise one wall of the binding pocket, while residues in the A284–GGRYDGL291 peptide found in AS9–AH5 comprise the floor and back wall of the histidine binding pocket. In addition to van der Waals contacts provided by these residues, hydrogen bonds to the histidine side chain are provided by Glu131 and Tyr264 (Figure 6B). In total, these interactions provide both shape and electrostatic complementarity, as well as satisfying the hydrogen bonding capabilities of the histidine ring. Topologically, the pocket is analogous to those found in AspRS (Cavarelli *et al.*, 1994; Poterszman *et al.*, 1994), SerRS (Belrhali *et al.*, 1994) and LysRS (Onesti *et al.*, 1995).

Comparison of all HisRS

The structure of HisRS from *E.coli* allows an improved alignment of all known HisRS primary structures (Figure 4) and provides clues on the structural basis of the prokaryotic–eukaryotic split. Homologies between prokaryotic and eukaryotic HisRS are concentrated in the catalytic domains, with only 28.5% identity between *E.coli* and *S.cerevisiae* over 235 residues. The eukaryotic enzymes are much more closely related to each other, with an overall identity of 43.5% between HisRS proteins from *S.cerevisiae* and *H.sapiens* (50% over the catalytic domain and 19% over the C-terminal extension). The greater extent of homology among all HisRS over residues in the catalytic domain (1–235, omitting the insertion domain) suggests that the catalytic domain has been largely retained over evolution. Many of the strictly

conserved residues play important functional roles in adenylate binding, the dimerization interface or maintaining the orientation of secondary structure elements that comprise the active site. Based on the high degree of conservation of crucial residues in motif 2, this provides further evidence of a strong structural homology between HisRS and the yeast translational regulatory protein GCN-2 (Wek *et al.*, 1989). However, GCN-2 cannot function as an aaRS, for it lacks the key residues in motif 3 and in the histidine A region.

Major differences between prokaryotic and eukaryotic HisRS include the N-terminal domain particular to eukaryotes and variations in sizes of selected loop regions. Based on circular dichroism studies (Raben *et al.*, 1994), the variable N-terminal domain (18 residues in *Caenorhabditis elegans* and 50 in *S.cerevisiae*) may be a coiled-coil domain that resembles the coiled-coil motif in SerRS. However, the addition of this domain to HisRS may have been a relatively recent occurrence, as this extra domain is completely encoded by the first two exons in the human gene, and related sequences have been detected in other eukaryotic synthetases, including the human tryptophanyl-, glycyl- and chimeric glutamyl/prolyl-tRNA synthetase (Cerini *et al.*, 1991; Fett and Knippers, 1991; Nada *et al.*, 1992). Other differences between prokaryotes and eukaryotes include: the absence of helix AH2A in eukaryotes; a larger motif 2 loop in eukaryotes, variability in the size of the insertion domain; and considerable variability in the size of the AS8–AS9 loop. Other than a conserved GExExxxG motif, there is little overall homology among the C-terminal domains of all HisRS, as

Fig. 6. (A) Electron density in the active site area of HisRS with bound histidyl-adenylate. The $2(F_{\text{obs}} - F_{\text{calc}})$, ϕ_{calc} map is contoured at 1.1σ level. (B) Interactions between the active site and histidyl-adenylate. Shown are the secondary structure elements constituting motifs 2 and 3, strands AS8 and AS9, with the adenylate and side chains of key residues rendered in a ball-and-stick representation. Main chain interactions of Tyr122 and Thr281 with the adenylate are indicated. Important hydrogen bonds are drawn as green lines. (C) Schematic drawing of the interactions between the adenylate moiety and the protein. (A) was generated with MINIMAGE (Arnez, 1994) followed by MOLSCRIPT (Kraulis, 1991). Figures 1A and C, 5 and 6B were rendered with MOLSCRIPT followed by RASTER3D (Bacon and Anderson, 1988). Figures 2 and 3 were drawn with MOLSCRIPT alone.

Fig. 7. Proposed model of tRNA binding to HisRS. The molecular surface of the protein showing the electrostatic potentials calculated with DelPhi (Nicholls and Honig, 1991) was calculated and rendered with GRASP (Nicholls *et al.*, 1991). Negatively charged regions are in red and positively charged areas in blue. The tRNA is rendered as a green coil. Shown are (A) the same view as in Figure 1C and (B) rotated by 90° around the dimer axis.

compared with the homology between the catalytic domains.

Implications for tRNA recognition

Comparison of the structures of yeast AspRS (Ruff *et al.*, 1991) and GlnRS from *E.coli* (Rould *et al.*, 1989) in complex with their cognate tRNAs has led to the proposal that the active sites of class I synthetases are arranged to recognize the minor groove of the tRNA acceptor stem, while those of class II synthetases recognize the major groove. Using the coordinates of tRNA^{Phe} (Quigley and Rich, 1976; Hingerty *et al.*, 1978; Sussman *et al.*, 1978) as a basis for a model of tRNA^{His}, and both the yeast AspRS complex and the electrostatic potential surface (Nicholls and Honig, 1991) as guides for superposition, the interaction between HisRS and its cognate tRNA can be modelled by inserting the acceptor stem of tRNA^{His} into the catalytic cleft and packing the anticodon stem against the C-terminal domain (Figure 7). In this orientation, the C-terminal domain is located at the appropriate distance to make contact with the anticodon stem. tRNA^{Phe} was chosen as the basis for modelling tRNA^{His} because the two tRNAs possess identical base triplets while tRNA^{Asp} is

different (Giegé *et al.*, 1993). Although the retention of histidine identity by both tRNA^{His} amber and opal suppressors (Yan and Francklyn, 1994 and unpublished data) suggests that the QUG anticodon is not obligatory for histidine identity, the possibility that contacts to anticodon nucleotides contribute some binding specificity to the recognition of tRNA^{His} has not been ruled out. Moreover, phosphorothioate footprinting analysis shows that regions of the anticodon stem are protected by HisRS (W.Yan and C.S.Francklyn, unpublished).

The structure of HisRS from *E.coli* is also significant in light of the fact that it is the first structure of an aaRS that efficiently aminoacylates mini- and microhelix substrates. It has been argued previously that, owing to the importance of the G1:C73 base pair as an identity and recognition determinant, a substantial portion of the specific binding energy between HisRS and its cognate tRNA is provided by contacts at the acceptor stem (Francklyn *et al.*, 1992). The model based on rigid body docking suggests that contacts to the tRNA acceptor stem would be contributed by the motif 2 loop, which contains many polar residues, and the AS8/AS9 loop, which is disordered. The latter polypeptide segment would be required to undergo a conformational change in order to bind tRNA, disrupting the hydrogen bond between the ϵ -oxygen of Gln279 and the main chain amide of Gly120. Based on the relatively high disorder of this part of the protein (the high B-factors in the flanks and the fact that the loop itself could not be built), this is not unreasonable. Moreover, there is considerable sequence divergence between prokaryotic and eukaryotic HisRS at this part of the sequence, consistent with the idea that this region contacts the discriminator base, which changes from cytosine to adenine in the evolution from prokaryotes to eukaryotes (Sprinzl *et al.*, 1989). While obligatory, these interactions may not fully account for the ability of HisRS to aminoacylate mini- and microhelix substrates. Extra binding energy for these additional acceptor stem interactions (i.e. the contacts required for minihelix charging) may be provided by the disordered insertion domain (residues 170–230). Although no clear chain tracing of this region is possible, the electron density in this region is consistent with the behaviour of a flexible domain that closes down over the top of the tRNA during aminoacylation. The presence of such domains in class II aaRS may be dominant structural features which confer the ability to aminoacylate mini- and microhelix substrates. More concrete evidence for this model will come from analysis of crystals based on the HisRS:tRNA^{His} complex.

Materials and methods

Crystallization and data collection

Recombinant HisRS was purified from overproducing cells as described (Francklyn *et al.*, 1994). Protein was dialysed against a solution containing 10 mM PIPES pH 7.4 and 20 mM β -mercaptoethanol before crystallization. Crystals of HisRS complexed with histidine and ATP were grown at room temperature (25°C) in sitting or hanging drops containing equal volumes of the complex (10 mg/ml) and a reservoir solution consisting of 0.1 M Tris-HCl (pH 7.4), 0.5 M NaCl and 10% polyethylene glycol (PEG) 6000 (Francklyn *et al.*, 1994). Two crystal forms are obtained using these conditions, one monoclinic, space group P2₁, with unit cell dimensions $a = 61.4$ Å, $b = 110.7$ Å, $c = 196.8$ Å, $\beta = 98^\circ$, and the other triclinic, space group P1, with unit cell dimensions $a = 61.4$ Å, $b = 110.7$ Å, $c = 108.7$ Å, $\alpha = 115.0^\circ$, $\beta = 97.4^\circ$,

Table II. Summary of crystallographic analysis

Phasing			
Derivative	Native	MA ^a	TMLA ^b
Resolution (Å)	2.6	3.2	3.2
Unique reflections	54 820	33 659	29 682
Multiplicity	1.7	1.7	1.5
Completeness (%)	69.4	79.9	70.4
R_{merge} (%) ^c	5.7	4.4	4.6
Cullis R^d		0.66	0.95
Number of heavy atom sites		8	4
MIR analysis (12.0–3.2)			
Phasing power ^e		1.9	0.5
Mean overall figure of merit	0.385		
Refinement			
Resolution range (Å)	Reflections	R-factor	Free R-factor
8.00–4.84	7260	0.2329	0.3225
4.84–3.99	6743	0.1951	0.2853
3.99–3.54	6815	0.2260	0.3087
3.54–3.24	6932	0.2537	0.3142
3.24–3.02	6887	0.2742	0.3724
3.02–2.85	6689	0.2934	0.3603
2.85–2.71	6550	0.3190	0.3877
2.71–2.60	5085	0.3348	0.3792
8.00–2.60	52961	0.2462	0.3271
R.m.s. deviations in bond lengths (Å)			0.015
bond angles (°)			2.925
dihedral angles (°)			25.072
Number of water molecules			157
Number of non-hydrogen atoms			11 539

^aMA, mercury acetate.^bTMLA, trimethyllead acetate.^c $R_{\text{merge}} = \sum_{hkl} \sum_i |I_{hkl,i} - \langle I_{hkl} \rangle| / \sum_{hkl} \sum_i I_{hkl,i}$, where i are the observations of reflection hkl .^dCullis $R = \sum |F_{\text{PH}} \pm F_{\text{P}} - |F_{\text{H(calc)}}|| / \sum |F_{\text{PH}} \pm F_{\text{P}}|$.^ePhasing power = $[\sum |F_{\text{H(calc)}}|^2 / \sum (|F_{\text{PH}}| - |F_{\text{H(calc)}}|)^2]^{1/2}$.Cullis R , phasing power and figure of merit were calculated with MLPHARE (Otwinowski, 1991).

$\gamma = 90.0^\circ$. The triclinic data were indexed in this way so that the unit cell parameters correlate more closely to those of the monoclinic cell; the self-rotation functions are thus identical, which means that the asymmetric units in the two forms are related by a simple translation.

Crystals were stabilized in 0.1 M Tris–HCl pH 7.4, 15% PEG 6000 and 0.2 M NaCl. Mercury derivatives were prepared by soaking native crystals in 1 mM mercury acetate $[\text{Hg}(\text{OOC}-\text{CH}_3)_2]$ for 5–7 h. Lead derivatives were obtained by soaking native crystals in 25 mM trimethyllead acetate $[(\text{CH}_3)_3\text{PbOOC}-\text{CH}_3]$ for 4 h. Data were collected at 0°C on a MARResearch phosphor image plate system using Cu-K α X-rays ($\lambda = 1.541$ Å) generated by a Siemens rotating anode generator set at 40 kV and 100 mA. Diffraction images were processed with the MARXDS/MARSCALE software (Kabsch, 1988).

Heavy atom and phase determination

The positions of mercury atoms were first determined for the monoclinic crystals by using the automatic search program HASSP (Terwilliger and Eisenberg, 1987) and refined with MLPHARE (Otwinowski, 1991). Single isomorphous replacement (SIR) phases were calculated with MLPHARE. Electron density maps calculated with the fast Fourier transform routine in the CCP4 package (Collaborative Computational Project, 1994) showed molecular boundaries and some secondary structural features. Solvent flattening with SQUASH (Zhang, 1993) improved the maps, but not sufficiently to allow model building. Since the monoclinic crystal form is related to the triclinic form, and better diffraction data were available for the latter, the mercury positions determined for the former and difference amplitudes between native and mercury data calculated for the latter were used in a molecular replace-

ment experiment with the program AMoRe (Navaza, 1994). The resulting positions were refined against the triclinic data, and SIR phases calculated with MLPHARE. The lead positions were then determined by difference Fourier methods. The heavy atom positions were then refined together and used to determine MIR phases with a mean figure of merit (FOM) of 0.385 for reflections in the resolution range 12.0–3.2 Å (Table II), which corresponds to a mean phase error of $\sim 70^\circ$ (Blundell and Johnson, 1976).

Electron density map averaging

The resulting electron density map clearly showed molecular boundaries and some secondary structural features, but it was not interpretable. It was used to determine the molecular envelope by way of a correlated density map as described by Rees *et al.* (1990) and with the programs BONES and MAMA from the RAVE package (Kleijwegt and Jones, 1994). The non-crystallographic symmetry operators were determined from the mercury positions by superposition using the method of least squares. Ten cycles of electron density averaging, structure factor calculation and combination of calculated and MIR phases (SIGMAA, Read, 1986) at 10.0–3.5 Å with the program suites RAVE and CCP4 yielded an interpretable electron density map. Phases were then extended to 3.3 Å. The mean FOM for the combined phases at this point was 0.85, with an R-factor of 24.7%, free R-factor (Brünger, 1992b) of 34.5% (for 6% of the entire native data set; these reflections were selected randomly and were not used in any calculations) and a correlation coefficient between observed and calculated structure factor amplitudes of 87.3%.

Model building

The backbone of the monomeric core of the aspartyl-tRNA synthetase from yeast (Ruff *et al.*, 1991) was placed into the electron density map calculated using combined phases at 3.3 Å. The secondary structure elements were then fitted individually using O (Jones *et al.*, 1991), whereas the regions with no corresponding density were deleted. The resulting model was extended to the remaining monomers and used in another round of averaging and phase combination. Skeletonized electron density (calculated using BONES, Kleijwegt and Jones, 1994) in the C-terminal region strongly resembled the C-terminal domain of GlyRS (Logan *et al.*, 1995); the backbone of the latter was then used as a guide for building the model. After three rounds of model building, averaging and phase combination, the updated model was refined with the program X-PLOR (Brünger, 1992a) prior to structure factor calculations. At this point, the (working) crystallographic R-factor was 31.0% and the free R-factor was 35.3%. After two additional rounds, electron density averaging was no longer deemed necessary. Further model building proceeded using $2F_{\text{obs}} - F_{\text{calc}}$ maps.

Refinement

The model was refined with the program X-PLOR (Brünger, 1992a), first with strict non-crystallographic symmetry (NCS) constraints. These were replaced by NCS restraints in the later stages of positional and B-factor refinement. The resolution limit for the refinement was extended gradually to 3.0 Å, then to 2.8 Å and finally to 2.6 Å. The model underwent several rounds of manual rebuilding aided by $(2F_{\text{obs}} - F_{\text{calc}})\phi_{\text{calc}}$ electron density maps and stereochemical analyses using the program PROCHECK (Laskowski *et al.*, 1993) followed by refinement based on simulated annealing (Brünger *et al.*, 1987), energy minimization and individual B-factor optimization. The current atomic model refined at 2.6 Å resolution comprises four HisRS monomers, two of which contain 366 residues (4–177, 229–274, 279–424) and two contain 357 residues (4–166, 230–274, 279–424) out of a total of 424 residues; each monomer contains a histidyl-adenylate molecule. Also, there are 157 water molecules. The average temperature factor over all non-hydrogen atoms is 33 Å². The crystallographic R-factor is 25.1% for all reflections between 8.0 and 2.6 Å, with the working R-factor of 24.6% and the free R-factor of 32.7%. The r.m.s. coordinate error was estimated by the method of Luzatti (1952) to be 0.4 Å. The correlation coefficient between F_{obs} and F_{calc} for all reflections in the given resolution range is 0.895, with the free correlation coefficient of 0.805. The geometry is good, with r.m.s. deviation in bond lengths of 0.015 Å, r.m.s. deviation in bond angles of 2.93° and r.m.s. deviation in dihedrals of 25.1° (Table II). All ϕ/ψ angles are within the allowed regions of the Ramachandran plot, with 92% in the most favoured regions. The stereochemistry and geometry of the final model were analysed using the program PROCHECK and found to be better than the average of the reference structures. The coordinates will be deposited in the Brookhaven Protein Data Bank.

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