Microbial amidases belong to the thiol nitrilases family and have potential biotechnological applications in chemical and pharmaceutical industries as well as in bioremediation. The amidase from Pseudomonas aeruginosa is a 6x38 kD enzyme that catalyses the hydrolysis of a small range of short aliphatic amides. The hereby reported high resolution crystallographic structure shows that each amidase monomer is formed by a globular four-layer αββα sandwich domain with an additional 81 residues long C-terminal segment. This wraps arm-in-arm with a homologous C-terminal chain of another monomer, producing a strongly packed dimer. In the crystal, the biological active homo-hexameric amidase is built grouping three such dimers around a crystallographic three-fold axis. The structure also elucidates the structural basis for the enzyme activity, with the nitrilases catalytic triad at the bottom of a 13 Å deep, funnel-shaped pocket, accessible from the solvent through a narrow neck with 3 Å diameter. An acyl transfer intermediate, resulting from the purification protocol, was found bound to the amidase nucleophilic agent, Cys166. These results suggest that some of pocket defining residues should undergo conformational shifts to allow substrates and products to access and leave the catalytic pocket, for turnover to occur.

Bacterial amidases (acylamide amidohydrolases, EC 3.5.1.4) are widespread in nature, where they catalyze the hydrolysis of amides into the corresponding carboxylic acids and ammonia, RCONH₂ + H₂O ⇌ RCO₂⁻ + NH₄⁺ (1-3). The importance of these hydrolases in biotechnology is growing rapidly, since their potential applications span through chemical and pharmaceutical industries as well as in bioremediation (4,5). They also exhibit acyl transfer activity in the presence of hydroxylamine, RCONH₂ + NH₂OH ⇌ RCONHO⁻ + NH₄⁺ (6). The resulting hydroxamic acids are known for their chelating properties (7,8). Some of them are described as potent inhibitors of metalloproteases and have been investigated in therapeutic applications as anti-HIV (9) or antimalarial (10) agents. Additionally, they can be used in wastewater treatments as a way to eliminate metal ions (11).

The inducible aliphatic amidase, encoded by the amiE gene from Pseudomonas (P.) aeruginosa (12), is an homo-hexameric enzyme (13) that catalyses the hydrolysis of a small range of aliphatic amides (14) and shows a high inhibitory sensitivity toward urea and hydroxyurea (15,16). This aliphatic amidase belongs to nitrilases, a 13 branches superfamily of thiol enzymes involved in natural product biosynthesis and post-translational modification in plants, animals, fungi and some prokaryotes (1,17,18).

Site-directed mutagenesis studies have identified amino-acid residues involved in the enzyme activity or in substrate specificity (13,19,20). Single point mutations in P. aeruginosa amidase enabled the hydrolysis of longer aliphatic amides such as butyramide and valeramide (2,21) or aromatic amides like phenylacetamide (22) and acetanilide (23). Although the catalytic mechanism is still not fully understood (24-26), this enzyme has attracted a great attention because of the variations in its substrate and inhibitor specificities that can be generated through single
point mutations. Cys\textsuperscript{166} has been found to act as the nucleophile in the covalent catalysis of \textit{P. aeruginosa} amidase, within a catalytic triad, Glu - Lys - Cys, that has been consistently identified in all members of the nitrilase superfamily (13,26).

Microbial amidases with altered substrate specificity have attracted a growing interest in the last decade because they can be used in the detoxification of industrial effluents containing toxic amides such as acrylamide and formamide. Additionally, immobilized amidase can be used efficiently for production of acrylic acid from acrylamide, thus converting a toxic ambient contaminant into a widely used industrial raw material (27-29).

For efficient industrial applications, some critical catalytic properties of amidases such as thermostability, catalytic activity, enantioselectivity and substrate specificity require further improvements. Since microbial amidases are a class of enzymes that have potential value for the development of commercial bioprocesses (5), our laboratory began the structural characterization of wild-type amidase from \textit{P. aeruginosa}. A complete understanding of the structural characteristics responsible for activity and substrate activity will help in designing appropriate \textit{in vitro} mutations to create amidases with commercial interest. Moreover, the structural characterization of amidase may reveal new insights about the detailed catalytic mechanism of this enzyme.

Recently, a preliminary study on the phase problem solution of crystals from the amidase from \textit{Geobacillus (G.) pallidus} RAPc8 was described (30), where the amidase hexamer is reported to assume the form of a trimer of dimers exhibiting D3 point-group symmetry.

In this paper we describe the X-ray structural determination of amidase from \textit{P. aeruginosa}, the first detailed 3D report on an aliphatic amidase belonging to the second branch of the nitrilase superfamily.

**EXPERIMENTAL PROCEDURES**

**Bacterial Strains, Media and Growth Conditions** - The constitutive mutant strain (L10) of \textit{P. aeruginosa} was isolated by mutagenesis from the wild type strain 8602. Microbial L10 strain was grown overnight at 37 °C, 250 rpm, with orbital shaking in minimal salt medium containing sodium lactate and ammonium sulfate as described previously (23,31).

**Extraction and Purification of Wild-Type Amidase from \textit{P. aeruginosa}** - Wild-type amidase from \textit{P. aeruginosa} was extracted from cells and purified in a two step procedure involving affinity and gel filtration chromatography, as reported previously (31,32). The purified protein was concentrated to 25 mg/mL in 50 mM Tris-HCl pH 7.2 containing 5 mM DTT and 1mM EDTA, and stored at -20°C until use.

**Protein Crystallization, Data Collection, and Phase Problem Solution** - Protein crystals of \textit{P. aeruginosa} wild-type amidase were obtained as previously described (Andrade & Frazão et. al. Acta Crystallograph Sect F63 Struct Biol Cryst Commun., in press). Heavy atom crystals derivatives were screened using JBScreen Heavy (Jena Bioscience) by soaking amidase crystals in well solution complemented with 5 mM of several heavy metal salt solutions. Diffraction data from native and putative heavy atom derivatives were collected in-house and at ID29 and ID14-EH2 beamlines of ESRF, Grenoble, France, using the HKL suite (33) to process and merge intensity data. The structure was solved by the single isomorphous replacement with anomalous scattering method (SIRAS) using the HKL2MAP (34) graphics user interface. Native and putative derivative data sets were scaled together and analyzed with SHELXC (G. M. Sheldrick, personal communication), the heavy atom substructure was determined with SHELXD and the phase problem solved with SHELXE (35) using a potassium tetrachloroplatinate (II) derivative crystal.

**Model Building and Refinement** - ARPwARP (36) in CCP4 (37) was used for automated model building. The structure was refined anisotropically with REFMAC (38) followed by SHELXL (39). XFIT (40) from the XtalView package (41) was used to fit, complete and analyse the molecular model against sigmaA (42) electron density maps. Water molecules...
were inserted at 3 sigma peaks in difference Fourier m|Fo|-D|Fc| maps and kept if their atomic displacement parameters refined below 50 Å². The stereochemistry of the amidase model was assessed with PROCHECK (43). The refinement of the protein and solvent molecules included atomic anisotropy and riding protons (39). Statistics on diffraction data, refinement and stereochemistry are presented in Table I.

Other Methods - Solvent accessible areas were calculated with AREAIMOL and interatomic interactions with CONTACT, from the CCP4 suite (37), solvent accessible and molecular surfaces were calculated with SURFNET (44), and structure comparisons were performed with SSM (45) and MODELLER (46). Figures were prepared with XFIT (40), RASTER3D (47) and PyMol (48).

RESULTS AND DISCUSSION

Structure determination and quality - The native data was incompletely collected to 1.25 Å (51 % completeness in the highest resolution shell), but at 1.4 Å resolution shows a local completeness of 88.9 % and an accumulated completeness of 99.0 % (Table I for further data statistics).

The structure was determined by SIRAS from a platinum soaked crystal containing a single heavy atom site (the second highest site showed a relative occupancy of 0.2). The extension of the phases to 1.0 Å using the "free lunch algorithm" (49) as implemented in SHELXE (35) led to highly detailed model-unbiased maps that showed at atomic resolution a partially occupied reaction intermediate at the catalytic site, an acetohydroxamic acid derivative (see below).

There is one molecule in the asymmetric unit containing 341 out of the 346 sequenced residues, since no significant electron density is visible for the last 5 C-terminal residues, LEKEA, possibly disordered in the solvent media. 27 side-chain residues are modelled as discretely disordered conformers, 307 solvent molecules are modelled as waters and 1 as a sulphate anion. The anisotropic atomic refinement using all available data to 1.25 Å resolution converged to Rwork/Rfree 10.1/12.6 %,

Amidase architecture – The amidase monomer shows a four-layers αββα (33 % of α-helical chains and 22 % of β-strands) sandwich arrangement, with the two internal β-sheets, built up by seven and six strands, interconnected by two inter-sheets cross-overs (Figure 1). The structure topology corresponds to that of nitrilases, reference 3.60.110 in the CATH protein classification (50), with an additional N-terminal short β-strand (residues 1-3) and a C-terminal long chain (residues 260-341) composed by 5 short α-helices connected by unstructured segments.

The αββα sandwich shows recognizable pseudo-symmetry, with two sets of six strands (residues 13-158 and 159-258, respectively), related by a twofold rotation axis running through the middle of the sandwich, longitudinal to the strands directions (Figure 1B). The superposition of the two halves reveals that in spite of a very low sequence identity, 5%, in the structure-guided alignment, 71 homologous Cα’s overlap within a 3.5 Å distance cut-off with an r.m.s. deviation of 1.6 Å (Figure 1C). The presence of two similar domains in one protein was first described for bovine liver rhodanese (51) and was later found in many other structures, see e.g. (52), most of which showing low sequence similarity between the domains. These likely result from a gene duplication and fusion event of an ancestor protein.

A search for structural homologues of amidase with DALI (53) in the PDB (54) led to the available entries of nitrilase family members, namely from branch 6 of carbamylases 1ERZ (55) and 1FO6 (56), from branch 10 of Nit proteins 1EMS (57) and a putative protein CN hydrolase 1F89 (58). Their global 3D structure comparison is presented in Table II.

Amidase oligomerisation - The single, crystallographically independent amidase molecule packs against 5 neighbours (Figure 2 and Table III). Its long, atypical C-terminal chain segment wraps around the homologous C-terminal segment of its closest neighbour, producing a strongly packed dimer that occludes 30% of otherwise solvent accessible surface. Three such dimers are disposed around the crystallographic three-fold axis producing the biologically active homo-hexameric amidase.
particle. The recently reported quaternary structure of the amidase from *G. pallidus* (30), according to its putative molecular replacement solution, seems to show an equivalent arrangement. In this tri-dimeric association (Figure 2D) each monomer contacts four neighbours of the homo-oligomer occluding 49% of otherwise solvent exposed surface, and involving 771 inter-atomic interactions until 3.6 Å, of which 147 correspond to H-bonds to 3.2 Å (Table III).

*P. aeruginosa* is a mesophilic bacterium with optimal growth at 37°C (2). However, wild-type amidase exhibits a very high heat stability at 55°C (19), making this enzyme unusually thermostable for this class of bacteria. From an industrial point of view, thermozyymes offer major biotechnological advances over mesophilic enzymes (59), and a detailed understanding of the underlying interactions that define protein stability is essential to design more stable proteins. Thermostability seems to depend on several structural features, such as the hydrophobic effect, H-bonding and salt bridges, surface charged residues distribution, and packing effects and aminoacid composition in the protein interior (59,60). The unusually high stability of the homo-hexameric amidase particles (19) has raised the hypothesis that this property might have resulted from putative inter-molecular disulphide bridges. However, no disulphide bridge was detected in the 3D model and, furthermore, the distribution of the cystein residues precludes any inter-molecular disulphide bond, as the minimal distance between inter-molecular cysteine sulphur atoms is 10.5 Å. Additionally, the closest intra-molecular distance between cysteine C-alphas (residues 166 and 198) is *circa* 6 Å, but their side-chains point away from each other. These cysteine residues reside in two parallel β-strands and a disulphide bridge between them would always be highly improbable, as it would necessarily imply the disruption of H-bonds interconnecting the β-strands. The oligomer stability must therefore derive from other type of stabilization factors. Other research workers (20,26) have reported the structural role of Glu59 in the maintenance of the quaternary structure of the enzyme since the substitution Glu59 → Val resulted in the dissociation of hexamer into dimers with loss of amidase activity. The hexameric arrangement described above together with the tight packing through the long C-terminal arms embrace, unique among known structural homologues, may constitute the relevant factors promoting the observed remarkable thermostability of amidase.

**Amidase active site** - The *P. aeruginosa* aliphatic amidase belongs to the prototypical branch 2 of the nitrilase superfamily (17,18) catalysing the hydrolysis of a small range of short aliphatic amides. In nitrilase-related proteins the catalytic activity depends on a conserved catalytic triad, Glu59, Lys134 and Cys166 (*P. aeruginosa* amidase numbering) as deduced from recently obtained structures (55-58). Biochemical studies and site-directed mutagenesis on Glu59Gln, Lys134Asn and Cys166Ser confirmed this assumption giving rise to catalytic inactive variants (13,26). Our structure shows that these functional groups are localised within 5 Å from each other and, together with Met193 and conserved Tyr60, Trp138, Glu142, Gly191 and Tyr192 form a ca. 13 Å deep pocket with 23.9 Å3. This pocket is accessible from the surrounding media only through a funnel-shaped entrance (Figure 3A).

Trp138 residue has been found to play an important role both in enzyme catalysis as well as in structural stability of the aliphatic amidase since the substitution Trp138 → Gly is responsible for alterations in amidase substrate specificity as well as in enzyme conformation and stability (19). The substitution Trp138 → Gly or Trp138 → Ser resulted in mutant amidases with no affinity for urea whereas they exhibited reduced affinity for acetamide and acrylamide compared with the wild-type enzyme. On the other hand, these mutations in amidase allowed the hydrolysis of phenylacetamide as well as p-nitrophenylacetamide which are more bulkier substrates than either acetamide or acrylamide (19).

The catalytic triad is localized at the bottom of the pocket with Cys166, the nucleophile agent in nitrilase related proteins (1,13,18), at the top of a β-turn-α structural motif, known in the family as the nucleophile elbow (58). This residue is in an energized conformation, as judged by its
localization in a disallowed region of the Ramachandran plot (\(\Phi = 40^\circ\) and \(\psi = -109^\circ\)). Unexpectedly, a clear electron density cloud was found within the catalytic pocket, contiguous to the nucleophylic S\(_{\gamma}\) from Cys\(_{166}\) (Figure 3 B). This cloud was observed since the initial experimental Fourier maps and remained throughout the model refinement. As the protein had been crystallized in the absence of any substrate or inhibitor, such an electron density must therefore correspond to a ligand that could only have been provided from the purification procedure, whereby amidase elution through an affinity column (Epoxy-activated Sepharose 6B-acetamide) was carried out by using acetamide and hydroxylamine as eluting agents (32). The acyl transfer activity of amidase induced the formation of an intermediate to acetohydroxamic acid, labelled ACH\(_{401}\) in Figure 3 B and C, which shows to form H-bonds with the side chains of Glu\(_{59}\), Lys\(_{134}\) and Glu\(_{142}\), and with main-chain N of Asp\(_{167}\) and O of Gly\(_{191}\), and must have been retained at the catalytic pocket throughout the subsequent experimental procedures. In order to confirm the correctness of the assumed chemical nature of the observed adduct and to exclude the possibility of having introduced a model biased artefact with the structure refinement, model-unbiased maps were re-calculated directly from the experimental SIRAS phases using the "free lunch algorithm" (49) extended to 1.0 Å. The obtained maps, Figure 3 B and C, confirm the adduct connectivity to Cys\(_{166}\) and the acyl transfer reaction intermediate specific shape. Experimental observations of covalently bound intermediates were also reported in Rhodococcus ATCC 39484 nitrilase by mass spectroscopy analysis (61,62). The present report confirms the existence of such intermediates and maps them covalently bound specifically to the invariant Cys\(_{166}\). However, there is a striking difference between the nitrilase intermediate and that found in Ps. aeruginosa amidase. While the electron density maps of the here described structure at quasi atomic resolution show an intermediate with a central carbon in a clear tetrahedral sp\(^3\) conformation, the molecular mass of the intermediates in the nitrilase may be either thioimidate or acylenzyme species (61,62), where the carbonyl carbon binds to only three atoms. Short chain aliphatic amidases, as in Ps. aeruginosa, are known for their additional acyl transfer activity (32), and due to the presence of both an acyl donor (acetamide) and an acyl acceptor (hydroxylamine) in the purification step it is not surprising to find Cys\(_{166}\) ligated to the precursor of the resulting hydroxamic acid. In the hydrolysis reaction, however, there is not the possibility to form similar H-bonds between its intermediate and the invariant glutamic acids, thus explaining the different hybridization of the trapped carbonyl C atom.

The dependence of the amidase activity upon amino acid replacement of Glu\(_{59}\) and Lys\(_{134}\) can now be understood on the basis of the observed H-bonding network. The nucleophilic attack by the thiol of Cys\(_{166}\) to the carbonyl group of the substrate is facilitated by its interactions with the amino groups of Asp\(_{167}\) main-chain and of Lys\(_{134}\) side-chain, in particular this later, with its positive character increased by the assistance of two H-bonds from the carboxylic groups of Glu\(_{59}\) and Glu\(_{142}\). These not only assure the protonation of the NZ atom of Lys\(_{134}\) but also form two H-bonds with the oxygen of the hydroxamic group of the intermediate, and promote therefore its stabilization. Clarke (63) showed that in Ps. aeruginosa amidase both acetamide and propionamide are good substrates for hydrolysis and acyl transfer reaction but, surprisingly, propionamide is the most rapidly hydrolysed substrate whereas acetamide is the most rapidly transferred onto hydroxylamine. Our structure shows that not only Cys\(_{166}\) is under conformational strain (see above) but also that the methyl group of the acetamide intermediate is only at 3.39 Å of CE1 atom from Tyr\(_{192}\), "touching" the bottom of the reaction cavity. Therefore for a substrate like propionamide the acyl group must translate by about a bond distance, which not only will tune the strain at the nucleophile Cys\(_{166}\) (and eventually facilitate hydrolysis) but also may destabilize the H-bonds with the hydroxamic group (and unfavour the acyl reaction), in comparison to acetamide case.

The purification protocol and the retention of the reaction intermediate at the catalytic pocket may have played an important role in the high resolution quality of the produced crystals, when compared with those previously described in the literature (13). Enzymes with improved crystals
are often obtained through addition to the crystallization experiment either inhibitors or substrate analogues. Their accommodation into the active site, filling an otherwise void volume, promotes a more compact structure with a concomitant decay in its conformational flexibility and lower atomic displacement parameters, which results in higher resolution diffraction data.

**Conclusions** - Amidases convert amides into acids and are therefore of great interest in industrial applications. Here we report a quasi atomic resolution structural determination of the first aliphatic amidase structure. It shows the topology of the nitrilase family and hints for a probable gene-duplication occurrence due to its two-fold pseudo-symmetry. The amidase structure differs from other nitrilases by an additional short β-strand at the N-terminus and an additional long C-terminal chain, which wraps arm-in-arm with a neighbour to produce a strongly packed dimer. The biological active homo-hexamer amidase is formed by packing three such dimers around a three-fold rotation axis. The six amidase active-sites are accessed by funnel-shaped, 13 Å deep pockets, where the nitrilase catalytic triad resides. The structure shows that the catalytic pocket is shaped by conserved residues, and in its interior a ligand was found covalently bound to the sulphur atom of the nitrilase nucleophile Cys166. Covalently bound intermediates for a nitrilase reaction had already been identified by mass spectroscopy, and the additional acyl transfer capability of *Ps. aeruginosa* explains the differences between the present and the previously indentified intermediate. The structure also highlights the roles of the other catalytic residues. The constricted pocket access and the limited volume of the catalytic cavity determine the amidase specificity, although one should also consider the possibility of some mobility in the neighbouring residues, allowing the access of substrates and the release of reaction products. Such localized but mechanistically important conformational transitions are often induced by substrate binding and switched off by the release of products, enabling another cycle of the enzymatic reaction. The present structure opens the way for further studies on the amidase specificity determinants.
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FOOTNOTE

The atomic coordinates and structure factors of the aliphatic amidase from Pseudomonas Aeruginosa have been deposited in the Protein Data Bank with accession numbers nnnn and mmmm.

FIGURE LEGENDS

FIG. 1. Amidase monomer structure. A, Cartoon representation of P. aeruginosa amidase monomer coloured from blue (N-terminal) to red (C-terminal), with the molecule envelope in grey. The monomer shows a four-layer αββα sandwich architecture with an extended C-terminal region including several α helical segments. B, Topology diagram of amidase with triangles representing β-sheets and circles representing α-helices. As the two mixed β-sheets, represented in dark and light blue, are topologically equivalent, the monomer results from their duplication with additional secondary-structure elements in grey. C, Superposition of the two monomer halves N- and C- structural motifs, in light blue and red, respectively, also present in available structures of the nitrilase family.

FIG. 2. Amidase homo-hexamer. A, Cartoon of the biological active amidase hexamer, showing the strong dimeric association involving the wrapping of C-terminal chains. B, Representation of the hexamer solvent accessible surface, in the same orientation and with monomers colored similarly. C, View of the amidase hexamer down the crystallographic 3-fold axis. D, Schematic representation of the hexamer, in the same orientation, highlighting the packing relationships with 2- (arrows) and 3-fold (triangle) crystallographic rotation axes.

FIG. 3. Amidase catalytic pocket. A, Semi-transparent solvent accessible surface (grey) of the amidase catalytic cavity displaying at its bottom the clustered catalytic triad, Glu59, Lys134 and Cys166, together with other conserved cavity forming residues (1,26) (atoms as sticks with carbon in blue, oxygen in red, sulphur in orange, helical motifs in green and beta-chains in yellow). B and C, Experimental SIRAS electron density map of amidase at 0.7 σ (blue mesh) fitted with the anisotropically refined model (atomic anisotropic displacement parameters as transparent ellipsoids at 0.5 probability level, yellow for carbon red for oxygen, blue for nitrogen and green for sulphur) showing (B) the side-chain of Cys166, with its SG atom covalently bound to an acyl transfer reaction intermediate of acetamide into hydroxylamine, an acetohydroxamic acid derivative (ACH401) refined by SHELXL to 0.6 occupancy, and (C) a cross-eyes stereo view of the acyl transfer intermediate (from an approximately perpendicular view) with neighboring protein residues at H-bonding or van der Waals interacting distances. The central carbon of ACH401 assumes a distorted tetrahedral sp³ hybridization and is single bonded to the neighbouring oxygen, which shares a proton (dashed green) both with atom NZ of Lys134, at 2.64 Å, and with the amine nitrogen of Asp167, at 2.95 Å. Additionally, its hydroxamic oxygen forms two H-bonds (dashed green) with the carboxylic groups of Glu59 and Glu142, at 2.85 and 2.62 Å, respectively. The methyl group of the acetohydroxamic acid derivative is in close contact with the side-chain of Tyr192, at 3.39 Å (pointed orange) from its CZ atom.

FIG. 4. Reaction scheme. Usually accepted reaction scheme for acyl transfer reaction (63-65) on hydroxylamine and undesirable hydrolysis (65). Enz. stands for enzyme. The tetrahedral intermediate found in the present structural study is marked with a *.
Table I- Diffraction data and refinement statistics

| Data collection | Pt²⁺-Derivative | Native | Native |
|-----------------|-----------------|--------|--------|
| ESRF Station (Grenoble) | ID14-ch2 | ID29 | ID29 |
| Wavelength (Å) | 0.933 | 0.975637 | 0.975637 |
| Space group | P6₁ 22 | P6₁ 22 | P6₁ 22 |
| Unit-cell lengths (Å) | a = b = 102.6, c = 151.7 | a = b = 102.71, c = 151.32 | a = b = 102.71, c = 151.32 |
| Crystal solvent content (v/v, %) | 52 | 52 | 52 |
| Wilson B (Å²) | 39.6 | 8.5 | 8.5 |
| Mosaicity (%) | 0.93 - 1.25 | 0.88 - 1.76 | 0.88 - 1.76 |
| Resolution range (Å) | 29.62-2.40 (2.49-2.40) | 42.67-1.40 (1.43-1.40) | 42.67-1.25 (1.28-1.25) |
| No. reflections | 179807 | 1382592 | 1545027 |
| Redundancy | 9.6 | 15.1 | 13.4 |
| No. unique reflections | 18815 | 91754 | 115216 |
| Completeness (%) | a 99.9 (97.8) | 99.0 (88.9) | 89.0 (51.5) |
| | b 17.3 (4.9) | 28.5 (7.3) | 24.9 (3.0) |
| Rpim (%) | a, b 2.7 (11.7) | 2.1 (10.6) | 2.2 (26.0) |
| Rrim (%) | a, c 8.6 (31.8) | 8.6 (33.5) | 9.0 (56.8) |
| Rsym (%) | a, d 7.8 (27.4) | 8.4 (31.5) | 8.7 (49.7) |

Refinement

| | Nr. protein atoms | 2793 | 2793 |
| | Nr. water molecules | 307 | 307 |
| | Nr. sulphate anions | 1 | 1 |
| | Average protein a.d.p. (Å²) | 12.6 | 10.9 |
| | Average solvent waters a.d.p. (Å²) | 27.6 | 27.6 |
| | Resolution range (Å) | 42.67-1.4 | 42.67-1.25 |
| | Rwork/Rfree (%) | 10.4/10.7 | 10.1/12.6 |
| | Rfactor (%) | 10.4 | 11.1 |

No. Residues in Ramachandran regions

| | Most favoured (%) | 264 (91.0) | 264 (91.0) |
| | Additionally allowed (%) | 25 (8.6) | 25 (8.6) |
| | Disallowed (%) | 1 (0.3) | 1 (0.3) |

Protein stereochemistry

| | R.m.s bond distance (Å) | 0.013 | 0.013 |
| | R.m.s. angle distance (Å) | 0.030 | 0.030 |
| | R.m.s. plane distance (Å) | 0.031 | 0.031 |

The values in parentheses are for the outer resolution shell.

Rpim = Σ[I(h) - <I(h)>] / Σ[I(h)], where I is the observed intensity and <I> is the average intensity of multiple observations from symmetry-related reflections. Calculated with program RMERGE (66), it is an indicator of the precision of the final merged and averaged data-set.

Rrim = Rmeas = Σ[I(h)] / Σ[I(h)], where I is the observed intensity and <I> is the average intensity of multiple observations from symmetry-related reflections. Calculated with program RMERGE (66), it represents an indicator of the average spread of the individual measurements.

Rsym = Σ[I(h) - <I(h)>] / Σ[I(h)], where I is the observed intensity and <I> is the average intensity of multiple observations from symmetry-related reflections. Calculated with Scalepack (33).

a.d.p. stands for atomic displacement parameter

Rwork = Σ||Fobs|| - |Fcalc|| / Σ||Fobs||, where Fobs and Fcalc are the observed and calculated structure factors, respectively.

Rfree computed as in Rwork, but only for 1744 (1.5%) randomly selected reflections, which were omitted in refinement.

Rfactor computed as in Rwork, but using all available data within the resolution limits.
Table II  3D superposition analysis of available structures (54) from the nitrilase superfamily. Top-left to bottom-right diagonal, total number of residues; from the bottom-left corner to the diagonal, number of 3D homologous Cα positions to a 3.5 Å cut-off (identity %); from the diagonal to the top-right corner, Cα’s r.m.s. distances between 3D homologous Cα’s. Amidase, the present structure; 1F89, PDB entry code of a putative CN hydrolase protein from yeast; 1EMS, the Nit from worm; 1ERZ and FO6, N-carbamoyl-D-amino acid amidohydrolases from Agrobacterium sp. Strain KNK712 and from Agrobacterium radiobacter, respectively.

|        | Amidase | 1F89 | 1EMS | 1ERZ | 1FO6 |
|--------|---------|------|------|------|------|
| Amidase| 341     |      |      |      |      |
| 1F89   | 240 (22)| 271  |      |      |      |
| 1EMS   | 237 (20)| 237 (31)| 272 |      |      |
| 1ERZ   | 243 (17)| 234 (20)| 233 (20)| 303 | 0.5  |
| 1FO6   | 243 (18)| 234 (21)| 233 (19)| 301 (96)| 302 |

Table III- Interactions analysis between each monomer, A₁, with its packing neighbours, within the homo-hexamer, A₁' (the arm-in-arm mate), A₂, A₂', A₃ and A₃', or with a neighbour, B, from another neighbouring hexamer.

|        | A₁' | A₂ | A₂' | A₃ | A₃' | B   |
|--------|-----|----|-----|----|-----|-----|
| Monomers solvent accessible area occlusion upon oligomerisation, (Å²) | 4950 (30%) | 914 (6%) | 0 (0%) | 917 (6%) | 1094(7%) | 67(4%) |
| No. atomic interactions, to 3.6 Å | 449 | 77 | 0 | 77 | 168 | 23 |
| No. H-bonds (interactions between N and/or O atoms to 3.2 Å) | 82 | 22 | 0 | 11 | 32 | 23 |
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Figure 1
Figure 3
Figure 4
Structure of amidase from Pseudomonas aeruginosa showing a trapped acyl
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