CMLS, Cell. Mol. Life Sci. 60 (2003) 1764–1773
1420-682X/03/081764-10
DOI 10.1007/s00018-003-3189-2

CMLS Cellular and Molecular Life Sciences

Research Article

Crystal structure of *Enterobacter cloacae* 908R class C β-lactamase bound to iodo-acetamido-phenyl boronic acid, a transition-state analogue

J. Wouters [a,*], E. Fonzé [b], M.Vermeire [c], J.-M. Frère [b] and P. Charlier [b]

[a] Institut de Recherches Microbiologiques JM Wiame, 1 Av E. Gryzon, 1070 Brussels (Belgium),
Fax: +32 (2) 5276 72 73, e-mail: jwouters@dbm.ulb.ac.be
[b] Centre d'Ingénierie des Protéines and Laboratoire d'Enzymologie, Institut de Chimie B6, Université de Liège, Sart-Tilman, 4000 Liège (Belgium)
[c] Institut de Physique B5, Université de Liège, Sart-Tilman, 4000 Liège (Belgium)

Received 16 May 2003; accepted 4 June 2003

Abstract. The structures of the class C β-lactamase from *Enterobacter cloacae* 908R alone and in complex with a boronic acid transition-state analogue were determined by X-ray crystallography at 2.1 and 2.3 Å, respectively. The structure of the enzyme resembles those of other class C β-lactamases. The structure of the complex with the transition-state analogue, iodo-acetamido-phenyl boronic acid, shows that the inhibitor is covalently bound to the active-site serine (Ser64). Binding of the inhibitor within the active site is compared with previously determined structures of complexes with other class C enzymes. The structure of the boronic acid adduct indicates ways to improve the affinity of this class of inhibitors. This structure of 908R class C β-lactamase in complex with a transition-state analogue provides further insights into the mechanism of action of these hydrolases.

Key words. Class-C β-lactamase; *Enterobacter cloacae* 908R; boronic acid complex; iodo-acetamido-phenyl boronic acid (IAPB); transition-state analogue.

The expression of β-lactamases is one of the most common and well-studied forms of antibiotic resistance. Class C β-lactamases are among the most challenging of these enzymes because they are not significantly inhibited by clinically used β-lactamase inhibitors. Moreover, they have a broad spectrum of action and can hydrolyse a variety of substrates, including the third-generation cephalosporins.
Class C β-lactamases hydrolyse their substrates in a four-step process (fig. 1). Early mutagenesis [1, 3] and structural [4–6] studies identified key catalytic residues, including Ser64, Tyr150, Lys67 and Asn152.
Subsequent X-ray crystallographic studies investigated acyl-adduct complexes of the class C enzymes with β-lactam inhibitors and poor substrates such as aztreonam (PDB code 1FR6 [4]), moxalactam (PDB code 1FCO [7]), ceftazidime (PDB code 1IEL [8]), imipenem (PDB code 1LL5 [9]) and a cephem sulphone (PDB code 1GA0 [10]). These structures revealed key interactions that contribute to the formation of the acyl-enzyme intermediate. In *Escherichia coli* AmpC, one of the class C β-lactamases that has been extensively studied, binding to imipenem (PDB code 1LL5) differs from its binding to moxalactam (PDB code 1FCO) but is similarly to its binding to TEM1 (PDB code 1BT5), a class A β-lactamase. The less-constrained active-site geometry in class C enzymes thus allows different binding modes and, hence, different possible mechanisms of inhibition. For example, imipenem appears to act as an inhibitor because, once it has reacted

* Corresponding author.

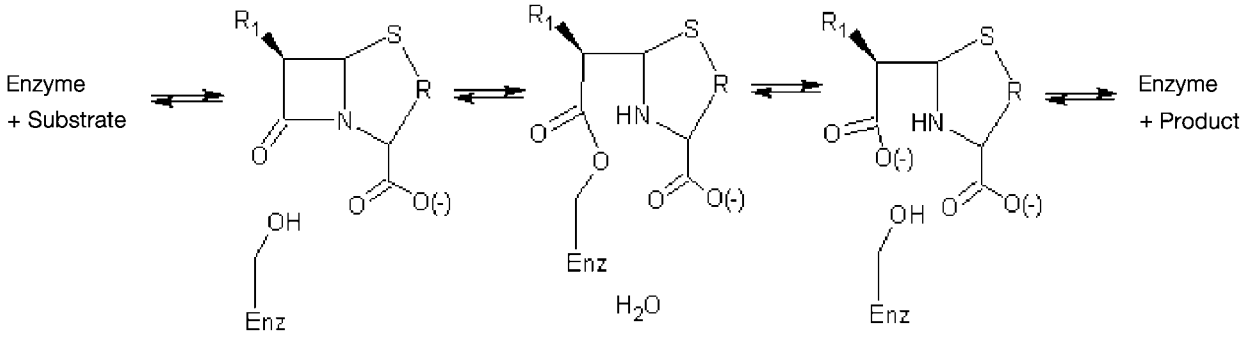


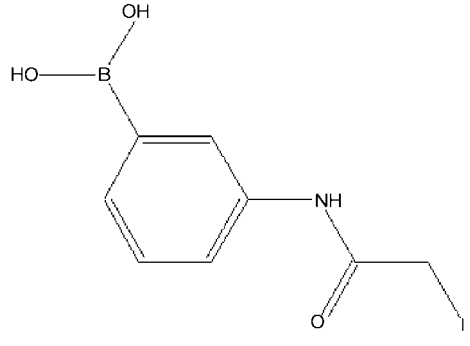
Figure 1. Four-step process for the hydrolysis of substrates by class C β-lactamases (Enz = enzyme).

with the enzyme, the acyl-enzyme adduct formed undergoes conformational changes that rotate the acyl centre away from the point of hydrolytic attack [9].

Recently, efforts to capture crystal structures of intermediates (pre-covalent enzyme-substrate and post-covalent enzyme-product) have been undertaken by studying complexes between the S64G mutant AmpC in complex with the β-lactam cephalothin in its substrate and product forms (1KVL, 1KVM [11]). These structures of milestones in the reaction pathway of AmpC provide insight into substrate recognition, catalysis, and product release. One major conclusion from these structural and mechanistic studies is that the ligand undergoes a dramatic conformational change as the reaction progresses.

Since the X-ray crystal structures of class C β-lactamases have been determined, these molecules are attractive targets for novel inhibitor discovery using structure-based methods [12,13]. The *E. coli* AmpC enzyme is one of the class C β-lactamase that has been extensively studied. Several classes of non-β-lactam inhibitors have been identified for this protein. Substituted boronic acids have been prepared leading to irreversible inhibitors with high affinity and specificity [14, 15]. Complexes with transition-state boronic acids [5, 8, 14, 16] and phosphonate inhibitors [17] confirmed the possibility of different binding modes within the binding site of class C β-lactamases. One concern with this type of boronic acid inhibitors, also observed with phosphonates [17], is that they form covalent adducts with activated serine nucleophiles, potentially reducing their selectivity versus other serine active enzymes like serine proteases. In an effort to design non-covalent, more selective inhibitors of class C β-lactamases, molecular docking calculation and virtual screening techniques have been used to identify original molecules, and novel non-covalent inhibitors of AmpC have emerged from this structure-based discovery process [18]. The structure of the enzyme with one of these new inhibitors, 3-[(4-chloroanilino)sulphonyl]thiopene-2-carboxylic acid, was determined by X-ray crystallography and closely resembled the docking prediction.

As part of this structural approach to class C β-lactamases giving access to mechanistic and structure-based drug design studies, we report here the crystal structures of the wild-type *Enterobacter cloacae* 908R class C β-lactamase, alone and in complex with iodo-acetamido-phenyl boronic acid (IAPB; scheme 1).



Scheme 1. The structure of iodo-acetamido-phenyl boronic acid (IAPB).

Material and methods

Crystallisation, data collection, phase determination and structure refinement

Crystals of *E. cloacae* 908R were prepared as described elsewhere [19]. Crystals of the complex with IAPB, were obtained by soaking a crystal of the apo enzyme in a saturated solution of the inhibitor in the mother liquor [0.1 M bicine buffer at pH 9.0 and 30% polyethylene glycol (Mr = 6000)] 30 min before starting data collection. Crystals were directly flash-frozen from this solution. X-ray diffraction data were collected at 100 K using a Siemens X1000 area detector and a Rigaku RU-200 rotating anode generator as X-ray source. The detector was at a distance of 130 mm with a 2θ angle of 20° and 22° for the 908R-IAPB complex and the free enzyme, respectively. The data collection step size was 0.2° between frames, with a count time of 70 s. per frame. Indexing, integration and scaling of data sets were carried out using the XENGEN 2.0 package [20].

Data collection and refinement statistics for the final models for the free *E. cloacae* 908R structure at 2.1 Å resolution and the IAPB-bound structure at 2.3 Å resolution are reported in table 1.

The structure of 908R class C β-lactamase was solved by molecular replacement using the program AMoRe [21] as implemented in the CCP4 program suite (Collaborative Computational Project, Number 4, 1994). Both structures were refined by alterning conjugated gradient least-squares cycles using the Shelxl program [22] and graphical modelling using the program Turbo-Frodo [23]. An initial model of the IAPB inhibitor was built and energy minimised using InsightII and Discover (MSI/biosym San Diego). In its isolated conformation, the boronic acid $B(OH)_2$ function is planar, as expected. In the complex with *E. cloacae* 908R, a tetrahedral co-ordination of the boron atom is observed affecting the geometry of the inhibitor. Those modified internal co-ordinates of the inhibitor were translated into 1–2 and 1–3 distance restraints used in the refinement process.

Table 1. Data collection and refinement statistics.

	Wild type	IAPB
Crystal data		
Space group	$P2_12_12$	$P2_12_12$
Cell dimensions (Å)	46.468	46.381
	83.589	83.763
	95.638	95.810
Data set		
Wavelength (Å)	1.54179	1.54179
Highest resolution (Å)	2.00	2.24
Total reflections	81043	48365
Unique reflections	21420	11810
Observed reflections [$I > 2\sigma(I)$]	19781	9588
Completeness (%)	82.7	88.6
R_{merge} (%)	10.9	12.8
Refinement		
Resolution range (Å)	10–2.00	10–2.24
Number of protein atoms	2757	2757
Number of ligand atoms	0	14
Number of water molecules	81	47
R_{cryst} (observed/all data) (%)	21.2/21.9	20.4/22.6
R_{free}[a] (%)	26.9	28.1
R.m.s. deviations from restraints		
Bond lengths (Å)	0.005	0.004
Bond angles (Å)	0.021	0.016
Average B factor		
All protein atoms (Å^2)	35.08	34.00
All ligand atoms (Å^2)	–	43.74
All water molecules (Å^2)	47.19	37.83
Ramachadran plot[b]		
Most favoured regions (%)	87.0	85.7
Additionally allowed region (%)	12.3	13.7
Generously allowed region (%)	0.7	0.7
Disallowed region (%)	0.0	0.0

[a] Free test subset represents 5% of total unique reflections.
[b] As defined by PROCHECK, Gly and Pro residues excluded.

Statistical search

Statistical searches in the Protein Data Bank were performed using Version 4.0 of the Relibase program for searching protein-ligand databases available on the net (http://relibase.ccdc.cam.ac.uk). The structure was visualised via a graphical Rasmol-type interface available with the on-line Relibase tools.

Results and Discussion

Apo structure (unliganded form) of *E. cloacae* 908R class C β-lactamase

The refined 2.3-Å structure of the *E. cloacae* P99 enzyme (PDB code 1BLS [17]) was used as a search model for a molecular replacement solution using data within the resolution range of 10 – 2.5 Å. The 361-amino-acid sequence of the molecular probe differs by only five mutations. One clear solution for both the rotation and translation functions was directly obtained with an R factor and a correlation coefficient of 0.28 and 0.804, respectively. The electron density calculated with the best solution was of very good quality and clearly showed the mutations to be introduced in the structure of *E. cloacae* 908R.

The overall structure of *E. cloacae* 908R is similar to those previously reported for other class C β-lactamases, including *E. coli* AmpC, *E. cloacae* P99 and *Citrobacter freundii* enzymes. It is composed of a mixed α/β secondary structure with nine-stranded antiparallel β sheets in the middle flanked by a row of three α helices on one side, and a helical domain with 11 helices on the other. The primary catalytic residue (Ser64), which is acylated in the course of the enzymatic hydrolysis, is positioned at the N-terminal end of a buried central helix (fig. 2).

Structural similarities are also found between the various class C β-lactamases, class A β-lactamases and β-lactam-sensitive D-analyl-D-alanine carboxy-peptidase/transpeptidase (DD-peptidase) [24].

In the apo structure of *E. cloacae* 908R, a water molecule occupies the so-called oxyanion hole and is held by two strong hydrogen bonds involving the main chain at residues Ser318 and Ser64 (table 2). The combined strength of both hydrogen bonds increases slightly from class C to class A and could be related to the rates of deacylation in the penicillins.

Complex with IAPB

Clear electron density connects the Oγ atom of the catalytic serine Ser64 to the tetrahedral boron atom of the phenyl boronic acid inhibitor, suggesting that a dative covalent bond is formed between IAPB and the enzyme (fig. 3). One of the oxygen atoms (O1) of the boron adduct sits in the 'oxyanion hole', forming direct hydrogen bonds to main-chain nitrogen atoms (table 2), as discussed below.

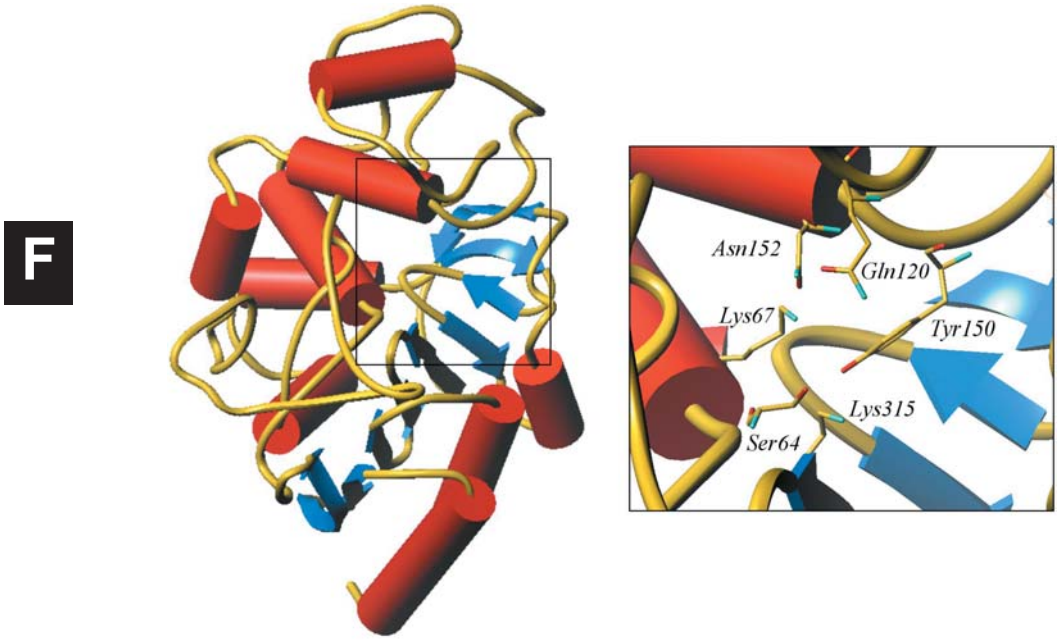


Figure 2. Overall structure of the *E. cloacae* 908R class C β-lactamase, with a closer view of the active site showing the active Ser64 and residues Lys67, Gln120, Asn152, Tyr150 and Lys315.

Table 2. Selected geometric features in the active site of *E. cloacae* 908R class C β-lactamase in its unliganded form or in complex with IABP.

	Distances (Å)	
	unliganded	in complex with IAPB
Og_Ser64–OH_Tyr150	2.84	3.21
OH_Tyr150–Nz_Lys315	2.81	2.70
OH_Tyr150–Nz_Lys67	3.26	3.23
Og_Ser64–Nz_Lys67	3.08	3.49
Og_Ser64–O_water*	2.58	–
N_Ser318–O_water*	2.50	–
O_Ser318–O_water*	3.11	–
N_Ser318–O1_IAPB	–	2.49
O_Ser318–O1_IAPB	–	2.42
Ne2_Gln120–Od1_Asn_152	2.86	3.38
Ne2_Gln120–O_IAPB	–	2.75

* Oxyanion water molecule.

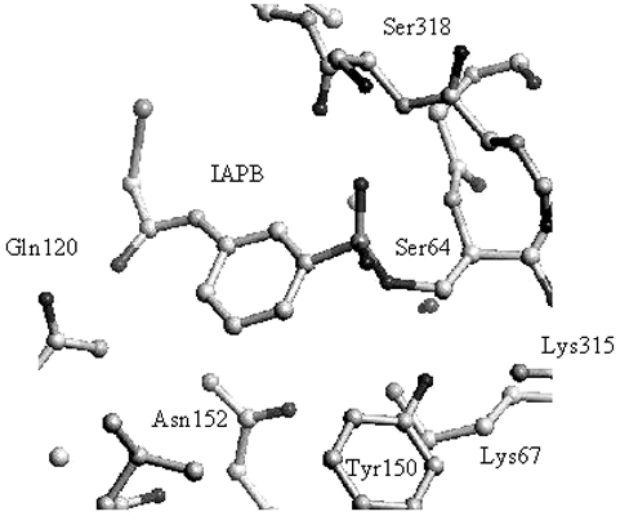


Figure 3. Interactions between IAPB and selected residues of the active site of *E. cloacae* 908R.

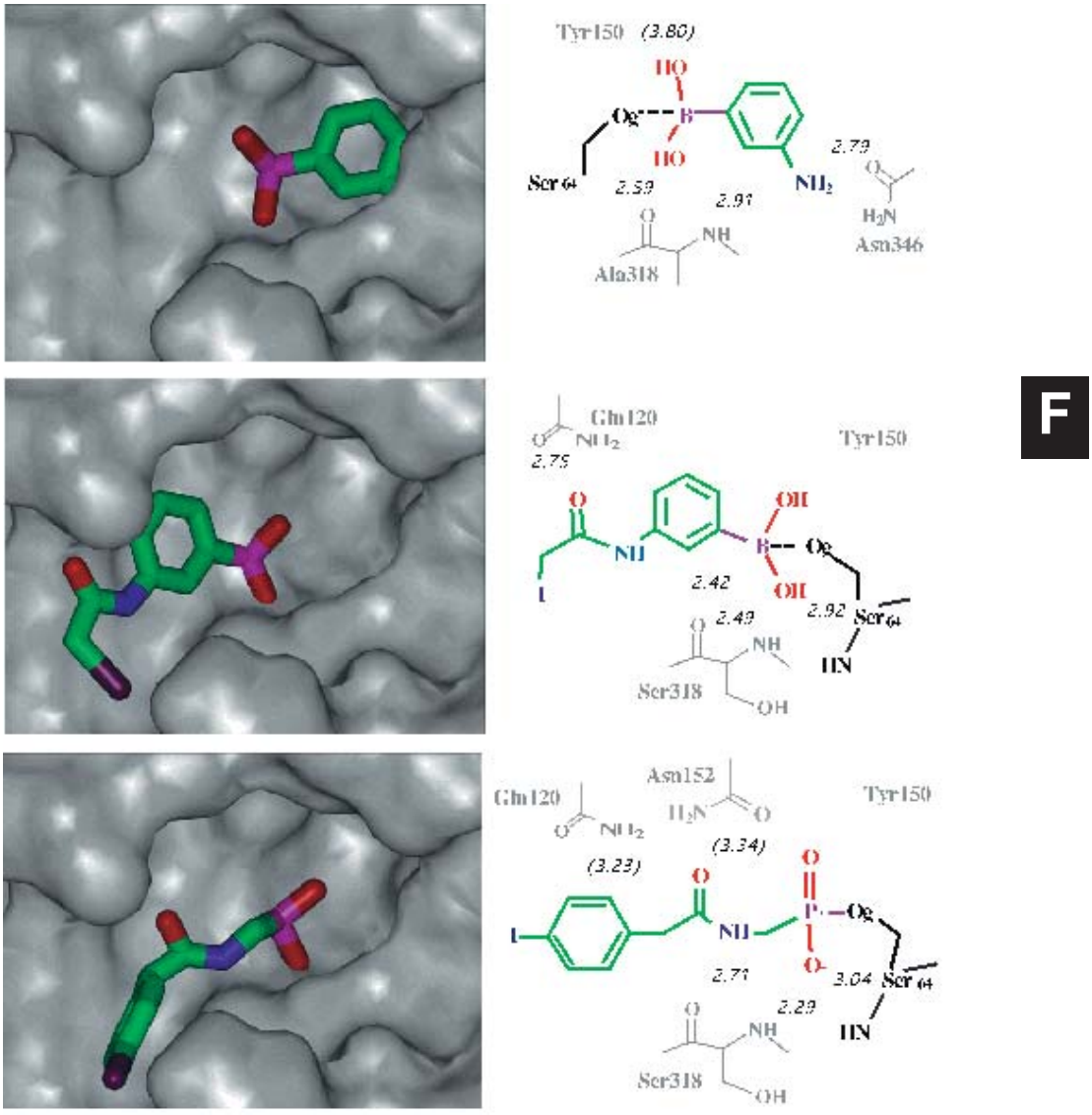
This covalent bond between IAPB and the protein is consistent with the proposed mechanism of inhibition and similar to that observed in other structures of AmpC complexed with aryl boronic acid, as discussed further in the text. The IAPB inhibitor makes a number of favourable interactions with the protein (table 2). Atoms within hydrogen-bonding distance of the inhibitor include the main-chain nitrogen of Ser64, and the backbone N and O atoms of Ser318, which interact with one of the boronic oxygens (O1).

In the apo structure, the conformation of Gln120 is such that it hydrogen bonds with Asn152 (table 2). In the complexed structure, Gln120 is displaced by the inhibitor and no longer interacts with Asn152. The lateral iodoacetamido chain is further stabilised by a hydrogen bond between the carbonyl of the carboxamido group and Gln120. Apart from these changes within the active site, comparison of the refined structures of 908R, unliganded and in complex with the boronic acid inhibitor, shows that neither backbone nor side-chain conformations change much upon complexation.

Interestingly, the relative orientation of the inhibitor inside the active site of *E. cloacae* 908R is distinct from

that observed in the complex of *E. coli* AmpC with m-aminophenyl boronic acid (fig. 4). In the latter structure, the m-amino group of the inhibitor makes an hydrogen bond with Asn346 and positions the phenyl ring close to Thr31. By contrast, IAPB points in the opposite direction, the lateral iodo-acetamido chain being stabilised by a hydrogen bond between the carbonyl of the carboxamido group and Gln120. The inhibitor fits the binding site of the protein well (molecular surface representation in fig. 4). The terminal iodo methyl group is less stabilised. Two distinct positions of the iodine atom were refined, both with half occupancy.

The structure of the *E. cloacae* 908R-IAPB complex resembles that of other class C β-lactamases in complex with other small boronic acids. The *E. cloacae* 908R-IAPB complex also shares similarities with the structure of a phosphonate derivative of the *E. cloacae* P99 β-lactamase (PDB code 1BLS; figure 4). In this structure, the inhibitor has phosphorylated the active-site serine with loss of the m-carboxyphenol leaving group. The resulting N-[(p-iodophenyl)acetyl]aminomethyl phosphate is positioned in the active site in such a way that the arylacetamido side chain is placed as anticipated from analogous β-lactamoyl complexes, with the amido group hydrogen bonded to the backbone carbonyl of Ser318 and to the amides of Gln120 and Asn152. One phosphonyl oxygen atom is hydrogen bonded to main-chain NH groups of Ser318 and Ser64, while the other oxygen is not within hydrogen-bonding distance of any amino acid. Tyr150 and Lys67 are closely associated with Oγ of



F

Figure 4. Comparison of the relative orientation of phenyl boronic acids in the active site of class C β-lactamases (molecular surface representation of the enzyme). Upper: *E. coli* AmpC with m-aminophenyl boronic acid. Middle: *E. cloacae* 908R with IAPB. Lower: *E. cloacae* P99 with N-[(p-iodophenyl)acetyl]aminomethyl phosphate.

Ser64. This arrangement, similar to that observed in the present structure, was interpreted in terms of the transition state for breakdown of the tetrahedral intermediate in the deacylation step of catalysis. In this mechanism, Tyr150 phenol seems the most likely general acid, and thus the corresponding phenoxide anion would be the general base catalyst in acylation [17]. This view is in complete agreement with recent mechanistic studies performed on AmpC [11].

Comparison with other class C β-lactamases complexes with aryl boronic acids

As small aryl boronic acids inhibit class C β-lactamases at sub-micromolar concentrations, different X-ray crystal structures of complexes with boronic acid inhibitors are available in the PDB data bank. Taking advantage of the large amount of structural data available for these enzymes, statistical searches in the PDB were performed with the Relibase program to compare the geometries of complexes and identify structural features of the binding site of class C β-lactamases (fig. 5). Complexes with aryl boronic acids were investigated. From the statistical search with Relibase, apart from the structure of cocaine esterase in complex with phenyl boronic acid (PDB code 1JU3 [25]), aryl boronic acid ligands are apparently not found in other structures deposited at the PDB. This does not mean that the aryl boronic fragment is specific for class C β-lactamase but probably reflects the fact that no one has yet attempted to obtain complexes between these compounds and other (serine active) enzymes.

Analysis of the structures of complexes between class C β-lactamases and aryl boronic acids reveals that, except for the complex with m-aminophenyl boronic acid, orientation of IAPB deduced from our structure is similar to that observed with other aryl boronic acid transition-state analogues. Indeed, comparison of the structures in figure 5 suggests that small aryl boronic acid inhibitors bind to a well-defined cleft in the different class C β-lactamases. This cleft has also been shown to bind the ubiquitous R1 side chain of β-lactams [15, 18]. Much of this cleft is left unoccupied by the small aryl boronic acids. With the exception of one complex (PDB code 3BLS), all boronic ligands adopt similar orientations in the protein binding sites. Combination of structure-based design and in-parallel synthesis allowed rapid exploration, in AmpC, of this binding site whose role in recognition had not been previously explored [15]. The resulting inhibitors differ considerably from β-lactams but nevertheless inhibit the enzyme well. The crystal structure of ETP [3-(4-benzenesulphonyl-thiophene-2-sulphonylamino)-phenylboronic acid, Ki 83 nM] in complex with AmpC (PDB code 1GA9) is the first exploration of this highly conserved cleft and provides valuable information for further design against class C β-lactamases.

Comparison of the present structure of 908R complexed to IAPB with complexes with other boronic acid inhibitors and class C β-lactamases further reveals consensus binding sites. These sites were already identified and characterised in previous studies, using a combination of crystallographic data and computational approaches [15, 18]. Among these binding sites, the highly conserved oxyanion hole recognises one hydroxyl group of the boronic acid. The aryl ring of aryl boronic acids is also stabilised by interactions with Asn152. Interestingly, in complexes with β-lactams, this conserved residue defines a recognition site that interacts with the carbonyl oxygen in the R1 side chain of β-lactams [18].

Statistical analysis of the geometry within these sites was performed on the structures retrieved with the Relibase (fig. 6). The geometry of the oxyanion described in this work (table 2) is consistent with the distribution of distances given in figure 6a (D1 and D2 define the distances between the hydroxyl group of the boronic inhibitor and the main-chain nitrogen and oxygen atoms, respectively, and correspond to N_Ser318–O1 and O_Ser318–O1 distances in the complex of 908R with IAPB). The geometry of the aryl-amide interaction involving residue Asn152 is given by the parameters D3, A1, A2 and T1 in figure 6b. These parameters underline contacts (less than 4 Å) between the side chain of residue 152 and the aromatic ring of the boronic inhibitors together with an axial approach (A1 and A2 close to 90° and T1 close to 180°). In the structure with IAPB, the corresponding parameters are: D1 = 3.63 Å, D2 = 3.92 Å, A1 = 130.2°, A2 = 98.7°, T1 = 161.4°. These values fall within the statistical distribution.

Conclusion

The structure of the complex between IAPB and *E. cloacae* 908R is consistent with the status of boronic acids as transition-state analogues.

Comparison with the structures of complexes with other transition-state boronic acids and β-lactams confirms the possibility of different binding modes within the binding site of class C β-lactamases. The less-constrained active-site geometry observed in different class C enzymes potentially allows different possible mechanisms of inhibition. Recent mechanistical studies based on X-ray structures on trapped reaction intermediates are in agreement with this view. From the point of view of drug design, this opens interesting perspectives for potentially modulating existing inhibitors to make them more selective for class C β-lactamases.

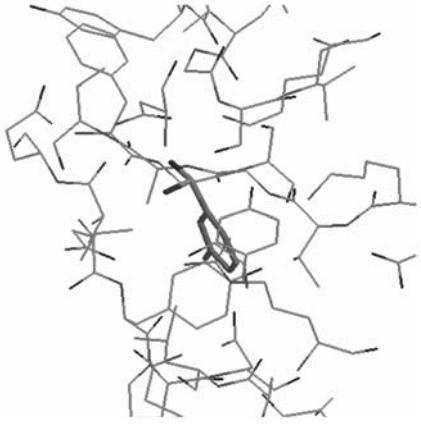
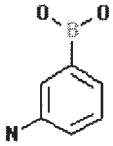
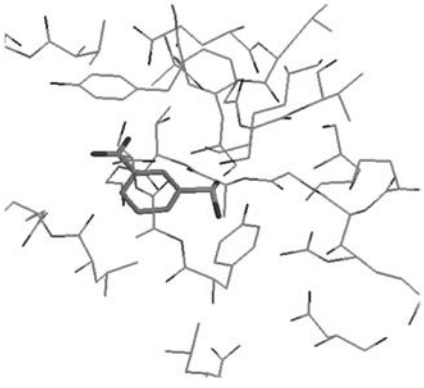
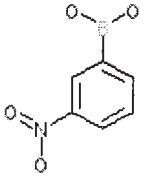
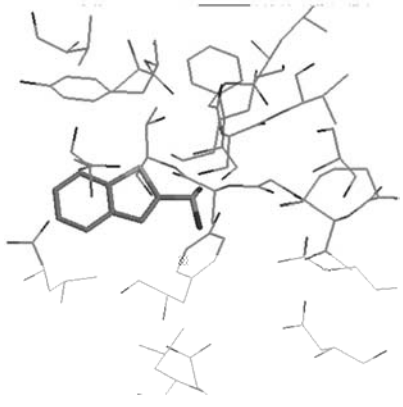
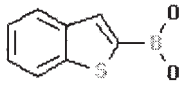
PDB code	Binding site[a]	Aryl boronic acid
3BLS		 APB
1KDS		 NPB
1C3B		 BTB[b]

Figure 5. Results of the Relibase search for structures of complexes with aryl boronic acids. [a] The same relative orientation of the binding site has been retained, with the catalytic Ser64 at the top, and Tyr150 on the right. Only the structure of the binding site in chain A is presented because the ligands in each active site (chains A and B) bind similarly. [b] The structure of benzothiophene boronic acid has been added to the selection by similarity with the initial search fragment ('benzene ring' plus 'boronic acid'). [c] Note the two distinct geometries of the $B(OH)_2$ groups: one, tetrahedral, covalently bound to the active-site catalytic serine and the other, planar, not forming any covalent bond within the binding site.

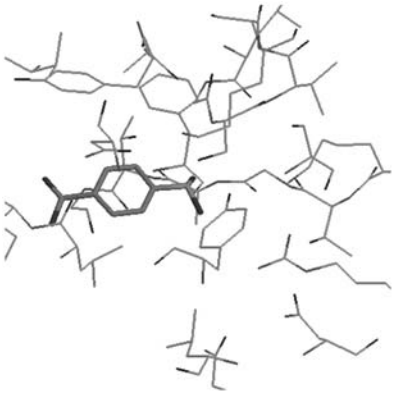
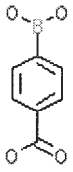
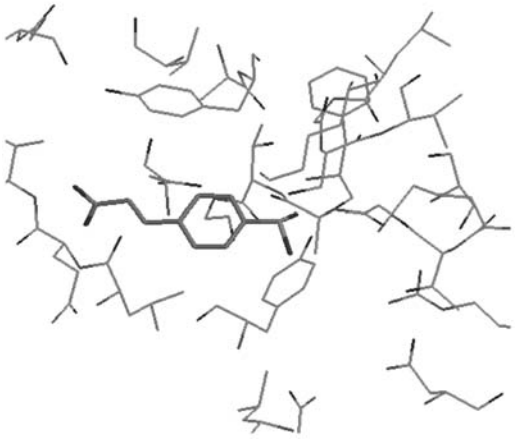
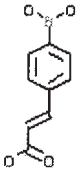
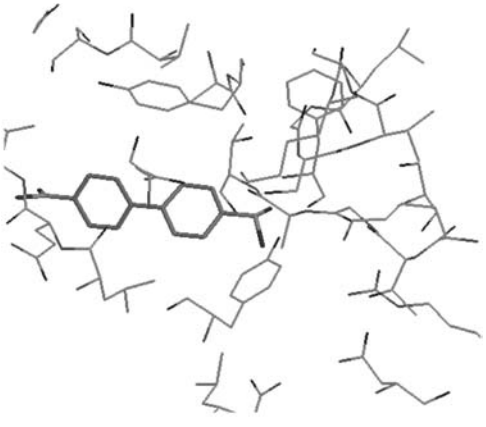
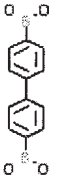
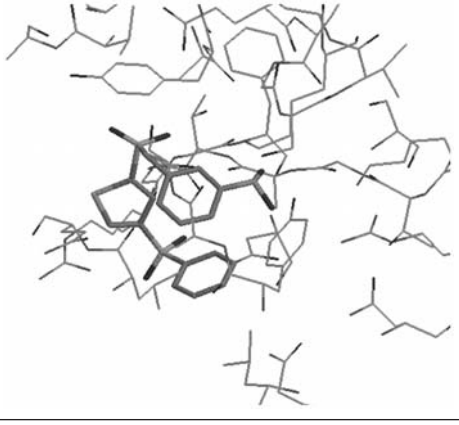
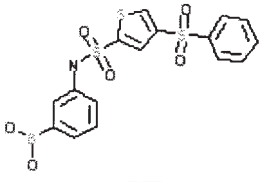
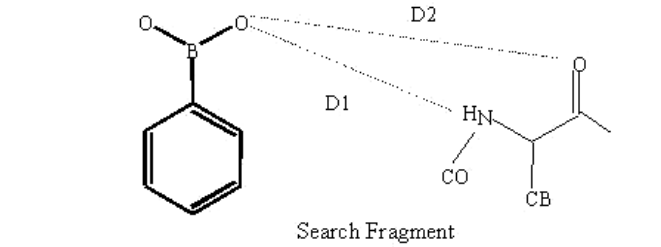
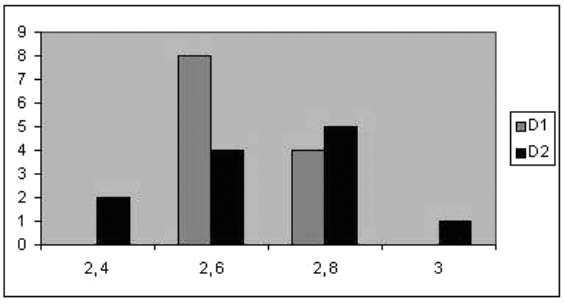
PDB code	Binding site[a]	Aryl boronic acid
1KDW		 4CB
1KE0		 CVB
1KE3		 BPB[c]
1GA9		 ETP

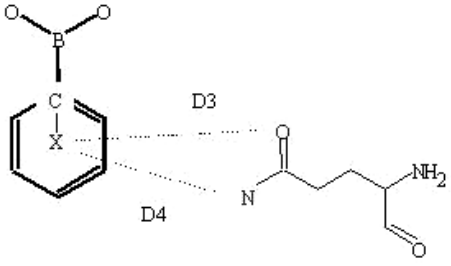
Figure 5 (continued).



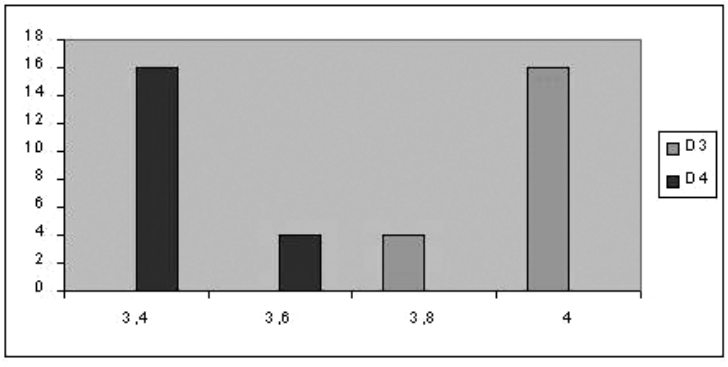
Search Fragment

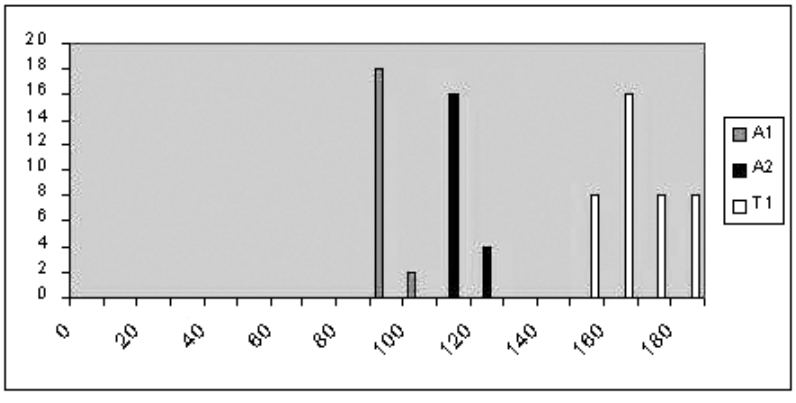


a



X = centroid of the Aryl ring
A1 = O-X-C angle
A2 = N-X-C angle
T1 = N-O-X-C torsion angle





b

Figure 6. Statistical analysis of the geometry of complexes between class C β-lactamases and boronic acid inhibitors. (**a**) First hydroxyl binding site, (**b**) Aryl-pi binding site.

1 Monnaie A., Dubus A. and Frere J.-M. (1994) The role of lysine-67 in a class C β-lactamase is mainly electrostatic. Biochem. J. **302**: 1–4

2 Dubus A., Normark S., Kania M. and Page M. G. P. (1994) The role of tyrosine 150 in catalysis of β-lactam hydrolysis by AmpC β-lactamase from *Escherichia coli* investigated by site-directed mutagenesis. Biochemistry **33**: 8577–8586

3 Dubus A., Normark S., Kania M. and Page M. G. P. (1995) Role of asparagine 152 in catalysis of β-lactam hydrolysis by *Escherichia coli* AmpC β-lactamase studied by site-directed mutagenesis. Biochemistry **34**: 7757 – 7764

4 Oefner C., D'Arcy A., Daly J. J., Gubernator K., Charnas R. L., Heinze I. et al. (1990) Refined crystal structure of a of β-lactamase from *Citrobacter freundii* indicates a mechanism for β-lactam hydrolysis. Nature **343**: 284–288

5 Usher K. C., Blaszczak L. C., Weston G. S., Shoichet B. K. and Remington S. J. (1998) Three-dimensional structure of AmpC β-lactamase from *Escherichia coli* bound to a transition-state analogue: possible implications for the oxyanion hypothesis and for inhibitor design. Biochemistry **37**: 16082–16092

6 Lobkovsky E., Moews P. C., Liu H., Zhao H., Frere J.-M. and Knox J. R. (1993) Evolution of an enzyme activity: crystallographic structure at 2-Å resolution of cephalosporinase from the ampC gene of *Enterobacter cloacae* P99 and comparison with a class A penicillinase. Proc. Natl. Acad. Sci. USA **90**: 11257–11261

7. Patera A., Blaszczak L. C., Stoichet B. (2000) Crystal structures of substrate and inhibitor complexes with AmpC β-lactamase: possible implications for substrate-assisted catalysis. J. Am. Chem. Soc. **122**: 10504–10512

8 Powers R. A., Caselli E., Focia P. J., Prati F. and Shoichet B. K. (2001) Structures of ceftazidime and its transition-state analogue in complex with AmpC β-lactamase: implications for resistance mutations and inhibitor design. Biochemistry **40**: 9207–9214

9 Beadle B. M. and Shoichet B. K. (2002) Structural basis for imipenem inhibition of class C β-lactamases. Antimicrob. Agents Chemother. **46**: 3978–3980

10 Crichlow G. V., Nukaga M., Doppalapudi V. R., Buynak J. D. and Knox J. R. (2001) Inhibition of class C β-lactamases: structure of a reaction intermediate with a cephem sulfone. Biochemistry **40**: 6233–6239

11 Beadle B. M., Trehan I., Focia P. J. and Shoichet B. K. (2002) Structural milestones in the reaction pathway of an amide hydrolase: substrate, acyl, and product complexes of cephalothin with AmpC β-lactamase. Structure **10**: 412–424

12 Shoichet B. K., McGovern S. L., Wei B. and Irwin J. J. (2002) Lead discovery using molecular docking. Curr. Opin. Chem. Biol. **6**: 439–446

13 Powers R. and Stoichet B. K. (2002) Structure-based approach for binding site identification on AmpC β-lactamase. J. Med. Chem. **45**: 3222–3234

14 Caselli E., Powers R. A., Blasczcak L. C., Wu C. Y. E., Prati F. and Shoichet B. K. (2001) Energetic, structural, and antimicrobial analyses of β-lactam side chain recognition by β-lactamases. Chem. Biol. **8**: 17–31

15 Tondi D., Powers R. A., Caselli E., Negri M.-C., Blázquez J., Costi M. P. et al. (2001) Structure-based design and in-parallel synthesis of inhibitors of AmpC β-lactamase. Chem. Biol. **8**: 593–610

16 Powers R. A., Blazquez J., Weston G. S., Morosini M. I., Baquero F. and Shoichet B. K. (1999) The complexed structure and antimicrobial activity of a non-beta-lactam inhibitor of AmpC beta-lactamase. Protein Sci. **8**: 2330–2337

17 Lobkovsky E., Billings E. M., Moews P. C., Rahil J., Pratt R. F. and Knox J. R. (1994) Crystallographic structure of a phosphonate derivative of the *Enterobacter cloacae* P99 cephalosporinase: mechanistic interpretation of a β-lactamase transition-state analog. Biochemistry **33**: 6762–6772

18 Powers R. A., Morandi F. and Shoichet B. K. (2002) Structure-based discovery of a novel, noncovalent inhibitor of Amp C β-lactamase. Structure **10**: 1013–1023

19 Wouters J., Charlier P., Monnaie D., Frère J.-M. and Fonzé E. (2001) Expression, purification, crystallization and preliminary X-ray analysis of the native class C β-lactamase from *Enterobacter cloacae* 908R and two mutants. Acta Crystallogr. D**57**: 162–164

20. Howard A. J., Gilliland G. L., Finzel B. C., Poulos T., Ohlendorf D. O. and Salemme F. R. (1987) The use of an imaging proportional counter in macromolecular crystallography. J. Appl. Crystallogr. **20**: 383–387

21. Navaza J. (1994) *AMoRe*: an automated package for molecular replacement. Acta Crystallogr. A**50**: 157–163

22 Sheldrick G. M. and Schneider T. R. (1997) SHELXL: high resolution refinement. Methods Enzymol. **277**: 319–343

23 Roussel A. and Cambillau C. (1992) Turbo-Frodo. Biographics, AFMB, Marseille, France

24 Knox, J. R., Moews P. C. and Frere J.-M. (1996) Molecular evolution of bacterial beta-lactam resistance. Chem. Biol. **3**:937–947

25 Larsen N. A., Turner J. M., Stevens J., Rosser S. J., Basran A., Lerner R. A. et al. (2002) Crystal structure of a bacterial cocaine esterase. Nat. Struct. Biol. **9**: 17–21