**SUPPLEMENTARY ONLINE DATA**

**Solution structures of the Bacillus cereus metallo-β-lactamase BcII and its complex with the broad spectrum inhibitor R-thiomandelic acid**

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**EXPERIMENTAL**

**Incorporation of the zinc atoms and the inhibitor molecule into CYANA during structure refinement**

The structure determination and automated assignment module used in the present study did not take into account the presence of the two metal atoms or, in the case of the complex, the inhibitor molecule. These were incorporated through the use of the generic linker system present in CYANA. (The NOE-based constraint sets used in refinement were obtained with automated NOE assignment without the use of the linker system.) The residue ‘ION’ from the standard library was modified to a ZN residue and accordingly the ‘METAL’ atomtypes entry in the standard library was modified to ZINC. Following the last residue of the protein sequence (Lys227) and the connecting linker residue (PL228), a 12 residue linker was used to connect to the first zinc atom ZN241, comprising ten LL5-type linker residues flanked by LL2-type linker residues. An identical segment was used to connect to the second zinc atom ZN254. The length of the linker segment was chosen to be of sufficient length to ensure minimum steric interference. The effect of the presence of the linker system, with the zinc atoms both fixed and unfixed, on the CYANA target function was examined with an identical set of constraints during refinement. No significant impact was detected by comparison with a refinement run lacking the linker system. The zinc atoms were fixed to the corresponding atoms of the metal-co-ordinating residues with the use of a set of six lower (lol) and upper (upl) distance bounds (for His[^6][^5], His[^6][^6], Asp[^6][^6], His[^6][^9] and Cys[^6][^6], lol = 2.2, upl = 2.4, and for His[^10][^10], lol = 2.3, upl = 2.5) loosely based on the distances observed in the di-zinc X-ray structure PDB code 1BVT; in addition, a Zn–Zn interatomic distance was included (lol = 3.65, upl = 3.85) (Table S1).

In a similar fashion, the inhibitor molecule was successfully incorporated into the linker system by using a novel residue definition including a cluster of pseudoatoms to ensure correct connectivity with the linker system. The PDB file of the inhibitor was read into MOLMOL [1] and the appropriate dihedral angles were defined (LB1, CH1, CH2, CH3 and LB2). The atom names and types were in accordance with the IUPAC nomenclature and the CYANA 2.1 naming conventions. The dihedral angle and the atom declarations were ordered so as to be compatible with the tree structure of the dihedral angles (SYBYL 7.2 interface to DYANA). Pseudoatoms were added for the thiomandelate aromatic ring protons (QG for HG1 and HG2, QD for HD1 and HD2, and QR for all the ring protons). The creation of the overlap atoms at the beginning and end of the residue definition (corresponding to N, CA and C in the case of amino acids) which ensure the connectivity of the new residue with the generic linker residues was essential since thiomandelate is not an amino acid derivative. A connecting system such as the one used in the creation of proxy residues [2] was used successfully.

**Structure calculation and refinement**

**Simulated annealing**

It has been reported, in the case of structural calculations performed with the program ARIA, that an increase in the number of simulated annealing steps can prove to be highly beneficial in achieving convergence, especially large proteins where chemical shift degeneracy is a significant issue [3,4]. In the present study we found that the use of extended torsion angle dynamics (TAD) steps (15 times the normal) can indeed be beneficial in terms of convergence and target function values. However, these calculations tended to become biased towards similar conformations, as reflected by the unusually low RMSD values observed even in some regions expected to be unstructured. This suggests that extended TAD steps can be valuable, providing converged and essentially correct structures and reducing exhaustive peak list analysis, but must be used with caution.

**Dihedral angle constraints**

We found that too general a use of dihedral angle constraints derived from the TALOS database may introduce erroneous features in regions of unusual or complex backbone structure distribution, for example around the zinc co-ordination sites. Consequently, the structures reported in the present study were calculated both with and without dihedral angle constraints and we focus on the structures obtained without these constraints. Table 1 in the main text gives the structural statistics for the structures calculated without dihedral angle constraints. Table S2 gives these statistics for structures calculated with these constraints. The structures calculated with and without dihedral angle constraints had very similar RMSD values (0.35–0.38 for backbone atoms) and similar Ramachandran statistics (>98% of residues in the core and allowed regions), but the structures calculated with dihedral angle constraints showed significantly more distance and angle violations.

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[^6]: 1H, 13C and 15N chemical shifts and NMR restraints have been deposited in the BioMagResBank (BMRB) under accession codes 19047 (BcII) and 19048 (BcII–R-thiomandelic acid) and co-ordinates have been deposited in the PDB under accession codes 2M5C (BcII) and 2M5D (BcII–R-thiomandelic acid).

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Figure S1  Comparison of zinc co-ordination in solution and in crystal

The co-ordination of the two zinc atoms by protein ligands in BclI in the free enzyme in crystal (A, PDB code 1BVT; B, PDB code 1BC2) and in solution (C) and in the BclI-thiomandelate complex in solution (D) are shown separately (A–D) and superimposed (E).
Figure S2 Constraints defining the conformation of the β3–β4 loop

The NOE constraints, including both those involving side chain atoms and those involving backbone atoms, are shown on backbone representations of the loop (residues 29–42) for (A) the free enzyme and (B) the R-thiomandelate complex. The two panels share the same orientation and the inhibitor molecule is presented in red. (C) Constraint distribution for residues 29–42, showing total number of constraints (C1) and long plus medium range constraints (C2); free enzyme constraints are shown in blue, complex constraints in red.

Figure S3 Local backbone RMSDs

Local backbone RMSDs are shown on the solution structures of the free enzyme (A) and the complex (B) in rainbow colours: violet (≤0.03 Å), blue (0.03–0.04 Å), cyan (0.05–0.06 Å), green (0.07–0.09 Å), yellow (0.1–0.2 Å), orange (0.2–0.3 Å) and red (>0.3 Å).
Figure S4  Zinc atoms and their protein ligands in the structures of the free enzyme and the R-thiomandelate complex

The bundles of structures shown were determined solely from the NOE-based constraints, before the introduction of zinc restraints in refinement. Structures are shown for the free enzyme (slate blue) and the complex (orange). The positions of the protein ligands after refinement are shown in blue (free enzyme) and red (complex) and the positions of the zinc atoms as blue (free enzyme) and orange (complex) spheres.

Table S1  Metal co-ordination constraints used (lower and upper limits) and corresponding distances on the X-ray structure (PDB code 1BVT) and both NMR structures

| Distance          | lol | upl | 1BVT | BcII | BcII-RTM |
|-------------------|-----|-----|------|------|----------|
| His86 NE2–Zn1     | 2.20| 2.40| 2.33 | 2.40–2.46| 2.42–2.47|
| His88 ND1–Zn1     | 2.20| 2.40| 2.24 | 2.40–2.48| 2.18–2.43|
| His149 NE2–Zn1    | 2.20| 2.40| 2.32 | 2.16–2.40| 2.27–2.42|
| Cys168 SG–Zn2     | 2.20| 2.40| 2.29 | 2.19–2.41| 2.24–2.44|
| His210 NE2–Zn2    | 2.30| 2.50| 2.41 | 2.50–2.53| 2.29–2.51|
| Asp90 OD2–Zn2     | 2.20| 2.40| 2.29 | 2.23–2.43| 2.19–2.43|
| Zn1–Zn2           | 3.65| 3.85| 3.75 | 3.82–3.88| 3.62–3.65|

Figure S5  Conformations of Lys171 and Asn180 in the BcII–thiomandelate complex

The side chains of Lys171 and Asn180 are shown in yellow and thiomandelate in red. The constraint network defining the positions of Lys171 and Asn180 is depicted with red lines and the side chains of the residues involved in these constraints are shown in blue. The distances between the NZ atom of Lys171 and the oxygen atoms of the carboxylate group of the inhibitor and the distances between the HD21 and HB3 atoms of the Asn180 and the QR pseudo atom of the aromatic ring of the inhibitor are indicated. The zinc atoms are shown as cyan spheres and the protein backbone is coloured grey.

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**Table S2** Structural statistics and agreement with experimental data for the refined BcII and BcII–R-thiomandelate structures calculated with external (TALOS) dihedral angle input

Target function, RMSD and energy are calculated for a bundle of 20 structures unless specified otherwise. RTM, R-thiomandelate.

| Parameter                              | CYANA/CANDID                  | BcII                        | BcII–R-thiomandelate |
|----------------------------------------|-------------------------------|-----------------------------|----------------------|
| Input peaks                            | 13,088                        | 12,499                      |
| Unassigned peaks                       | 10,82                         | 11,59                       |
| Assignment percentage                  | 91.7                          | 90.7                        |
| Target function (cycle 1)              | 233.87                        | 195.59                      |
| Target function (final)                | 5.39                          | 3.81                        |
| RMSD, Å (residues 7–227) cycle1        | 0.85/1.30                     | 0.95/1.39                   |
| RMSD, Å (residues 7–227) final        | 0.30/0.66                     | 0.31/0.67                   |
| NOE constraints                        |                               |                             |
| Backbone/heavy atoms                  | 7165                          | 6565                        |
| Short range (i = 1)                    | 3004                          | 2815                        |
| Medium range (1 < i ≤ 4)               | 1257                          | 1098                        |
| Long range (> 4)                       | 2004                          | 2652                        |
| Removed during refinement              | 104                           | 2                           |
| Short range 1 restraint                |                               |                             |
| Medium range 1–4 restraint             |                               |                             |
| Long range > 4 restraints              |                               |                             |
| Other constraints                      |                               |                             |
| Non-NOE constraints (zinc)             | 7                             | 9                           |
| RTM constraints                        | –                             | 13                          |
| Dihedral restraints                    | 206                           | 202                         |
| Hydrogen bonds                         | 0                             | 50                          |
| Stereospecific assignments             | 142                           | 111                         |
| Target function (refinement)           |                               |                             |
| Minimum                                | 2.52                          | 2.97                        |
| Average (20 structures)                | 3.1 ± 0.3                     | 3.3 ± 0.2                   |
| Average (50 structures)                | 4.3 ± 1.6                     | 3.5 ± 0.2                   |
| RMSDs of experimental restraints (20 structures) |             |                             |
| Upls                                   | 0.006 ± 0.001                 | 0.006 ± 0.0003              |
| Lows                                   | 0.002 ± 0.002                 | 0.005 ± 0.001               |
| Torsion angles                         | 0.23 ± 0.06                   | 0.21 ± 0.03                 |
| Van der Waals energy sums              | 14.3 ± 0.7                    | 14.1 ± 0.7                  |
| 50 structures                          | 16.19 ± 2.34                  | 14.88 ± 0.82                |
| Mean violations (20/50 structures)     |                               |                             |
| Distance > 0.5 Å                       | 0.3 ± 0.4/0.6 ± 0.7           | 0/0                         |
| Angle > 5°                             | 0.06 ± 0.22/1.0 ± 2.8         | 0/0                         |
| Van der Waals                          | 0/0                           | 0/0                         |
| Van der Waals                          | 0/0                           | 0/0                         |
| Ramachandran statistics (CANDID stage/refinement) |             |                             |
| Backbone/heavy atoms                  | 0.38/0.72                     | 0.38/0.72                   |
| Core (%)                               | 78.6/78.7                     | 78.2/78.6                   |
| Allowed (%)                            | 20.9/20.0                     | 21.3/22.0                   |
| Generously allowed (%)                 | 0.1/0.6                       | 0.1/0.8                     |
| Disallowed (%)                         | 0.5/0.6                       | 0.5/0.6                     |

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