Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Structure of the macrocycle thiostrepton solved using the anomalous dispersion contribution of sulfur

Charles S. Bond, Matthew P. Shaw, Magnus S. Alphey and William N. Hunter*

The Wellcome Trust Biocentre, University of Dundee, Dundee DD1 5EH, Scotland

Correspondence e-mail: w.n.hunter@dundee.ac.uk

Received 13 December 2000
Accepted 14 February 2001

PDB Reference: thiostrepton, 1e9w.

The structure of a tetragonal crystal form of thiostrepton has been solved using the anomalous dispersive effects of five S atoms from high-redundancy data collected to 1.33 Å resolution at the Cu $K\alpha$ wavelength. Data measured to 1.02 Å resolution with a synchrotron source were used for refinement. Details of the molecular structure, intramolecular and intermolecular interactions are given.

1. Introduction

Thiostrepton, a thioazole-containing antibiotic first isolated from *Streptomyces azureus* (Pagano *et al.*, 1956; Vandeputte & Dutcher, 1956), inhibits ribosome function (Blyn *et al.*, 2000), displaying activity against Gram-positive bacteria (Kutscher *et al.*, 1958) and *Plasmodium falciparum*, the causal agent of malaria (McConkey *et al.*, 1997).

The mechanism of action involves thiostrepton binding at the 'GTPase centre' of the large subunit 23s rRNA at a domain composed of a four-way junction of double-helical RNA (Rosendahl & Douthwaite, 1994; Wimberly *et al.*, 1999) and interferes with the normal assembly of the L11 subunit–rRNA complex (Conn *et al.*, 1999; Porse *et al.*, 1998).

An incomplete structure from monoclinic crystals was published previously (Anderson *et al.*, 1970), but no coordinates were deposited. We chose to determine the structure in order to place a coordinate set in the public domain and to investigate the use of the anomalous dispersive signal from sulfur at a single wavelength for phase determination in a similar manner to that applied to crambin (Hendrickson & Teeter, 1981).

2. Materials and methods

2.1. Crystallization and data collection

Thiostrepton (100 mg; CALBIOCHEM) was dissolved in 6.5 ml chloroform:isoamyl alcohol (24:1), aliquoted in 1 ml volumes into glass vials and 100 µl of glycerol and 200 µl of ethanol were added. The vials were covered with Parafilm and tetragonal crystals grew at room temperature within days.

Crystals were cooled to 100 K (Oxford Cryosystems) and data to 1.33 Å were collected using a Rigaku rotating-anode apparatus (Cu $K\alpha$; λ = 1.5418 Å) and an R-AXIS IV image plate. Two passes of 352° in 8° oscillations with 5 min and 30 min exposures were made to ensure high redundancy and good measurement of both high- and low-resolution data. High-resolution data (1.02 Å resolution) were later collected at BM14 of ESRF (λ = 0.73 Å) on a MAR CCD with 1° rotation and 30 s exposure for each frame, measuring 61° of data. All data were processed and scaled using *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997); relevant details are presented in Table 1.

2.2. Structure solution and refinement

All five sulfur sites were found with *SnB* (Weeks & Miller, 1999) using the anomalous dispersive signal in the Cu $K\alpha$ data. 1000 trial structures were refined with 50 cycles of the *SnB* procedure. The anomalous signal ($R_{\rm anom}$ = 2.9%) was larger than the predicted value [2.4%; r.m.s.($|\Delta F|$)/r.m.s.($|F_o|$) $\simeq (N_{\rm ano}/2)^{1/2} \times 2f''_{\rm max} \times (3.14\ {\rm MW})^{-1/2}$; Smith, 1991]. A histogram of the *SnB* minimal function $R_{\rm min}$ (DeTitta *et al.*, 1994) showed a bimodal distribution, with 39 of 1000 solutions in the lower group (0.309–0.355).

The five sulfur positions were used to calculate phases (*MLPHARE*; Otwinowski, 1991). Histogram matching and solvent flattening were performed with *DM* (Cowtan, 1994) to solve phase ambiguities ($\langle\Delta\varphi\rangle$ = 71.5°) and to produce an interpretable electron-density map (Fig. 1) of high quality in $P4_32_12$, into which a model was built (*O*; Jones *et al.*, 1991).

To simplify the application of geometric restraints in the refinement, we defined a nomenclature using 16 fragments (Table 2; Fig. 2*a*) based on the original degradation analysis (Anderson *et al.*, 1970). The Cambridge Structural Database (Fletcher *et al.*, 1996) provided geometric data to construct a dictionary of bond-length restraints. A model was then refined using *SHELXL* (Sheldrick & Schneider, 1997) with data to 1.33 Å. Once

Table 1
Summary of data-collection, phasing and refinement statistics.

Data statistics		
Radiation source	Cu anode	ESRF
Crystal dimensions (mm)	0.20 × 0.10 × 0.05	0.15 × 0.15 × 0.20
Resolution (Å)	1.33	1.02
Space group	$P4_32_12$	$P4_32_12$
Unit-cell parameters (Å)	$a = b = 26.62$, $c = 27.46$	$a = b = 26.58$, $c = 27.44$
No. of measurements	91497	22556
No. of unique reflections	2446	5232
Redundancy	37.4	4.3
Completeness (%)	96.6 (57.9)†	96.7 (74.6)‡
$I/\sigma(I)$	85.9 (5.7)†	33.8 (5.4)‡
R_{merge} (%)	4.2 (16.0)†	3.3 (15.2)‡
Solvent content (%)	15.1	14.8
MLPHARE statistics		
FOM (acentrics)	0.36	
R_{Cullis}	0.63	
FOM (overall)	0.27	
DM statistics		
FOM (overall)	0.67	
Refinement statistics		
R_{cryst} (%)		11.73
R_{free} (%)		14.19
No. of water molecules		4

† Values in parentheses refer to the highest resolution bin, 1.33–1.36 Å. ‡ Values in parentheses refer to the highest resolution bin, 1.02–1.04 Å.

high-resolution data (1.02 Å) became available, the restraints were removed, except those for residue 16 which shows some disorder. H atoms were added in riding mode and anisotropic temperature-factor refinement of non-H atoms was carried out. Difference maps indicated the presence of four well ordered water molecules which were included in the model. The final maps also indicated the presence of disordered solvent which could not be modelled.

3. Results

We measured a good-quality high-redundancy data set, though not at a resolution (1.33 Å) for which direct methods could normally be applied to phase a complete structure. Nevertheless, S-atom positions were readily identified with *SnB* from the anomalous dispersion signal. Density modification, particularly the application of histogram matching (Cowtan, 1994), allowed the determination of the space-group enantiomorph and the generation of an interpretable electron-density map (Fig. 1).

Thiostrepton ($C_{72}H_{88}N_{19}O_{19}S_5$; Fig. 2) is a complex macrocyclic compound. While parts of the molecule are readily identifiable amino acids (Ala, Ile, Thr), others have been heavily modified. The molecule is composed of loop 1 (residues 1–6), loop 2 (residues 7–12) and a tail (residues 13–16). The dimensions of the molecule are approximately 25 × 16 × 12 Å. Residue 8 and its interactions with components of loops 1 and 2 provide the hydrophobic core of the molecule and further hydrophobic interactions with symmetry-related molecules help to stabilize the conformation of the tail residues. A detailed listing of hydrogen-bonding interactions is given in Table 3 and most are depicted in Fig. 2(*b*). Five intramolecular hydrogen bonds contribute to the conformation of the molecule, in particular linking residue 8 with residues 1, 2, 5 and 13. A water molecule (W1) binds in a hydrophilic cleft making five contacts to thiostrepton and one to another water (W2).

Table 2
Chemical structure of thiostrepton.

Residue	Description
1	Thiazole-2-carbaldehyde
2	Threonine
3	Dehydrobutyrine
4	Thiazole-2-carbaldehyde
5	Thiostreptine fragment
6	Thiazole-2-carbaldehyde
7	Thiostreptoic acid fragment
8	Quinaldic acid
9	Isoleucine
10	Alanine
11	Pyruvic acid
12	Alanine
13	Tetrahydro-pyridin-3-ylamine
14	Thiazole-2-carbaldehyde
15	Pyruvic acid
16	Pyruvic acid

Table 3
Intramolecular and intermolecular hydrogen bonds.

Asymmetric unit.

Donor	Acceptor	Distance (Å)
2 OG1	8 O16	2.78
5 O7	4 O7	3.60
5 O4	W1 O	2.69
7 N7	W1 O	3.34
8 O15	1 O7	2.80
8 O16	5 O4	2.72
13 N7	7 N1	2.77
13 N7	8 O12	3.14
W1 O	6 N1	3.31
W1 O	7 O10	2.91
W1 O	8 N1	2.92
W1 O	W2 O	2.97
W2 O	9 O	2.71
W3 O	9 O	2.92
W4 O	11 O	2.97

Symmetry related.

Donor	Acceptor	Distance (Å)
3 N	12 O	2.86
4 C4	15 O	2.95
5 O7	14 O7	3.83
9 N	6 O7	3.20
12 N	2 OG1	2.81
16 N	2 O	3.52
16 NT	8 O15	3.14
W1 O	W2 O	2.97
W2 O	9 O	2.71
W3 O	9 O	2.92

4. Discussion

The original structure determination (Anderson *et al.*, 1970) was achieved using monoclinic crystals in space group *C*2, with unit-cell parameters $a = 21.757$, $b = 22.846$, $c = 23.523$ Å, $\beta = 103.93^\circ$ and data measured to 1.0 Å resolution. Four sulfur positions were identified from a Patterson synthesis and the heavy-atom method was then used to generate the model. Refinement

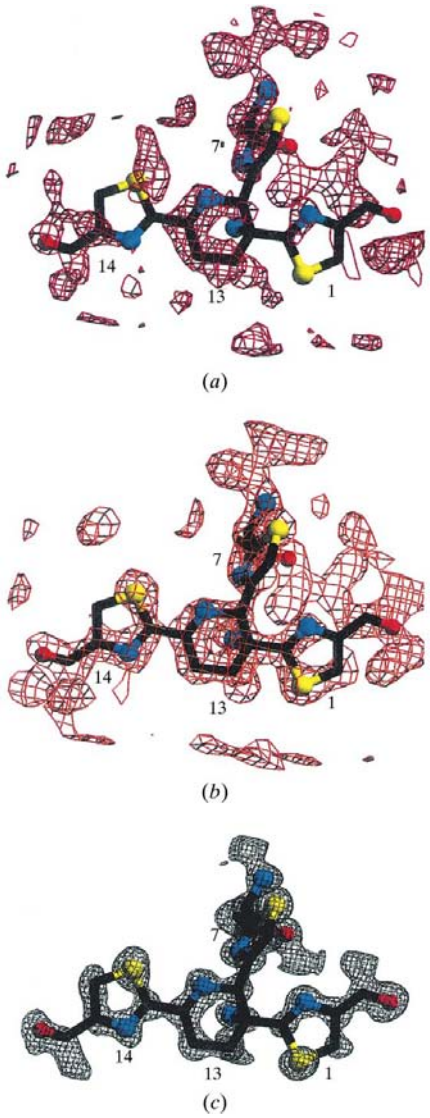

Figure 1
Electron density (1.6σ) at three stages of the analysis. In each case, the final model is shown with N, O and S atoms depicted as spheres coloured blue, red and yellow, respectively, with C positions shown as black sticks superimposed with the final model for part of thiostrepton. (*a*) The first map at 1.33 Å resolution generated from *MLPHARE*. (*b*) The map to 1.33 Å resolution after density modification (*DM*). (*c*) The final map at 1.02 Å resolution.

converged with an R_{cryst} of 0.152 for 4520 reflections. The final model is missing the terminal residue, which in our nomenclature corresponds to residue 16. In both structures, the surrounding solvent is highly disordered. Our work has not significantly improved on the original structure; rather, it has confirmed the chemical and three-dimensional structural results of Anderson *et al.* (1970) and provided coordinates for thiostrepton which are now in the public domain.

Thiostrepton is a microcosm of the protein world, containing C, H, N, O and S atoms in residues linked by peptide bonds, bound water molecules and a hydrophobic core. In using the sulfur anomalous signal to determine its structure, we contribute further proof-of-principle to the routine phasing of protein crystal data in this manner as previously demonstrated for crambin (Hendrickson & Teeter, 1981) and obelin (Liu *et al.*, 2000). The presence of S atoms in most protein molecules combined with the increasing ease of collecting highly redundant high-resolution protein data presage the widespread application of this approach in the future.

5. Conclusions

The anomalous signal from five S atoms in the antibiotic thiostrepton has been used to solve the structure of a tetragonal crystal form. The coordinates, which were not available from an earlier structure determination (Anderson *et al.*, 1970), are now placed in the public domain. The in-house data may be suitable for methods development and is available from the authors.

This work was funded by the Wellcome Trust, BBSRC and a BBSRC–Pfizer CASE award (MPS). We wish to acknowledge the use of the EPSRC's Chemical Database

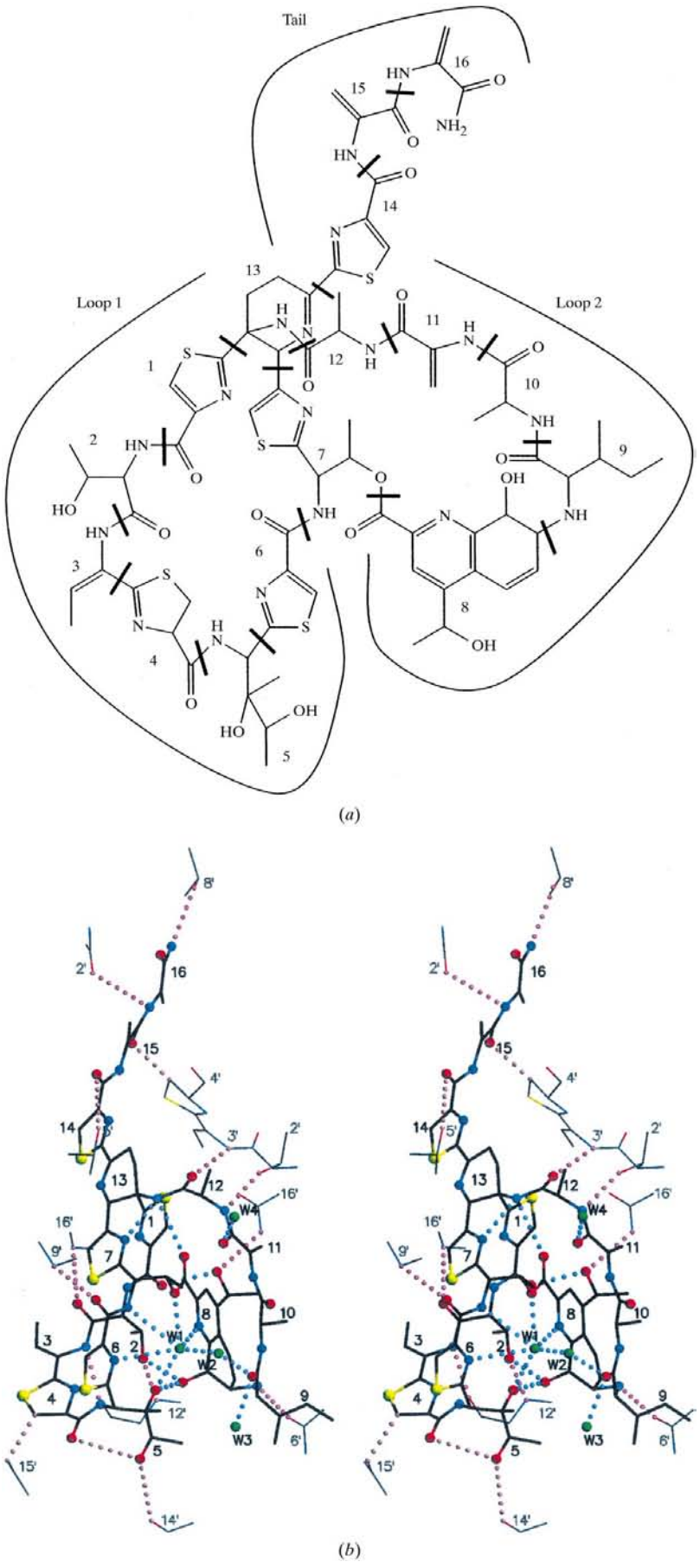

Figure 2
(*a*) The chemical structure of thiostrepton. Numbered residues are separated by a black bar. (*b*) Stereoview of thiostrepton depicting the intramolecular (cyan dashed lines) and selected intermolecular (purple dashed lines) hydrogen-bonding interactions. The same colour scheme as Fig. 1 is used, with the addition that water molecules are depicted as green spheres. Components of some symmetry-related molecules that participate in hydrogen-bonding interactions are shown as sticks with carbon positions in grey and labelled with the symbol ′. H atoms and the symmetry-related hydrogen-bonding partners for waters have been omitted for the purpose of clarity. (This figure was generated using *MOLSCRIPT*; Kraulis, 1991.)

Service at Daresbury and thank Professor A. H. Fairlamb for the gift of thiostrepton, the ESRF for synchrotron time and staff at Pfizer, in particular Mr A. Tucker, for support.

References

Anderson, B., Hodgkin, D. C. & Viswamitra, M. A. (1970). *Nature (London)*, **225**, 233–235.

Blyn, L. B., Risen, L. M., Griffey, R. H. & Draper, D. E. (2000). *Nucleic Acids Res.* **28**, 1778–1784.

Conn, G. L., Draper, D. E., Lattman, E. E. & Gittis, A. G. (1999). *Science*, **284**, 1171–1174.

Cowtan, K. D. (1994). *Jnt CCP4/ESF–EACBM Newsl. Protein Crystallogr.* **31**, 34–38.

DeTitta, G. T., Weeks, C. M., Thuman, P., Miller, R. & Hauptman, H. A. (1994). *Acta Cryst.* A**50**, 203–210.

Fletcher, D. A., McMeeking, R. F. & Parkin, D. (1996). *J. Chem. Int. Comput. Sci.* **36**, 746–749.

Hendrickson, W. A. & Teeter, M. M. (1981). *Nature (London)*, **290**, 107–113.

Jones, T. A., Zou, J. Y., Cowan, S. W. & Kjeldgaard, M. (1991). *Acta Cryst.* A**47**, 110–119.

Kraulis, P. J. (1991). *J. Appl. Cryst.* **24**, 946–950.

Kutscher, A. H., Sequin, K., Rankow, R. M. & Piro, J. D. (1958). *Antibiot. Chemother.* **8**, 576–583.

Liu, Z. J., Vysotski, E. S., Chen, C. J., Rose, J. P., Lee, J. & Wang, B. C. (2000). *Protein Sci.* **9**, 2085–2093.

McConkey, G. A., Rogers, M. J. & McCutchan, T. F. (1997). *J. Mol. Biol.* **272**, 2046–2049.

Otwinowski, Z. (1991). *Proceedings of the CCP4 Study Weekend. Isomorphous Replacement and Anomalous Scattering*, edited by W. Wolf, P. R. Evans & A. G. W. Leslie, pp. 80–86. Warrington: Daresbury Laboratory.

Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.

Pagano, J. F., Weinstein, M. J., Stout, M. A. & Donovick, R. (1956). *Antibiot. Ann.* **1956**, 554–559.

Porse, B. T., Leviev, I., Mankin, A. S. & Garrett, R. A. (1998). *J. Mol. Biol.* **276**, 391–404.

Rosendahl, G. & Douthwaite, S. (1994). *Nucleic Acids Res.* **22**, 357–363.

Sheldrick, G. M. & Schneider, T. R. (1997). *Methods Enzymol.* **115**, 319–343.

Smith, J. L. (1991). *Curr. Op. Struct. Biol.* **1**, 1002–1011.

Vandeputte, J. & Dutcher, J. D. (1956). *Antibiot. Ann.* **1956**, 560–561.

Weeks, C. M. & Miller, R. (1999). *J. Appl. Cryst.* **32**, 120–124.

Wimberly, B. T., Guymon, R., McCutcheon, J. P., White, S. W. & Ramakrishnan, V. (1999). *Cell*, **97**, 491–502.