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Structural Basis for the Aldolase and Epimerase Activities of *Staphylococcus aureus* Dihydroneopterin Aldolase

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Abstract

Dihydroneopterin aldolase (DHNA) catalyzes the conversion of 7,8-dihydroneopterin (DHNP) to 6-hydroxymethyl-7,8-dihydropterin (HP) and also the epimerization of DHNP to 7,8-dihydromonapterin (DHMP). Although crystal structures of the enzyme from several microorganisms have been reported, no structural information is available about the critical interactions between DHNA and the trihydroxypropyl moiety of the substrate, which undergoes bond cleavage and formation. Here, we present the structures of *Staphylococcus aureus* DHNA (SaDHNA) in complex with neopterin (NP, an analog of DHNP) and with monapterin (MP, an analog of DHMP), filling the gap in the structural analysis of the enzyme. In combination with previously reported SaDHNA structures in its ligand-free form (PDB entry 1DHN) and in complex with HP (PDB entry 2DHN), four snapshots for the catalytic center assembly along the reaction pathway can be derived, advancing our knowledge about the molecular mechanism of SaDHNA-catalyzed reactions. An additional step appears to be necessary for the epimerization of DHMP to DHNP. Three active site residues (E22, K100, and Y54) function coordinately during catalysis: together, they organize the catalytic center assembly, and individually, each plays a central role at different stages of the catalytic cycle.

Keywords

aldolase; dihydroneopterin aldolase; dihydroneopterin; dihydromonapterin; pterin

Introduction

Folates are essential for life.¹ Mammals obtain folates from their diet, whereas most microorganisms must synthesize folates *de novo*. Therefore, the folate biosynthetic pathway is an ideal target for antimicrobial agents. Inhibitors of folate enzymes dihydropteroate synthase and dihydrofolate reductase are currently used as antibiotics.² However, the resistance to these and other antimicrobial agents has increased dramatically during the past two decades. The crisis is further aggravated by the fact that most of new antibiotics are chemical modifications of the chemical structures of existing drugs. These compounds act against old

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targets and are therefore less effective tackling the widespread antibiotic resistance problem. Thus, new antimicrobial targets and agents are urgently needed. Dihydroneopterin aldolase (DHNA) is a folate pathway enzyme and is not a target for existing antibiotics. Therefore, DHNA is a new molecular target in a biosynthetic pathway proven to be susceptible to antimicrobial agents.

DHNA catalyzes two reactions, the epimerization of 7,8-dihydroneopterin (DHNP) to 7,8-dihydromonapterin (DHMP)³ and the conversion of DHNP or DHMP to 6-hydroxymethyl-7,8-dihydropterin (HP) with the generation of glycoaldehyde (GA)⁴ as shown in Figure 1(a). DHNA is a unique aldolase; it requires neither metal ions nor the formation of a Schiff base between the enzyme and the substrate.⁴ Although the epimerase reaction catalyzed by DHNA is very similar to the reaction catalyzed by L-ribulose-5-phosphate 4-epimerase,⁵ DHNA is different in structure and has no apparent sequence identity with other epimerases. While other aldolases and epimerases catalyze one type of reaction, DHNA catalyzes both. Therefore, it is of great interest to elucidate the structure and catalytic mechanism of DHNA.

To date, crystal structures have been determined for DHNA enzymes from *Staphylococcus aureus* (SaDHNA),^{6,7} *Arabidopsis thaliana*,⁸ *Mycobacterium tuberculosis* (MtDHNA),⁹ and *Streptococcus Pneumoniae*.¹⁰ The active site of the enzyme has been identified by the crystal structures of enzyme-product complexes SaDHNA•HP (PDB entry 2DHN)⁶ and MtDHNA•HP (PDB entry 1NBU).⁹ An active site lysine residue (K100) has been proposed to function as the general base and a bound water as a proton donor, leading to the first reaction scheme predicted for DHNA.⁶ Recently, our biochemical and biophysical studies have yielded further insights into the mechanism of DHNA, including the important roles of active site glutamate (E22) and tyrosine (Y54) residues,^{11,12} and the reversible nature of DHNA-catalyzed epimerization reaction.¹³ Here, we present two crystal structures, which make it possible to derive the critical interactions between DHNA and the trihydroxypropyl moiety of the substrate, providing further structural insights into the mechanism of DHNA-catalyzed reactions.

Results and Discussion

Neopterin and Monopterin Are DHNA Inhibitors

Neopterin (NP) and monopterin (MP) are oxidized forms of DHNP and DHMP, respectively (Figure 1). The oxidation results in the formation of a double bond between C7 and N8, which may make the protonation of N5 much harder so that NP and MP may not undergo chemical reaction catalyzed by DHNA. An SaDHNA•NP structure (PDB entry 1U68) was reported recently, but the occupancy factors for the 6-trihydroxypropyl moiety of NP are zero, suggesting this moiety was not observed.⁷ Our crystal structures of SaDHNA•NP and SaDHNA•MP are determined at 1.70 and 1.68 Å, respectively, containing well defined ligand molecules, showing that NP and MP are indeed inhibitors of DHNA. Substrate analogs can be efficient inhibitors. We believe that NP (MP) is an excellent analog of DHNP (DHMP) for three reasons. First, NP (MP) sufficiently resembles DHNP (DHMP) (Figure 1, panels (a) and (b)), comparable with other systems such as ATP analogs versus ATP. Second, the NH group at position 8 of DHNP (DHMP) has no hydrogen bond interaction with the protein. Therefore, the oxidation does not change the interactions between the protein and the pterin moiety of the ligand, as shown by SaDHNA•HP (PDB entry 2DHN) and our new structures. Third, the oxidation at positions 7 and 8 of DHNA (DHMP) does not affect the structure and not likely the conformation of the trihydroxypropyl moiety. Thus, the catalytically competent interactions between the trihydroxypropyl moiety and the enzyme are not likely to be disturbed.

The SaDHNA•NP Structure Mimics the Enzyme-DHNP Complex

DHNA functions as an octamer,⁶ containing eight subunits and eight active sites; each active site is located between two adjacent subunits (Figure 1(c)). The asymmetric unit of the SaDHNA•NP structure consists of four independent DHNA molecules (referred to as Mol A through D), each associated with an NP molecule. All four NP molecules are well defined with an example shown in Figure 2(a). Thus, the octamer of SaDHNA•NP contains two sets of four nonidentical active sites. Figure 2(b) depicts the catalytic center between Mol A and Mol B with the bound NP. The catalytic center assembly between Mol C and Mol D is similar as that shown in Figure 2(b). For Mol A and Mol C, a catalytic water molecule is hydrogen bonded to atom N5 of associated NP. The B-factors of the water oxygen atoms (30.0 and 25.1 Å²) are comparable with those of atom N5 of the NP molecules (27.0 and 27.7 Å²). However, this water molecule is not observed for Mol B and Mol D, for which the reason is not clear.

As previously observed for HP,⁶ the NP molecule is bound in a deep and narrow pocket with extensive electrostatic interactions with the enzyme (Figure 2(b)). Among 18 non-hydrogen atoms of the ligand, eight are involved in electrostatic interactions with six residues (A18, E22, N71, L73, E74, and K100) from one subunit and three (V52, H53, and Y54) from the partner subunit, either directly or bridged by water molecules. Residues E22, Y54, E74, and K100 are conserved, while A18, N71, L73, and V52 interact with the ligand with their carboxyl oxygen or amide nitrogen. The hydrophobic interactions between the protein and the ligand involve residues A18, V48, T51, H53, Y54, L72, P103, I105, and I5, among which Y54 and L72 are conserved (Figure 2(b)).

Although the pattern of pterin recognition for NP is similar to that previously reported for HP (PDB entry 2DHN), our SaDHNA•NP structure reveals interactions between the trihydroxypropylene moiety and the enzyme, especially the hydrogen bond between the 2'-hydroxyl group of NP and the ε-amino group of K100 (Figure 2(b)). It has been proposed that the deprotonation of the 2'-hydroxyl group serves as the initial reaction step for both the aldolase and epimerase activities of DHNA.^{3,4} Therefore, the interaction between K100 and 2'-hydroxyl indicates that K100 is in position to act as the general base, in agreement with site-directed mutagenesis data.^{6,11} Taken together, our SaDHNA•NP structure likely mimics the enzyme-substrate complex.

The SaDHNA•MP Structure Mimics the Enzyme-DHMP Complex

The asymmetric unit of the SaDHNA•MP structure consists of a single DHNA and one MP. Therefore, the octamer of SaDHNA•MP contains eight identical subunits with eight identical active sites. The structure of one catalytic center is depicted in Figure 3. The pattern of recognition for the pterin ring system in SaDHNA•MP is the same as in SaDHNA•NP, but the interactions between the trihydroxypropylene moiety of MP and the protein are significantly different. In the SaDHNA•NP structure, the ε-amino group of K100 interacts with the 2'-hydroxyl group of NP (Figure 2(b)), whereas in the SaDHNA•MP structure, K100 is hydrogen bonded to the 3'-hydroxyl group of MP (Figure 3(b)). Mimicking the enzyme-DHMP complex, our SaDHNA•MP structure indicates that a proton cannot be readily abstracted by K100 from the 2'-hydroxyl group of DHMP.

Four Snapshots along the Catalytic Pathway

The conformation of the SaDHNA monomer does not change significantly between different liganded states as indicated by the Cα-trace alignment between the monomers (Table 1). The previously reported SaDHNA•HP (PDB entry 2DHN, Figure 4(c)) and apo-SaDHNA (PDB entry 1DHN, Figure 4(d)) structures are the enzyme-aldolase product complex and the ligand-free enzyme, respectively. The SaDHNA•NP structure may represent the enzyme-substrate

complex (Figure 4(a)) while the SaDHNA•MP structure may represent the enzyme-epimerase product complex (Figure 4(b)). Therefore, two snapshots along the reaction pathway are available and two more can be derived for the elucidation of the catalytic mechanism of SaDHNA.

In addition to the catalytically important K100, another two conserved residues, E22 and Y54' (where the prime indicates the residue is from the partner subunit), have been identified and demonstrated to be catalytically important.^{11,12} The carboxyl group of E22 is hydrogen bonded to the ϵ -amino group of K100 and the 1'-hydroxyl group in all three complexes, whereas Y54' and K100 interact with the ligand differently (Figure 4). The ϵ -amino group of K100 and the hydroxyl group of Y54' are hydrogen bonded to the 2'-hydroxyl of NP (Figure 4(a)), but to the 3'-hydroxyl of MP (Figure 4(b)). In addition, the hydroxyl group of Y54' is hydrogen bonded to all three ligands, but K100 does not interact with HP (Figure 4(c)). Also note that in the NP complex, Y54' is not hydrogen bonded to K100 (Figure 4(a)), whereas in the HP complex, a strong hydrogen bond is formed between the two side chains (Figure 4(c)). These features of the catalytic center arrangement provide the structural basis for the mechanism of SaDHNA action.

Proposed Mechanism for SaDHNA-catalyzed Reactions

The mechanism of DHNA-catalyzed reactions is general acid and base catalysis in nature, and the protonation of N5 by a general acid and the deprotonation of 2'-hydroxyl by a general base lead to the formation of the reaction intermediate.^{3,6,11} Our SaDHNA•NP structure shows that the catalytic water molecule hydrogen bonded to N5 is in position for the role of the general acid, while K100 is in position for the role of the general base (Figure 4(a)). Assuming K100 is unprotonated as suggested by the observed pH optimum ~9.5 for DHNA-catalyzed reactions,^{4,6} the catalytic center assembly of the SaDHNA•DHNP complex can be derived and schematically illustrated (Figure 5), showing a proton-conducting wire connecting the N5 and the 2'-hydroxyl of DHNP via the ϵ -amino group of K100 and the catalytic water molecule. Thus, the proton of the 2'-hydroxyl group is readily abstracted by K100 and transferred along the wire to N5, resulting in the cleavage of the C1'-C2' bond and the formation of the reaction intermediate. Similarly, the catalytic center assembly of the SaDHNA•DHMP complex can be derived from the SaDHNA•MP structure (Figure 5). However, for the proton transfer from 2'-hydroxyl to N5 to occur, the breakage of two hydrogen bonds (from the 3'-hydroxyl group to Y54' and to K100, respectively) followed by a ~180° rotation (around the C1'-C2' bond) is required, assuming that the crystal structure represents a more stable conformation of the complex and that the 2'-hydroxyl of MP has to be aligned with the ϵ -amino group of K100 for catalysis. This additional step is, in fact, reflected in the kinetic properties of the enzyme. The k_{cat}/K_m ($\text{s}^{-1}\text{M}^{-1}$) value for SaDHNA is 1.8 toward DHMP but 9.7 toward DHNP,¹¹ suggesting a higher energy barrier for SaDHNA-catalyzed reactions of DHMP. From the intermediate state, the reaction can proceed in three directions: a simple reverse of the proton transfer leads to the re-formation of DHNP; a similar reverse of the proton transfer with a "twist" that results in the chirality change of C2' in the trihydroxypropyl moiety of the product leads to the formation of DHMP; and the deprotonation of N5 and the protonation of C1' (DHNP notation) of the reaction intermediate via an extended proton-conducting wire that involves Y54' leads to the formation of HP (Figure 5). The critical role of Y54 in the formation of HP from the reaction intermediate is based on our recent site-directed mutagenesis and biochemical studies.^{11,12}

The structural data indicate that during catalysis three catalytically important residues (E22, K100, and Y54')^{6,11,12} function coordinately: together, they organize the catalytic center assembly, and individually, each plays a central role at different catalytic stages. E22 is

constantly hydrogen bonded to both K100 and the 1'-hydroxyl group of the ligand (DHNP, DHMP, or HP), and thereby plays a critical role in the organization of the catalytic center assembly throughout the catalytic cycle. However, it is not part of the proton-conducting wires and may not be directly involved in proton transfer. K100 is a critical component of two proton-conducting wires (Figure 5). The first wire (composed of K100, the bound water molecule, and the substrate) plays a critical role in the generation of the reaction intermediate. The second wire (composed of Y54', K100, the bound water molecule, and the reaction intermediate) plays a critical role in the conversion of the reaction intermediate to HP. The role of Y54' is two-fold. It helps orient the 2'-hydroxyl of the substrate for the proton abstraction by K100 to generate the reaction intermediate, and consequently, becomes part of the second proton-conducting wire and plays a critical role for the protonation of the enol intermediate to generate HP (Figure 5).

Materials and Methods

Cloning, Overproduction, and Purification

The SaDHNA gene was cloned into the expression vector pET17b by standard PCR-based methods and over-expressed in the *Escherichia coli* strain BL21(DE3). The correct coding sequence was confirmed by DNA sequencing. The production of SaDHNA was induced when the OD600 of the culture reached 0.8-1.0 by the addition of IPTG to a final concentration of 0.5 mM. The culture was further incubated for 4 h and harvested by centrifugation. The *E. coli* cells were re-suspended in 20 mM Tris-HCl, pH 8.0 (buffer A) and lysed with a French press. The lysate was centrifuged for 20 min at ~27,000 g. The supernatant was loaded onto a DEAE-cellulose column equilibrated with buffer A. The column was washed with buffer A until OD280 of the effluent was <0.05 and eluted with a 0-500 mM linear NaCl gradient in buffer A. Fractions containing DHNA were identified by OD280 and SDS-PAGE and concentrated to ~15 mL with an Amicon concentrator using a YM30 membrane. The concentrated protein solution was centrifuged, and the supernatant was applied to a Bio-Gel A-0.5m gel column equilibrated with buffer A containing 150 mM NaCl. The column was developed with the same buffer. Fractions from the gel filtration column were monitored by OD280 and SDS-PAGE. Pure DHNA fractions were pooled and concentrated to 10–20 mL. The concentrated DHNA was dialyzed against 5 mM TrisHCl, pH 8.0, lyophilized, and stored at –80 °C.

Complex Formation, Crystallization and Data Collection

MP and NP were purchased from the Schircks Laboratories. The crystals of both complexes, SaDHNA•MP and SaDHNA•NP, were obtained via co-crystallization using the hanging-drop technique at 19±1 °C. The protein solution was mixed and incubated with the ligand prior to crystallization experiments. The drops contained equal volumes of protein and reservoir solutions. For SaDHNA•MP, the protein solution contained 10 mg/mL protein and 25 mM MP in 10 mM Tris-HCl (pH 8.0). The well solution contained 1.4 M sodium acetate and 0.2 M imidazole in 0.1 M sodium cacodylate (pH 6.5). Microcrystals (tetragonal bipyramids) appeared within an hour, and reached the size of 0.20 × 0.20 × 0.35 mm after one week. For SaDHNA•NP, the protein solution contained 10 mg/mL protein and 50 mM NP in 10mM Tris-HCl (pH 8.0). The well solution contained 0.8 M sodium-potassium tartrate in 0.1 M Na-Hepes (pH 7.5). Crystals (bipyramidal tetragonal blocks) appeared in a week, and grew to the size of 0.15 × 0.15 × 0.20 mm after a few months.

X-ray diffraction data were collected at 100 K with an ADSC Quantum-4 CCD detector mounted on the synchrotron beamline X9B at National Synchrotron Light Source, Brookhaven National Laboratory. The SaDHNA•MP crystal was tetragonal (I422) and diffracted to 1.68-

Å resolution. The crystal of SaDHNA●NP crystal was tetragonal (P4₂), twinned, and diffracted to 1.70-Å resolution. Data processing was carried out with DENZO and SCALEPACK.¹⁴ Data collection and processing details are summarized in Table 2.

Structure Solution and Refinement

The structure of the SaDHNA●MP complex was determined by difference Fourier synthesis using the apo-DHNA structure (PDB entry 1DHN) as the starting model after the solvent molecules were removed. The SaDHNA●NP structure was solved by molecular replacement using PHASER.^{15–17} Four structures, including apo-SaDHNA (PDB entry 1DHN), SaDHNA●HP (PDB entry 2DHN), SaDHNA●NP (PDB entry 1U68), and SaDHNA●MP, with solvent and ligand molecules removed, were used to derive the search model ensemble. The MR solution, containing four DHNA molecules, was subjected to rigid body refinement, energy minimization, and grouped B-factor refinement followed by a difference Fourier synthesis, which revealed the location of the NP molecules.

Both structures were refined using CNS¹⁸ and SHELXL.¹⁹ The crystal of SaDHNA●NP was twinned, following the matrix (1 0 0, 0 –1 0, 0 0 –1). The twinning fraction was first estimated to be 0.485 and refined to and kept at 0.5. Model building was carried out using the graphics package O.²⁰ Three side chains (R11, K30, and K68) were disordered in the SaDHNA●MP structure and were refined as two conformations with equal probabilities. Illustrations were prepared with program packages MOLSCRIPT,²¹ BOBSCRIPT,²² and PyMOL.²³ The asymmetric unit of SaDHNA●MP contains a complete DHNA polypeptide chain (residues 1 to 121), one MP molecule, one acetate ion, and 174 water molecules. The asymmetric unit of SaDHNA●NP contains four DHNA molecules, four NP molecules, and 642 water molecules. The statistics for both structures are shown in Table 2.

Atomic coordinates and structure factors have been deposited with the Protein Data Bank under accession codes 2NM2 for SaDHNA●NP and 2NM3 for SaDHNA●MP.

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Abbreviations used

DHNA	dihydroneopterin aldolase
SaDHNA	<i>Staphylococcus aureus</i> DHNA
DHNP	7,8-dihydroneopterin

DHMP	7,8-dihydromonapterin
NP	neopterin
MP	monapterin
HP	6-hydroxymethyl-7,8-dihydropterin
GA	glycoaldehyde
RMSD	root-mean-square deviation

Figure 1.

(a) The aldolase and epimerase reactions catalyzed by DHNA. (b) Chemical structures of NP and MP. (c) Two views (side, on the left; top, on the right) of the SaDHNA•HP octamer (PDB entry 2DHN). The hollow cylinder has the crystallographic 422 symmetry and a height of 70 Å, an outer diameter of 65 Å, and an inner diameter of 13 Å. The N- and C-termini are located on the top and bottom of the octamer, the “head-to-head” assembly of two tetrameric rings. The helices are illustrated as cylinders, strands as arrows, and loops as tubes. The subunits are distinguished with colors and outlined with transparent molecular surfaces. The HP molecules are shown as stick models in atomic color scheme (Carbon in black, nitrogen in blue, and oxygen in red).

Figure 2.

Stereoviews showing the catalytic center of SaDHNA•NP. (a) Representative $2F_o - F_c$ electron density (green net) contoured at 1.2σ for the NP molecule. The electron density map is shown as green nets. (b) The catalytic center assembly of SaDHNA•NP. Amino acid residues and the ligand are illustrated as ball-and-stick models with atomic color scheme (carbon in black, nitrogen in blue, and oxygen in red). The primary and symmetry-related DHNA molecules are shown in cyan and orange, respectively. NP is highlighted with thicker bonds and bigger atoms. The electrostatic interactions between the protein and the ligand are indicated with dashed lines.

Figure 3.

Stereoviews showing the catalytic center of SaDHNA•MP. (a) The $2F_o - F_c$ map (green net) contoured at 1.2σ for the bound MP molecule. (b) The catalytic center assembly of SaDHNA•MP. Amino acid residues and the ligand are illustrated as ball-and-stick models in atomic colors (carbon in black, nitrogen in blue, and oxygen in red). The primary and symmetry-related DHNA molecules are shown in cyan and orange, respectively. MP is highlighted with thicker bonds and bigger atoms. The electrostatic interactions between the protein and the MP molecule are indicated with dashed lines.

Figure 4.

Catalytic center assemblies in (a) SaDHNA•NP, (b) SaDHNA•MP, (c) SaDHNA•HP (PDB entry 2DHN), and (d) apo-SaDHNA (PDB entry 1DHN). Ligands and amino acid residues are illustrated as stick models in atomic colors (carbon in gray, nitrogen in blue, and oxygen in red), and the catalytic water molecule as a red sphere. Key hydrogen bonds are indicated with dashed lines. To indicate the ligand-binding site, the SaDHNA•HP structure is also shown in panel (d) as a line model in cyan.

Figure 5.

Proposed mechanism for SaDHNA-catalyzed aldolase and epimerase reactions. The protein-DHNP and protein-DHMP complexes are derived from the SaDHNA•NP and SaDHNA•MP structures (Figure 4, panels (a) and (b)), respectively, while the protein-HP complex is the SaDHNA•HP structure (Figure 4(c)). Dashed lines represent hydrogen bonds and curved arrows indicate the direction of electron transfer. Brackets indicate intermediate states.

Table 1

Root-mean-square Deviation (RMSD) Values for Superimposed SaDHNA Monomers on the Basis of C α Positions.

	HP	MP	NP-A	NP-B	NP-C	NP-D
apo ^a	0.20	0.26	0.60	0.51	0.53	0.58
HP ^b		0.26	0.59	0.50	0.51	0.57
MP ^c			0.59	0.53	0.54	0.61
NP-A ^d				0.66	0.72	0.72
NP-B					0.65	0.71
NP-C						0.69

^a apo-SaDHNA (PDB entry 1DHN);

^b SaDHNA●HP (PDB entry 2DHN);

^c SaDHNA●MP; and

^d SaDHNA●NP molecules A, B, C, and D.

Table 2
X-ray Data and Refinement Statistics for SaDHNA●NP and SaDHNA●MP.

	SaDHNA●NP		SaDHNA●MP	
Symmetry	P4 ₂		I422	
Space group	a = 60.0, b = 123.1 Å		a = 61.2, b = 124.5 Å	
Unit cell parameters	$\alpha = \beta = \gamma = 90^\circ$		$\alpha = \beta = \gamma = 90^\circ$	
Data statistics	Overall	Last shell	Overall	Last shell
Resolution range (Å)	30.0–1.70	1.76–1.70	30.0–1.68	1.74–1.68
Completeness (%)	92.1	88.3	98.0	88.7
Redundancy	3.8		10.5	
$I/\sigma(I)$	18.5	2.0	31.3	2.1
R_{scaling}^a	0.053	0.359	0.058	0.388
Crystal solvent content (%)	30.4		31.0	
Refinement statistics				
Reflections used for refinement	40008		12928	
Reflections used for R_{free}	2047		720	
Crystallographic R^b	0.220		0.227	
R_{free}	0.262		0.262	
Number of atoms / average B factors (Å ²)				
Proteins	3868 / 27.2		980 / 26.9	
Ligands	72 / 28.3		22 / 36.8	
Water molecules (oxygen atoms)	642 / 31.5		174 / 47.1	
RMSD from ideal geometry:				
Bond distances (Å)	0.007		0.007	
Angle distances (Å)	0.024		0.023	
Ramachandran plot:				
Most favored ϕ/ψ angles (%)	82.1		98.1	
Additional allowed ϕ/ψ angles (%)	17.2		1.9	
Generously allowed ϕ/ψ angles (%)	0.7		0.0	
Disallowed ϕ/ψ angles (%)	0.0		0.0	
Estimated coordinate error (Å)	0.250		0.245	

^a $R_{\text{scaling}} = \Sigma |I - \langle I \rangle| / \Sigma I$.

^b Crystallographic $R = \Sigma_{\text{hkl}} ||F_{\text{O}}| - |F_{\text{C}}|| / \Sigma_{\text{hkl}} |F_{\text{O}}|$.