Structure of *P. denitrificans* Amicyanin Mutant P94F

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Introduction

Amicyanin is an obligatory intermediate in the transfer of electrons from methylamine dehydrogenase (MADH) to any one of several *c*-type cytochromes in *Paracoccus denitrificans* [1]. The wild-type amicyanin has a redox midpoint potential of 300 mV. In complex, this midpoint potential decreases slightly [2] so that electrons may move through amicyanin to the *c*-type cytochrome. However, the single site mutant of amicyanin where Pro94 was changed to Phe has a midpoint potential of approximately 400 mV [3]. Such a midpoint potential increase would make it very difficult for the *c*-type cytochrome to oxidize amicyanin.

It has also been observed that in amicyanin and similar blue copper proteins such as plastocyanin, upon reduction of Cu(II) to Cu(I), a histidine side chain coordination bond between nitrogen and copper is broken [2, 4]. A determination of the crystal structure of the P94F mutant of amicyanin in both the oxidized and reduced states could possibly explain why the midpoint potential increases significantly as a result of this mutation. Here we report on the structure of the oxidized P94F mutant of amicyanin determined from data collected at BIOCARS (Bio Consortium for Advanced Radiation Sources).

Materials and Methods

The P94F mutant of amicyanin was crystallized by the sitting drop method from 100 mM sodium citrate (pH 5.5) and 2.25 M ammonium sulfate. The P94F mutant of amicyanin crystallizes in the orthorhombic space group $P2_12_12_1$ with cell parameters of a = 51.03 Å, b = 59.50 Å, c = 74.35 Å, and $\alpha = \beta = \gamma = 90°$. A crystal was frozen in a nitrogen stream from Oxford Cryosystems at a temperature of 100K by using paraffin oil as a cryoprotectant. Data were recorded at BIOCARS beamline 14-BM-C by using an ADSC-Q4 CCD detector and processed by using DENZO and SCALEPACK [5].

The P94F mutant of amicyanin was solved by molecular replacement by using the Molrep package in CCP4 [6]. There are two molecules in the asymmetric unit. Initial stages of refinement were performed by CNS [7] against data to 2.0-Å resolution. The model was then refined by using SHELX [8] against data to 1.0-Å resolution, alternating cycles of least-squares refinement with model building in XtalView [9]. Refinement statistics are included in Table 1.

Results

We have obtained a model of a mutant of amicyanin refined against data to high resolution. Studies of amicyanin indicate that there are several loops that have variable positions, implying flexibility [10]. In comparing the two molecules of P94F amicyanin in the asymmetric unit, we see that there are flexible loop regions (Fig. 1), with a root mean square deviation of C-α positions over the entire 105-residue polypeptide chain of 0.53 Å. The regions of apparent flexibility appear to be as follows: the nine N-terminal residues, the loop between Ile15 and Val22, the loop between Arg48 and His53, and the loop between Leu62 and Leu67. The differences in this last loop appear to be a result of a crystal lattice interaction between the main chain near Glu64 on Molecule 1 and the main chain near His36 on Molecule 2. Glu64 of Molecule 2 does not appear to be involved in any crystal lattice interactions. Another effect of the P94F mutation is that the side chain of Phe94 pushes the side chain of Met71 away from its usual position observed in wild-type amicyanin.

The crystal packing of the P94F mutant of amicyanin has a significantly different arrangement than that found in wild-type amicyanin [11]. The contact between asymmetric units appears to be controlled by an intermolecular van der Waals contact between the aromatic rings of phenylalanine at position 94 (Fig. 2). The closest approach of one molecule to the other at the side chain of Phe94 is 3.60 Å. Also, the side chain of

Table 1. Refinement statistics for P94F amicyanin.

Parameter	Value
Data range	27.6–1.0 Å
No. of reflections (working)	89,387
No. of reflections (free)	7,833
R_{work}	0.116
R_{free}	0.148
No. of non-H atoms	
Protein	1,639
Water	486
Sulfate	25
H atom model	Riding
B factors	Anisotropic

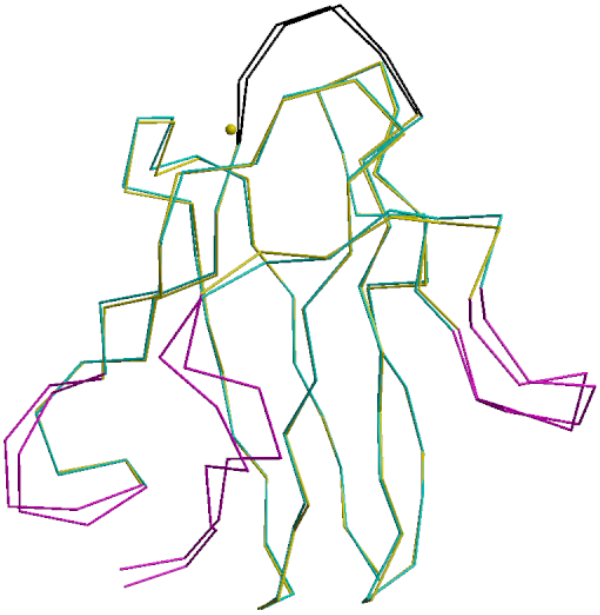

FIG. 1. Molecule 2 (cyan) of P94F amicyanin superposed on Molecule 1 (yellow). The N-terminal region, the loop between Ala15 and Val22 (right), and the loop between Leu62 and Leu67 (left) are in magenta, and the loop between Arg48 and His53 (top) is in black. The figure was rendered by using XtalView [9] and Raster3D [13].

Met71 contacts the side chain of Met71 in the other molecule, with the closest approach being 3.95 Å. As a result of the contact between these hydrophobic side chains, the outer nitrogen atom on the side chain of the copper ligand His95 of Molecule 1 forms a hydrogen bond with the carbonyl group of Phe94 on Molecule 2, and vice versa.

Discussion

In the wild-type amicyanin, the side chain of His95 contacts only solvent and is free to move upon reduction of Cu(II) to Cu(I) [10, 11]. It is likely that if a crystal of P94F amicyanin is reduced by an appropriate reducing agent, His95 is not free to move and the coordination bond cannot be broken. In other words, the crystal lattice interactions would prevent us from observing the conformational effects of reduction of P94F amicyanin, since the oxidized and reduced structures would be identical. From the 1.0-Å structure of the P94F mutant of amicyanin, we conclude that the crystal lattice interactions that are a direct consequence of the mutation of Pro94 to Phe preclude a useful study of the interesting redox properties of this particular mutant. Other mutations of Pro94 have been made that have the same redox properties [12] but are not likely to cause the same crystal

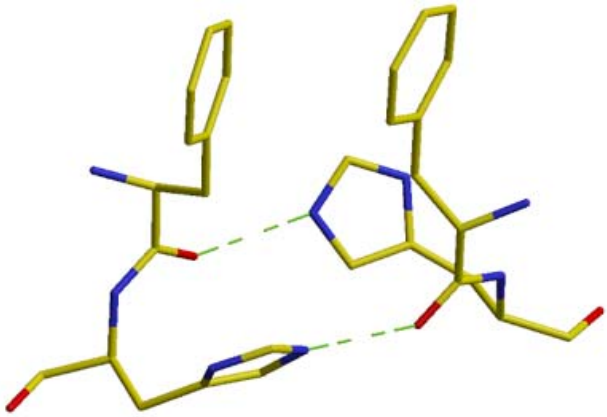

FIG. 2. Intermolecular contact at the crystal contact between Phe94 and His95. Hydrogen bonds are formed between the Nε atom of His95 and the carbonyl O atom of Phe94, and the closest approach between side chain atoms in Phe94 is 3.60 Å. It is clear that the hydrogen-bonding of the His95 side chain to the backbone of the other molecule should prevent the ring-flipping of His95 upon reduction of Cu(II) to Cu(I) within the crystal. The figure was rendered by using XtalView [9] and Raster3D [13].

lattice interactions caused by the presence of phenylalanine at residue 94.

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