Crystal Structure Determinations of Oxidized and Reduced Plastocyanin from the Cyanobacterium *Synechococcus* sp. PCC 7942[†,‡]

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ABSTRACT: The crystal structures of oxidized and reduced plastocyanins from *Synechococcus* sp. PCC 7942 have been determined at 1.9 and 1.8 Å resolution, respectively, at pH 5.0. The protein consists of only 91 amino acid residues, the smallest number known for a plastocyanin, and apparently lacks the mostly conserved acidic patch that is believed to be important for recognition with electron-transfer partners. The protein has two acidic residues, Glu42 and Glu85, around Tyr83, which is thought to be a possible conduit for electrons, but these are neutralized by Arg88 and Lys58. Residue Arg88 interacts with Tyr83 through a $\pi-\pi$ interaction in which the guanidinium group of the former completely overlaps the aromatic ring of the tyrosine. Reduction of the protein at pH 5.0 causes a lengthening of one Cu−N(His) bond by 0.36 Å, despite the small rms deviation of 0.08 Å calculated for the backbone atoms. Moreover, significant conformational changes of Arg88 and Lys58, along with the movement of a water molecule adjacent to the OH group of Tyr83, were observed on reduction; the guanidinium group of Arg88 rotates by more than 11°, and the water molecule moves by 0.42 Å. The changes around the copper site and the alterations around Tyr83 may be linked to the reduction of the copper.

Plastocyanin is a small (91−105 amino acids) copper-binding protein, which functions as an electron-transfer shuttle from cytochrome *f* of the b_6f complex to $P700^+$ of photosystem I in chloroplasts of algae and higher plants and also in many cyanobacteria (*1*−*3*). Plastocyanin is the sole protein responsible for this task in the photosynthetic electron-transfer system in higher plants. However, algae and cyanobacteria lacking plastocyanin use cytochrome c_6, previously also called cytochrome c_{552} or c_{553}, to transport electrons from cytochrome *f* to $P700^+$ (*4*). Some organisms contain the genetic information for both plastocyanin and cytochrome c_6. In these organisms, the presence of copper in the growth medium determines which gene is utilized (*5*−*9*).

Synechococcus sp. PCC 7942 was thought to be one of the cyanobacteria which lacks plastocyanin and has cytochrome c_6 as its sole electron-transfer agent to reduce $P700^+$. Previous studies on the immunological detection of plastocyanin in *Synechococcus* sp. PCC 7942 reported the absence of the plastocyanin polypeptide in the cell extracts (*7*, *10*−*14*). Recently, the *petE* gene of *Synechococcus* encoding plastocyanin has been cloned (*15*). The plastocyanin gene of *Synechococcus* sp. PCC 7942 is expressed in *Escherichia coli*, and the protein is correctly processed (*16*).

The crystal and solution structures of plastocyanins from a number of higher plants and eukaryotic algae have been determined (*17*−*25*). Recently, two structures of plastocyanins from cyanobacteria were reported (*26*−*27*). One is that of plastocyanin from *Anabaena variabilis* which possesses 105 amino residues, and the other is a triple mutant of the plastocyanin from *Synechocystis* sp. PCC 6803 which has 98 amino residues. Despite the sequence divergence among plastocyanins from cyanobacteria, algae, and higher plants, their three-dimensional structures are remarkably similar. The overall topology of plastocyanin consists of an eight-stranded β-barrel structure made up of two β-sheets that resembles the known structures of other cupredoxins such as azurin, amicyanin, and pseudoazurin (*28*). Plastocyanins from higher plants and green algae generally have a short (one-turn) α-helix from residues 52 to 55 between the two β-sheets (*3*). However, the two cyanobacterial plastocyanins contain a slightly longer (two-turn) α-helix from residues 47 to 54 amino residue (*26*, *27*). In the *Synechocystis* plastocyanin the highly conserved Gly49 residue is deleted, and in *Anabaena* plastocyanin Lys49 replaces Gly49. The lengthening of the α-helix in the cyanobacteria plastocyanins may be the cause of the change at residue 49. The copper atom of plastocyanin is located in the northern end of the protein and is coordinated by two histidines (His37 and His87, the

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[‡]Atomic coordinates have been deposited in the Brookhaven Protein Data Bank with PDB codes 1BXU and 1BXV.

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latter has a solvent-exposed imidazole ring), a methionine (Met92), and a cysteine (Cys84). The geometry of the active site is that of an irregular, or distorted, tetrahedron. The electron-transfer kinetics of plastocyanins have been investigated in detail, and two distinct surface patches for docking with physiological redox partners have been proposed (*2, 29*−*30*). One is the hydrophobic patch around the solvent-exposed His87, and the other is the negatively charged acidic patch around Tyr83 located on the east side of the molecule. The latter consists of two acidic clusters from residues 42 to 44 and 59 to 61 in higher plant plastocyanins. In the algal plastocyanins, residues 58 and 59 are deleted. To compensate, the algal plastocyanins have two negatively charged residues, Asp53 and either Asp85 or Glu85. The highly conserved Tyr83 is located on the east side of the molecule and is ca. 15 Å away from the copper atom. Both patches have been considered to be essential electron-transfer sites on the basis of kinetic studies (*31, 32*). The primary structure of *Synechococcus* sp. PCC7942 plastocyanin shows that it has deletions at positions 43−48, 50−51, and 59−61 relative to the plant proteins (*16*), resulting in a protein with only 91 amino acid residues. The protein is the shortest known to date among the plastocyanins. The acidic amino acid residues around the remote electron-transfer site of Tyr83 are replaced with neutral or positive amino acid residues such as in *Anabaena* and *Synechocystis* plastocyanins (*33, 34*), in which the acidic patch was also reported to be lacking (*26, 27*).

The structure of the copper-binding site of oxidized poplar plastocyanin at pH 6.0 is almost identical to that of the reduced polar plastocyanin at pH 7.8, with the largest deviation being 0.15 Å in the length of the Cu−$N_{\delta1}$(His87) bond (*19*). A remarkable change at the reduced copper center is observed at low pH values (*19*). The $N_{\delta1}$(His87) of the reduced protein becomes protonated with decreasing pH and moves slightly up and away from the copper atom, whereas the copper atom moves down and away from His87 so that it is eventually only trigonally coordinated by His37, Cys84, and Met92. At pH 5.1, the copper atom and its three-liganding atoms are essentially coplanar, the $N_{\delta1}$(His87) is protonated, and the distance between this nitrogen atom and the copper atom has increased to 3.05 Å (*3, 19*). A similar effect has also been observed in crystallographic studies on pseudoazurin and amicyanin (*35, 36*).

In this study the X-ray structural analyses of the cyanobacterial plastocyanin from *Synechococcus* sp. PCC 7942 have been performed in both the oxidized and reduced states at pH 5.0. We describe herein the structure of the smallest plastocyanin, which lacks an acidic patch and exhibits conformational changes around the copper atom and Tyr83 upon reduction different from those observed previously.

MATERIALS AND METHODS

Data Collection and Processing. The crystals of *Synechococcus* plastocyanin were obtained as described previously (*37*). Two forms of crystals were grown depending on the protein concentrations used. Tetragonal crystals were obtained using 1.8−2.0 M ammonium sulfate as precipitant when the protein solution was concentrated to 30−60 mg mL^{-1}. The typical size of the crystals was about 0.5 × 0.5 × 0.3 mm^3. The crystals belong to tetragonal $P4_1$ with unit cell parameters of $a = b = 43.1$ Å and $c = 56.9$ Å. High-resolution X-ray diffraction data were collected with synchrotron radiation at the $BL6A_2$ station of 2.5 GeV energy produced by the storage ring in the Photon Factory, the National Laboratory for High Energy Physics, Tsukuba, Japan. Diffraction patterns were recorded on a Fuji Imaging Plate (IP, 200 × 400 mm, Fuji Photo Film) (*38*) using Sakabe's Weissenberg camera for macromolecules (*39*) with an aperture collimator of 0.1 mm diameter and a cylindrical cassette of 286.5 mm radius filled with helium gas. The intensity data were processed using DENZO and scaled with the program SCALEPACK (*40*). Among 39 058 accepted observations up to 1.9 Å resolution, 8053 independent reflections were obtained, the completeness of which was 99.1% with an R_{merge} value of 7.1%. The reduced crystals of plastocyanin were obtained by soaking the oxidized crystals in the crystallization solution (0.1 M potassium phosphate, pH 5.0) containing 10 mM sodium L(+)-ascorbate. After 10 min the blue crystals became colorless and remained so for more than 10 days when in a glass capillary with the mother solution containing 10 mM sodium L(+)-ascorbate. X-ray diffraction data up to 1.8 Å resolution were obtained on the reduced form of the crystal. Among 43 919 accepted observations, 9373 independent reflections were obtained, the completeness of which was 96.2% with an R_{merge} value of 6.1%.

Structure Analysis and Refinement. The crystal structure of *Synechococcus* plastocyanin was solved by the molecular replacement method. Because the protein consists of only 91 amino acid residues, the smallest number known for a plastocyanin, the search model was based upon the structure of the green algal plastocyanin from *Ulva pertusa*, which possesses 97 amino acid residues (PDB code 1IUZ) (*41*). Nonconserved residues were replaced by alanine, and the loop region corresponding to the deleted residues in *Synechococcus* plastocyanin was removed in the model. Molecular replacement calculations were performed with the program AMoRe (*42*) from the CCP4 program package (*43*), but it was not possible to find the appropriate translation function solution. The translation search was continued using the program X-PLOR (*44*), which gave a clear peak with a correlation coefficient value of 0.387 and an *R*-factor of 46.6% (15−4 Å). After rigid-body refinement, the *R*-factor was reduced to 41.9% (8−3 Å). The model was refined with the programs X-PLOR (*44*) and REFMAC in the CCP4 package (*45*). Five percent of the reflections were set aside for R_{free} calculations (*46*). After one round of the simulated annealing protocol, multiple cycles of model fitting and maximum-likelihood refinements with bulk solvent correction were alternated. Ordered water molecules were included by selecting the peaks on the basis of $F_o - F_c$ difference Fourier maps contoured at 2.5σ and $2F_o - F_c$ maps contoured at 1.2σ. The quality of the final model was assessed from Ramachandran plots, and the analysis of the model geometry was carried out with the program PROCHECK (*47*). The plot indicated that 89.2% of the residues lay in the favorable regions and 10.8% in the allowed regions. The final *R* and R_{free} factors of the oxidized structure for all reflections between 10.0 and 1.9 Å resolutions were 0.151 and 0.187, respectively. The root-mean-square deviations from ideal geometry of the bond lengths and angles were 0.016 Å and 2.5°, respectively. A similar procedure was applied to the refinement of the reduced structure. The final *R* and R_{free}

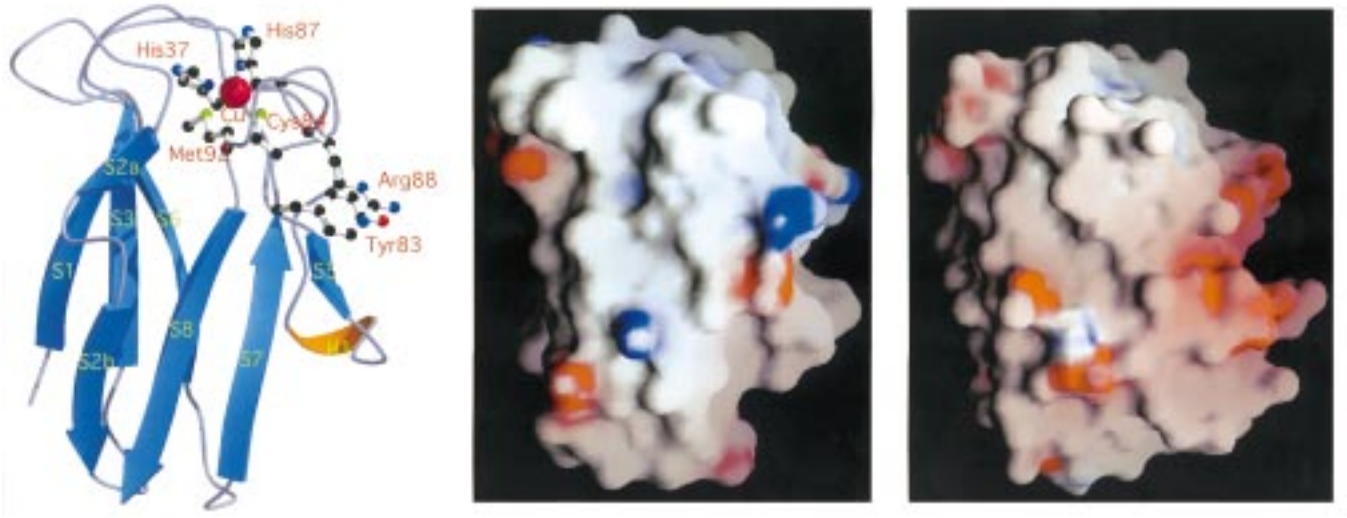

FIGURE 1: Ribbon presentation of the oxidized plastocyanin from *Synechococcus* sp. PCC 7942 viewed from the side of the molecule, drawn using MOLSCRIPT (*51*) and RASTER3D (*52*) (a, left). The copper ion is shown with a sphere in magenta at the top of the model. The four ligands, His37, Cys84, His87, and Met92, are shown in a ball-and-stick representation. The remote electron-transfer gate of Tyr83 and the cationic residue Arg88 are also shown in a ball-and-stick format. Electrostatic potential mapping on the molecular surface of plastocyanin from (b, center) *Synechococcus* and (c, right) poplar viewed from the corresponding direction. The protein molecule is shown by a solid surface, colored according to the calculated electrostatic potential and contoured from −20 (intense red) to +20 kT/e (intense blue). These were drawn using GRASP (*53*). The *Synechococcus* protein has deletions at positions 43−48, 50−51, and 60−61 compared to those of higher plant plastocyanins, and it apparently lacks the acidic patch conserved in eukaryotic plastocyanins.

Table 1: Data Collection and Final Refinement Statistics for the Oxidized and Reduced Plastocyanin from *Synechococcus* sp. PCC 7942

	oxidized form	reduced form
(a) Data Collection		
source	synchrotron	
camera	Weissenberg BL-6A$_2$	
crystal to detector distance	286.5	
method	oscillation	
no. of crystals	1	1
no. of frames	45	37
resolution range (Å)	30.0−1.9	30.0−1.8
reflections, measured/unique	39058/8053	43919/9373
completeness (%), overall/ outer shell	99.1/97.3 (1.97−1.9 Å)	96.2/77.1 (1.86−1.8 Å)
R_{merge}, overall/outer shell	7.1/12.3 (1.97−1.9 Å)	6.1/20.3 (1.86−1.8 Å)
(b) Final Refinement Statistics		
resolution (Å)	10.0−1.9	10.0−1.8
no. of atoms	776	777
no. of copper ions	1	1
no. of water molecules	82	83
no. of reflections		
working set	7192	7575
test set for R_{free}	785	837
R (%)	15.1	15.9
R_{free} (%)	18.7	19.5
av temperature factors (A^2)		
all atoms	17.7	21.2
main chain	13.1	19.0
side chain	21.8	23.5
metal ion	12.2	26.0
solvent atoms	37.4	45.4
rmsd from standard geometries		
bond length (Å)	0.016	0.016
bond angles (deg)	2.5	2.4
dihedral angles (deg)	23.4	28.8
improper angles (deg)	1.7	1.7
Ramachandran plot		
most favored (%)	89.2	87.8
allowed	10.8	12.2

factors for all reflections between 10.0 and 1.8 Å resolutions were 0.159 and 0.195, respectively. The root-mean-square deviations from ideal geometry of the bond lengths and angles were 0.016 Å and 2.4°, respectively. The results of data collection and refinement are summarized in Table 1.

RESULTS

Quality of the Final Model. The final model of oxidized plastocyanin from *Synechococcus* is made up of one monomer per asymmetric unit with 91 amino residues, 82 water molecules, and a single copper ion. The model remained close to the standard geometry throughout the refinement. The final model of the reduced structure includes 83 water molecules. The mean positional errors of the atoms estimated by Luzzati plots are 0.195 Å for the oxidized protein and 0.186 Å for the reduced protein (*48*). For well-defined parts of the structure, especially the β-strands, the internal side chains, and the region around the metal site, the errors are likely to be lower. The quality of the final model is summarized in Table 1. The program PROCHECK (*47*) was used to analyze conformational variations from the standard structures. A Ramachandran plot (*49*) shows that all non-glycine residues have dihedral angles falling in (or near) energetically preferred regions.

Overall Structure. A ribbon drawing of *Synechococcus* plastocyanin is presented in Figure 1a. The approximately spherical plastocyanin molecule has the overall dimensions of 40 Å × 32 Å × 28 Å. The molecule has eight β-strands forming two β-sheets. β-sheet I consists of four β-strands: S1, residues −1 to 5; S2a, residues 14 to 15; S3, residues 26 to 31; and S6, residues 69 to 73. β-sheet II contains four β-strands: S2b, residues 18 to 21; S4, residues 37 to 41; S7, residues 78 to 83; and S8, residues 93 to 98 (Figure 2). The structure of *Synechococcus* plastocyanin has a short 3_{10}-helix from Pro53 to Leu55 instead of the α-helix of higher plant plastocyanins, probably as a consequence of deletions in the amino acid sequence at positions 43−48, 50−51, and 60−61. The amino acid sequence alignment of plastocyanin

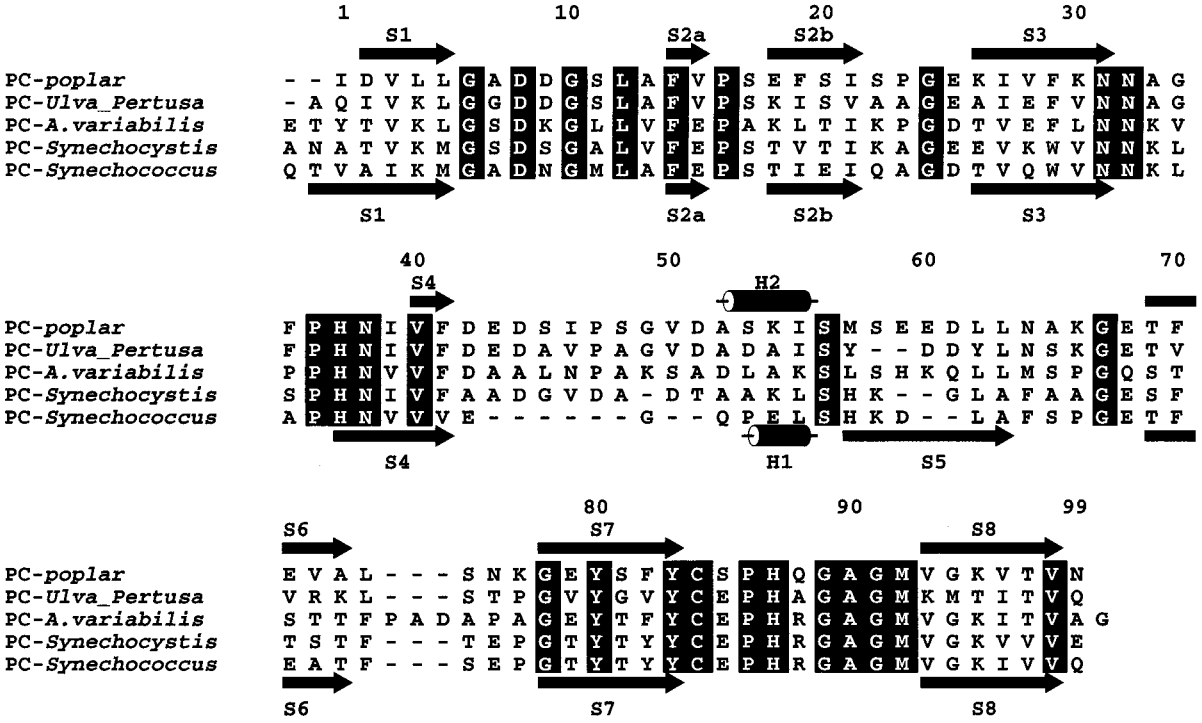

FIGURE 2: Amino acid sequence alignment of plastocyanins from a higher plant (poplar), a green algae (*U. pertusa*), and three cyanobacteria (*A. variabilis*, *Synechocystis*, and *Synechococcus*). Invariant residues in all plastocyanins are shown in black. Secondary structural elements in *Synechococcus* plastocyanin are also indicated. H1 indicates the 3_{10}-helix found in the *Synechococcus* protein from residues 53 to 55, while eukaryotic plastocyanins have a conventional α-helix from residues 52 to 55. The numbering of residues is as for that of poplar plastocyanin.

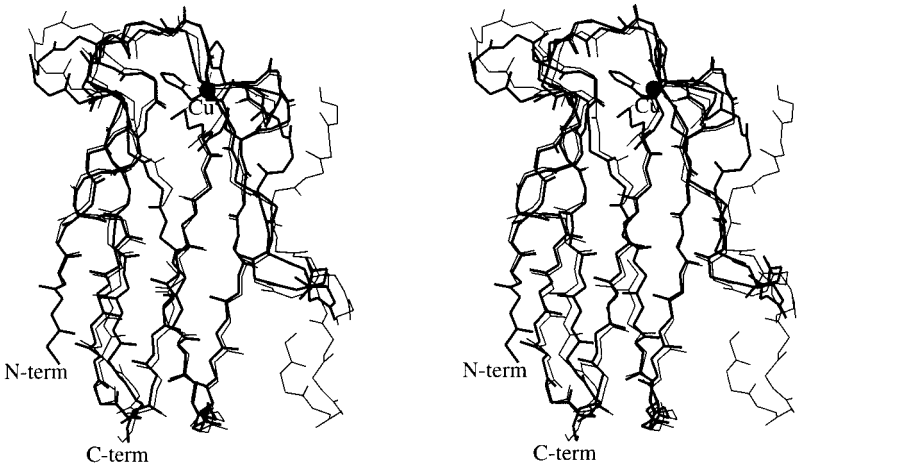

FIGURE 3: Stereoview of the backbone structures of *Synechococcus* plastocyanin (thick lines) superimposed on the higher plant plastocyanin (poplar, thin lines).

from *Synechococcus*, *Synechocystis*, *A. variabilis*, *U. pertusa*, and poplar is shown in Figure 2. The number of identical residues are 55/91 (60%) for *Synechococcus* and *Synechocystis*, 47/91 (52%) for *Synechococcus* and *A. variabilis*, 41/91 (45%) for *Synechococcus* and *U. pertusa*, and 39/91 (43%) for *Synechococcus* and poplar. Despite sequence divergence among plastocyanins of cyanobacteria, algae, and higher plants, the backbone structures are remarkably conserved except for the one-turn helix and β-strand S5 (Figure 3). The averaged rms deviations for the backbone atoms excluding the helix and the loops are 0.37 Å when the oxidized form of *Synechococcus* plastocyanin is compared with that of *Synechocystis*, 1.05 Å when compared with cyanobacterial plastocyanin from *A. variabilis*, 0.53 Å when compared with the green alga *U. pertusa*, and 0.50 Å when compared with the higher plant plastocyanin from poplar. The largest differences are at residues Gly49 (8.9 Å) and Lys59 (6.7 Å) and are caused by the deletions in the *Synechococcus* protein.

Characteristics of the Molecular Surface. In Figure 1b,c electrostatic potential maps of the molecular surfaces of *Synechococcus* and poplar plastocyanins are shown. Two distinct surface patches for electron transfer with physiological redox partners have been proposed for plastocyanin. One is the hydrophobic patch around the solvent-exposed His87, and the other is the negatively charged acidic patch around Tyr83 (*2, 29*−*32*). The hydrophobic patch is located at the north end of the molecule, colored in white (Figure 1c). The negatively charged patch, consisting of Asp and Glu residues at positions 42−44 and 59−61 (*3, 19*) in higher plant

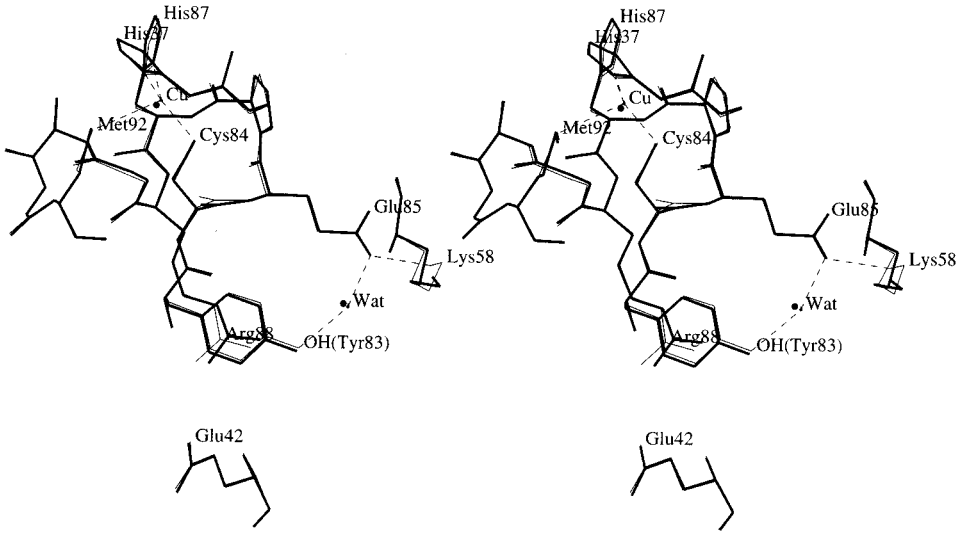

FIGURE 4: Stereoview of the structure around the Cu site and Tyr83 in the oxidized (thin line) and reduced (thick line) plastocyanin from *Synechococcus* sp. A quite small rms deviation of 0.08 Å is calculated for the backbone atoms and 0.38 Å for all atoms. The Cu−His87 distance is significantly lengthened by the reduction of the copper center (+0.36 Å) in contrast to others. The concerted movements around Arg88 and Lys58 at distances of 12.7 and 13.1 Å from the copper ion, respectively, are remarkable. The guanidinium group of Arg88 is completely overlapped by the aromatic bonds of Tyr83 in the oxidized protein, but upon reduction the guanidinium group rotates by more than 11°. The dihedral angle between the O(Wat310)−OH(Tyr83) and C_ζ(Tyr83)−$C_{\epsilon 1}$(Tyr83) bonds changes from −9.67° to 0.45° upon reduction.

plastocyanins, is located at the east end of the molecule (Figure 1c). The plastocyanin from *Synechococcus* has the shortest sequence known (91 residues) and lacks the negatively charged region at positions 43−48, 50−51, and 60−61 (Figure 2). Among the six negatively charged residues that are conserved in the acidic patch of plastocyanin from eukaryotic organisms, only two, Glu42 and Asp59, the latter of which is far from Tyr83, are conserved in the protein from *Synechococcus*. The acidic residue Glu85 is located near Tyr83 but the N_ζ group of Lys58 hydrogen bonds to the $O_{\epsilon 1}$ of Glu85 atom (Figure 4). Therefore, *Synechococcus* plastocyanin has no acidic patch.

Another striking aspect of the structure of the oxidized *Synechococcus* plastocyanin is the observation that the aromatic ring of Tyr83 is involved in a $\pi-\pi$ interaction with the cationic guanidinium group of Arg88. The dihedral angle of the planes of these two groups is 176.5°, and the distance between two planes is 3.6 Å. The guanidinium group is completely overlapped by the aromatic ring of Tyr83, which demonstrates that a $\pi-\pi$ interaction is probably dominant in the oxidized form. A similar interaction between Tyr83 and Arg88 was also found in the structure of the triple mutant of *Synechocystis* plastocyanin, but in that case the aromatic ring was not so completely overlapped by the guanidinium group. Another type of interaction, cation$-\pi$, is thought to be dominant in the plastocyanin from *Synechocystis.* It seems that the loop region from residues 42 to 48 of *Synechocystis* plastocyanin swells toward Tyr83 and results in the movement of the aromatic ring of this residue that reduces the overlap with the guanidinium group. Three acidic residues, Asp42, Asp44, and Glu85, are also neutralized, in part, by the positively charged Lys60 and Arg88 in *Synechocystis* plastocyanin. The electrostatic potential map of the molecular surfaces of all cyanobacteria plastocyanins is neutral around Tyr83. The acidic patch that is believed to be important in recognition of electron-transfer partners has surely been acquired through evolution.

Conformational Changes in the Vicinity of the Copper Site upon Reduction. A remarkable change at the copper center, from tetrahedral to trigonal geometry, was observed in reduced poplar plastocyanin at pH values below 6.0 (*19*) and also in *Alcaligenes faecalis* pseudoazurin and *Paracoccus denitrificans* amicyanin at pH 4.4 (*35*, *36*). However, only small changes between the oxidized and reduced poplar plastocyanins have been observed upon reduction at pH 7.8 (*19*). In this work the reduction of the protein was performed at pH 5.0. The rms deviation of the backbone structures between the two oxidation states of the protein is just 0.08 Å. When all atoms are included in the calculation, the rms deviation between the two structures is 0.39 Å. However, the errors are lower for well-defined parts of the structure, especially the β-strands, the internal side chains, and the region around the metal site. The conformational differences of the copper geometries in the oxidized and reduced forms are shown in Figure 4, and the bond lengths and angles at the copper centers are summarized in Table 2. According to this detailed structural comparison between the oxidized and reduced *Synechococcus* plastocyanins, the Cu−His87 distance is significantly lengthened by the reduction of the copper center (+0.36 Å) in contrast to the small changing in the other Cu−ligand bonds [Cu−His37 (+0.12 Å), Cu−Cys84 (+0.03 Å), and Cu−Met86 (−0.13 Å)]. The cuprous center also has a distorted tetrahedral geometry. A water molecule locates to make a hydrogen bond to N_ϵ(His87) in the oxidized form. This water molecule did exist in the reduced form. The quite large increase in Cu−His87 distance without a flip of the imidazole ring at pH 5.0 is a different case with the changes observed in the higher plant plastocyanins.

Other Redox-Linked Conformational Changes. Significant differences are found in the vicinity of Tyr83 between the structures of *Synechococcus* plastocyanins in its two oxidation states (Figure 4). In the oxidized protein water molecule 310 links the OH group of Tyr83 to the $O_{\epsilon 1}$ of Glu85; the

Table 2: Bond Lengths and Bond Angles of the Copper Site

	sPC[a]		poplar				
	ox. pH 5.0	red. pH 5.0	ox. pH 6.0	red. pH 7.0	rPC	ePC	cPC
			Bond Lengths (Å)				
Cu−N(His37)	1.97	2.09	1.91	2.12	2.02	1.89	2.03
Cu−N(His87)	2.01	2.37	2.06	2.39	2.01	2.17	2.07
Cu−S(Cys84)	2.14	2.17	2.07	2.16	2.11	2.12	2.25
Cu−S(Met92)	2.93	2.80	2.82	2.87	2.89	2.92	2.65
			Bond Angles (deg)				
N(His37)−Cu−N(His87)	101.4	93.4	97.2	99.1	99.6	104.4	105.5
N(His37)−Cu−S(Cys84)	131.0	140.3	131.7	136.3	131.3	125.3	129.6
N(His37)−Cu−S(Met92)	86.0	91.9	88.5	87.9	87.7	89.7	85.3
N(His87)−Cu−S(Cys84)	121.4	108.5	121.0	109.8	121.9	120.3	115.3
N(His87)−Cu−S(Met92)	98.8	99.9	100.6	106.0	100.3	102.3	106.1
S(Cys84)−Cu−S(Met92)	107.7	115.4	109.9	113.4	106.8	108.1	109.1

[a] Abbreviations: sPC, *Synechococcus* plastocyanin; rPC, *C. reinhardtii* plastocyanin; ePC, *E. prolifera* plastocyaninp; cPC, *Synechocystis* plastocyanin.

latter is also hydrogen bonded to N_ζ of Lys58. A $\pi-\pi$ interaction is found between Arg88 and Tyr83. The temperature factors of water molecule 310, the OH group of Tyr83, the $O_{\epsilon 1}$ of Glu85, and the N_ζ Lys58, are 28.2, 21.6, 21.8, and 31.1 Å^2, respectively, which are all relatively small. However, a significant conformational change of Lys58 and Arg88 and a movement of water molecule 310 occur upon reduction at pH 5.0. While the guanidinium group of Arg88 completely overlaps the aromatic ring of Tyr83 in the oxidized state, the guanidinium group tilted by more than 11° upon reduction. The $\pi-\pi$ interaction is thought to be weakened in the reduced structure of *Synechococcus* plastocyanin. Water molecule 310 moves 0.42 Å, and the dihedral angle between the O(Wat310)−OH(Tyr83) bond and the C_ζ(Tyr83)−$C_{\epsilon 1}$(Tyr83) bond changes from −9.7° to −0.45°.

DISCUSSION

Synechococcus plastocyanin, with only 91 residues, possesses the smallest number amino acids known for a plastocyanin. The alignment of the primary structures of the plastocyanins (Figure 2) shows that there are large sequence differences around the acidic patch in the cyanobacterial proteins as compared to their algal and higher plant counterparts. The overall structure of *Synechococcus* plastocyanin is that of a β-sandwich very similar to those of the other plastocyanins. The local area of the polypeptide chains on the east side of *Synechococcus* plastocyanin is different from those in eukaryotic plastocyanins. *Synechococcus* plastocyanin has a deletion of a total of 10 residues at positions 43−48, 50−51, and 59−60. All plastocyanins from green algae and higher plants have Gly49 at their one-turn helix. In the cyanobacterial plastocyanin from *Synechocystis* Gly49 is lacking, and the α-helix extends from Asp47 to Lys54, resulting in a complete two-turn helix (*27*). In *A. variabilis* plastocyanin, a lysine residue is found at position 49 and a two-turn helix is formed. We have recently found a complete three-turn helix in a fern plastocyanin, which also possesses an alanine at position 49 and has a three amino acid residue insertion before Ala49 (*50*). *Synechococcus* plastocyanin retains Gly49, but the protein lacks 10 amino residues around Gly49 and has a 3_{10}-helix present instead of one-turn α-helix found in all the eukaryotic plastocyanins. The backbone structures of plastocyanins differ dramatically from each other in the vicinity of the helix due to the replacements, insertions, or deletions of amino acids around position 49.

In higher plant plastocyanins the conserved residues from positions 42 to 44 and from positions 59 to 61 form the acidic patch around Tyr83, which is believed to be important for recognition with electron-transfer partners. However, only two acidic residues are found around Tyr83 in *Synechococcus* plastocyanin, namely, Glu42 and Asp59. The positively charged Arg88 is involved in a $\pi-\pi$ interaction with the aromatic ring of Tyr83. A similar interaction was also found in *Synechocystis* plastocyanin (*27*); however, the guanidinium group completely overlaps the aromatic ring of Tyr83 in *Synechococcus* plastocyanin. The acidic residue Glu85, which is adjacent to the copper ligand Cys84, is situated close to Tyr83 and is conserved in the green algal plastocyanin. In the cyanobacterial plastocyanins Lys58 neutralizes the negative charge of this residue. Indeed, the negative charge present around Tyr83 in *Synechococcus* plastocyanin is neutralized, in part, by the positively charged Lys58 and Arg88.

The bonds of the guanidinium group of Arg88 are almost parallel to the aromatic ring of Tyr83, which suggests that a $\pi-\pi$ interaction is dominant between these two side chains in the oxidized protein. Upon reduction at pH 5.0, a tilt of 11 ° in the guanidine plane occurs and the $\pi-\pi$ interaction is weakened. In the oxidized state, water molecule 310 links the OH group of Tyr83 to the $O_{\epsilon 1}$ of Glu85, which is also hydrogen bonded to Lys58. A small but significant conformational change occurs at Lys58 upon reduction, together with the movement of the water molecule. These small changes around Tyr83 may be linked to the reduction of the copper center.

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