Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Structure of cytochrome c_6 from *Arthrospira maxima*: an assembly of 24 subunits in a nearly symmetric shell

Cheryl A. Kerfeld,[a]* Michael R. Sawaya,[a] David W. Krogmann[b] and Todd O. Yeates[a]

[a]Molecular Biology Institute, University of California, Los Angeles, Box 951570, Los Angeles, California 90095-1570, USA, and [b]Department of Chemistry and Biochemistry, Purdue University, West Lafeyette, Indiana 47907, USA

Correspondence e-mail: kerfeld@mbi.ucla.edu

Received 24 December 2001
Accepted 10 April 2002

PDB References: cytochrome c_6, 1kib, r1kibsf.

Cytochrome c_6 from the cyanobacterium *Arthrospira maxima* is present in isoforms that can be resolved by size-exclusion chromatography. One isoform crystallized in space group $I4_132$ with eight protein molecules in the asymmetric unit and a total of 384 molecules in the unit cell. Within the crystal, the molecules are arranged as clusters of 24 cytochrome c_6 molecules. Each cluster is a hollow shell with approximate octahedral (432) symmetry. Structural and biochemical studies of cytochrome c_6 isolated from other cyanobacteria and algae have led to the suggestion that cytochrome c_6 forms oligomers. The cytochrome c_6 complex described here is the largest assembly of cytochrome c_6 molecules observed thus far.

1. Introduction

Cytochrome c_6 is a small (~90 amino acids) high-potential class I *c*-type cytochrome found in cyanobacteria and algae. In cyanobacteria, cytochrome c_6 functions as an electron carrier in both photosynthesis and respiration, carrying electrons from the cytochrome b_6f complex to photosystem I (Golbeck, 1994; Kallas, 1994) or to cytochrome *c* oxidase (Morand *et al.*, 1994; Kerfeld & Krogmann, 1998). Multiple putative oligomeric states of cytochrome c_6 observed in structural studies (Kerfeld *et al.*, 1995; Schnackenberg *et al.*, 1999) have been suggested to be important in influencing the docking of oxidized or reduced cytochrome c_6 with its multiple redox partners.

The filamentous cyanobacterium *Arthrospira maxima* contains isoforms of cytochrome c_6 separable by gel filtration. One isoform eluted at an estimated molecular weight of only 8.6 kDa, yet formed highly symmetric crystals (space group $I4_132$) with eight molecules in the asymmetric unit. Here, we report the structure of this form of *A. maxima* cytochrome c_6 and show that the protein molecules are arranged in the crystal to form nearly symmetric hollow shells. The observation of this unusual multimeric form of *A. maxima* cytochrome c_6 adds further support to the arguments that cytochrome c_6 may form biologically relevant oligomers.

2. Materials and methods

2.1. Protein preparation and characterization

Purified cytochrome c_6 was separated into five distinct gel-filtration peaks as previously described in Sawaya *et al.* (2001). Native and SDS-gel electrophoresis was carried out using the Pharmacia Phast System. Low molecular weight SDS–PAGE standards (BioRAD, Hercules, CA, USA) were used for molecular-weight estimation. Gels were stained either with

Coomassie blue or for heme-dependent peroxidase activity (Thomas *et al.*, 1976).

2.2. Crystallization, structure determination and refinement

Following a gel-filtration experiment on cytochrome c_6, the fourth peak resolved (form 4) was concentrated to 8 mg ml^{-1} in 5 m*M* Tris pH 7.5, 100 m*M* NaCl and crystallized by vapor diffusion over a reservoir containing 0.1 *M* Tris pH 7.8, 2.4 *M* ammonium sulfate and 5% methylpentanediol (MPD). The crystals diffracted X-rays to 3.5 Å and were indexed in the cubic space group $I4_132$, with unit-cell parameters $a = b = c = 237$ Å. Data were collected on a Rigaku R-AXIS II imaging plate equipped with an RU-200 rotating-anode X-ray generator (Molecular Structure Corporation, The Woodlands, TX, USA). Data were collected at room temperature. Crystal decay limited collection to 55° of data. Attempts to merge data sets between crystals resulted in unacceptably high values of $R_{\rm merge}$. The data set chosen for structure determination was the best compromise between completeness and $R_{\rm merge}$.

The structure was solved by molecular replacement using *EPMR* (Kissinger *et al.*, 1999). The search model was *A. maxima* cytochrome c_6 whose structure had been determined in an orthorhombic crystal form from a different size-exclusion resolved peak (form 3; Sawaya *et al.*, 2001). The structure was refined with the standard protocols of *X-PLOR*/*CNS* (rigid-body refinement, conjugate-gradient minimization, simulated annealing and grouped *B*-factor refinement; Brünger *et al.*, 1998), using all data in the resolution range 20–3.5 Å. *RAVE* (Kleywegt & Jones, 1994) was used for non-crystallographic symmetry averaging among the eight molecules in the asymmetric unit. Atomic models were constructed and visualized using *O* (Jones *et al.*, 1991).

3. Results and discussion

3.1. Protein isoforms

Size-exclusion chromatography at pH 8 of purified *A. maxima* cytochrome c_6 reproducibly resolves the protein into five peaks (Sawaya *et al.*, 2001). The protein in each peak migrates as a single band of ∼9 kDa in SDS–PAGE, with a trace of a 23 kDa form (Fig. 1*a*). Both the 9 and 23 kDa bands stain for heme-dependent peroxidase activity (Fig. 1*b*). The five size-exclusion-resolved forms have the characteristic absorption spectrum of reduced cytochrome c_6 (data not shown). All of the forms migrate as multiple bands in native PAGE, with variable amounts of monomer. Native PAGE analysis of the two most abundant peaks, forms 3 and 4, is shown in Fig. 1(*c*). By comparison to an isoform of *A. maxima* cytochrome c_6 isolated by a different procedure (Krogmann, unpublished work), form 4 cytochrome c_6 appears to migrate as if its dominant oligomeric component were larger than 30 kDa (Fig. 1*c*).

Mass spectrometry revealed that the different size-exclusion-resolved forms contain distinct mixtures of two different isoforms of the protein. The fourth peak resolved in size-exclusion chromatography (form 4) is mainly unmodified protein, 9852 Da (corresponding to the molecular weight based on the results of protein sequencing), with a small amount of a 9868 Da isoform. In contrast, the protein form found in the third peak (form 3) contained only the +16 Da form of the protein. Multiple mass forms of cytochrome c_6 differing by 16 Da attributed to methionine oxidation have also been observed in *Synechococcus elongatus* (Sutter, 1997) and in *Microcystis aeruginosa* (Krogmann & Kerfeld, unpublished results). Because *A. maxima* cytochrome c_6 form 3 and form 4 isoforms are not distinct in amino-acid composition, they appear to be differentially modified post-translationally.

3.2. Structure determination and refinement

Form 3 and form 4 cytochrome c_6 formed remarkably different crystals despite their nearly identical crystallization conditions: the only difference is the substitution of 5% glycerol for MPD in the form 3 mother liquor (Sawaya *et al.*, 2001). The slight difference is critical to crystallization; form 4 will not crystallize in the presence of glycerol, nor will form 3 crystallize in the presence of MPD. Form 3 cytochrome c_6

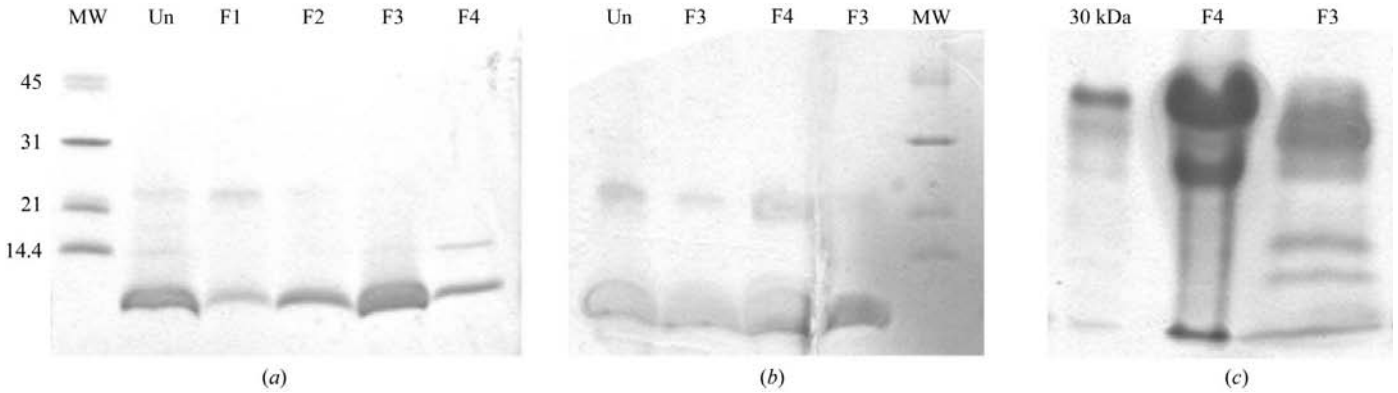

Figure 1
(*a*) Coomassie-stained SDS–PAGE (20%) of purified *A. maxima* cytochrome c_6 before size-exclusion chromatography (Un) and forms 1–4 (F1–4). MW, molecular-weight markers; sizes are given in kDa. (*b*) Heme-stained SDS–PAGE (20%) of unfractionated cytochrome c_6 and forms 3 and 4. Lanes 4 and 5 are Coomassie-stained form 3 and molecular-weight markers as in (*a*), lane 1. (*c*) 20% Native PAGE of *A. maxima* cytochrome c_6 retained by a 30 kDa molecular-weight cutoff Microcon filtration device (30 kDa) and forms 3 and 4.

crystallized in space group $P2_12_12_1$, with three molecules in the asymmetric unit. The structure of the orthorhombic crystal form was readily determined by molecular replacement (Sawaya *et al.*, 2001) using the *Chlamydomonas reinhardtii* cytochrome c_6 (Kerfeld *et al.*, 1995) as the search model. In contrast, despite the very similar crystallization conditions, form 4 cytochrome c_6 crystallized in the rare cubic space group $I4_132$, with eight molecules in the asymmetric unit. The unit-cell volume of the form 4 crystals is almost 50 times the volume of the unit cell of the form 3 cytochrome c_6 orthorhombic crystal.

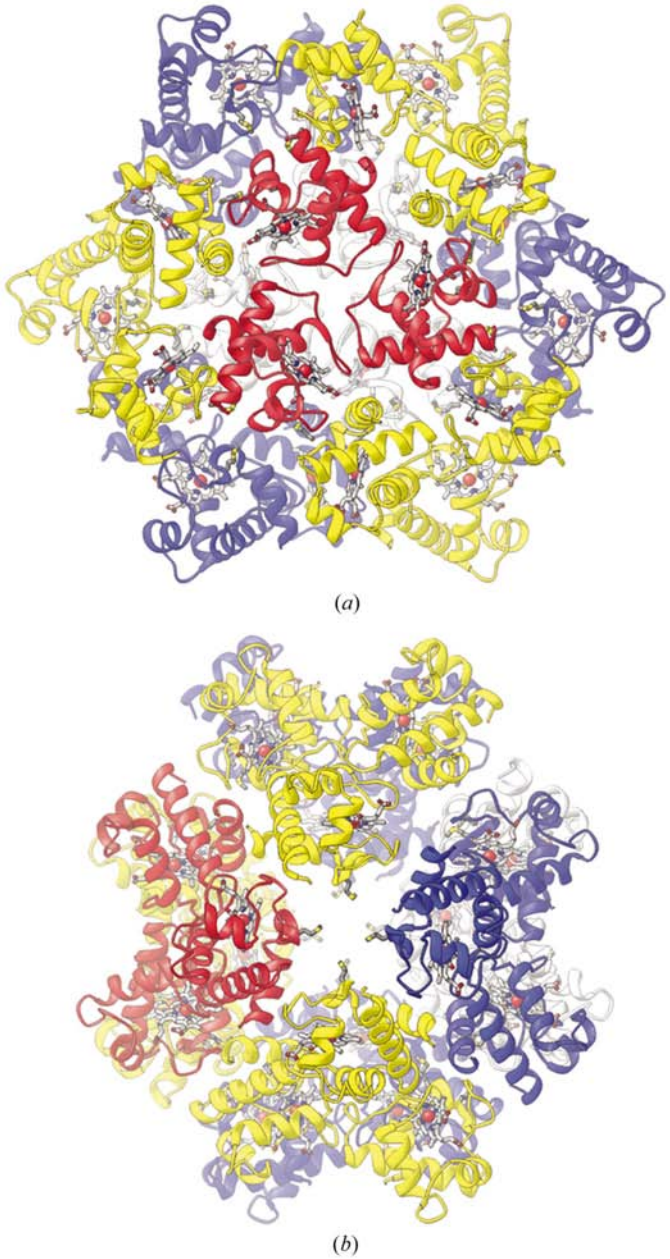

Figure 2
The *A. maxima* cytochrome c_6 24-molecule cluster, viewed down the (*a*) threefold and (*b*) pseudo-fourfold axis. In (*b*) the side chains of Met19 which flank the pore are shown. This figure and Figs. 3 and 5(*a*) were drawn with *RIBBONS* (Carson, 1997).

Table 1
Data-collection and refinement statistics.

Data collection	
Resolution† (Å)	3.5
$\langle I/\sigma(I)\rangle$‡	9
$\langle I/\sigma(I)\rangle$ (highest resolution shell)	3.4
$R_{\rm merge}$§ (%)	15.0
$R_{\rm merge}$ (highest resolution shell) (%)	39.1
Redundancy	7
Overall completeness (%)	91
Completeness in highest resolution shell (%)	95.5
Model refinement	
$R_{\rm work}$¶ (%)	20.2
$R_{\rm free}$†† (%)	22.3
No. of reflections	13268
No. of atoms	5525
Weighted r.m.s.d. from ideality	
Bonds (Å)	0.012
Angles (°)	1.45
Average *B* factor (Å^2)	
Main chain	39.6 (r.m.s.d. 1.21)
Side chain	43.1 (r.m.s.d. 2.35)

† Using all data [no $\langle I/\sigma(I)\rangle$ cutoff] between 20 and 3.5 Å. ‡ $\langle I/\sigma(I)\rangle$ is the average ratio of the observed intensity to the estimated standard deviation for the highest resolution shell. § $R_{\rm merge} = 100(\sum_i |I_i - \langle I\rangle|/\sum_i I_i)$, where the sum is taken over the unique reflections and $\langle I\rangle$ is the mean value of the multiple measurements of the *i*th intensity. ¶ $R_{\rm work} = 100(\sum |F_o - F_c|/\sum |F_o|)$. †† $R_{\rm free}$ was calculated as $R_{\rm work}$ using 5% of the reflections.

The high symmetry of the space group ($I4_132$) and the number of molecules in the asymmetric unit of the form 4 cytochrome c_6 crystals made molecular replacement challenging, despite the availability of an excellent search model, *A. maxima* cytochrome c_6 obtained from the orthorhombic crystal form. Attempts to solve the structure using *AMoRe* (Navaza, 1994), *X-PLOR*/*CNS* (Brünger *et al.*, 1998) and *GLRF* (Tong & Rossmann, 1997) failed. In addition to using a monomer search model, dimer and trimers of cytochrome c_6, based on assemblies observed in previous crystallographic studies of cytochrome c_6 from other species, were also tested as search models without success.

Molecular replacement was eventually successful using the program *EPMR* (Kissinger *et al.*, 1999). Obtaining a correct solution depended on the use of the bump-distance constraint option. The optimal value for the bump distance was 20 Å, slightly smaller than the average diameter of the protein (25 Å). The asymmetric unit was built one molecule at a time using *EPMR* until six molecules were located. The last two molecules were placed manually in a difference Fourier map. Strict NCS constraints were used for three rounds of refinement. Strong NCS restraints were used for two further rounds of refinement. Data-collection and refinement statistics are summarized in Table 1.[1]

3.3. Crystal packing

The most striking feature of the crystal structure of *A. maxima* form 4 cytochrome c_6 is the packing of the molecules within the unit cell. The primitive unit cell contains 192 molecules of cytochrome c_6; the body-centering operation

[1] Supplementary data have been deposited in the IUCr electronic archive (Reference: GR2240). Services for accessing these data are described at the back of the journal.

adds an additional 192 molecules. Within a single unit cell, the molecules are arranged into 16 identical hollow clusters, each composed of 24 molecules (Fig. 2). The diameter of the cluster at various points ranges between 75 and 120 Å.

The 24 protein molecules in a cluster are arranged so that they nearly obey octahedral (432) point symmetry. On average, subunit rotations of less than 4° are required to make the cluster obey perfect octahedral symmetry. Each pseudo-octahedral shell is generated by a crystallographic threefold axis operating on an asymmetric unit consisting of eight independent molecules. Within the cluster, a grouping of the molecules into trimers is evident. There are four crystallographically distinct trimers which are nearly identical to each other (Fig. 3). The asymmetric unit of the crystal contains two intact trimers and one third of the other two trimers, which both sit on the crystallographic threefold axis. Overall, the cluster approximates an assembly of eight nearly identical trimers, each positioned at a vertex of a cube.

The cluster is hollow. The diameter of the internal cavity varies between 50 and 65 Å. There is a pronounced asymmetry to the charge distribution: 16 of the 17 charged side chains are displayed on the exterior (Fig. 4). Arg68 is the only charged residue accessible in the interior of the shell. The Arg68 side chains of three adjacent cytochrome c_6 molecules form part of the trimer interface, resulting in eight patches of positive charge in the otherwise uncharged interior of the shell. Arg68 is highly conserved in the primary structures of cytochrome c_6 molecules (see Table 9 in Kerfeld *et al.*, 1995) and has been implicated in the electron transfer from cytochrome c_6 to PSI (Molina-Heredia *et al.*, 2001).

In an *A. maxima* cytochrome c_6 monomer, two edges of the heme, the heme CB carbon atom and a D propionate oxygen atom, are exposed on the surface. For this reason they are proposed to be important in electron transfer. In the shell assembly, the carboxylate oxygens of the heme propionate D remain exposed to solvent on the exterior (Figs. 2*a* and 4). In contrast, the edge of the C-ring of the heme is exposed on the inside of the cluster (Fig. 4). The edge-to-edge distance between nearest neighbor hemes in the interior of the shell is 17.4 Å, which is presumably close enough to allow the transfer of electrons (Page *et al.*, 1999).

Entrance into the inside of the shell is restricted to six pores of about 13 Å diameter arranged as on the faces of a cube (Fig. 2*b*). Four cytochrome c_6 molecules surround the pore, with the side chains of Met19 and Arg22 projecting into the opening. These two residues are hydrogen bonded to one another through main-chain atoms. Trp89, absolutely conserved in the primary structures of cytochrome c_6 (see Table 9 in Kerfeld *et al.*, 1995), also lines the pore and is exposed to solvent. It has been suggested previously that Met residues (in addition to the Met that functions as the sixth axial ligand) in cytochrome c_6 might function as endogenous antioxidants for Trp89 (Yamada *et al.*, 2000). The proximity of Met19 to Trp89 may allow it to protect Trp89 from oxidation by scavenging reactive oxygen species (Levine *et al.*, 1996).

3.4. Other oligomeric forms of cytochrome c_6 and putative functions

As noted above, the basic building block of the cubic assembly of the *A. maxima* cytochrome c_6 appears to be a

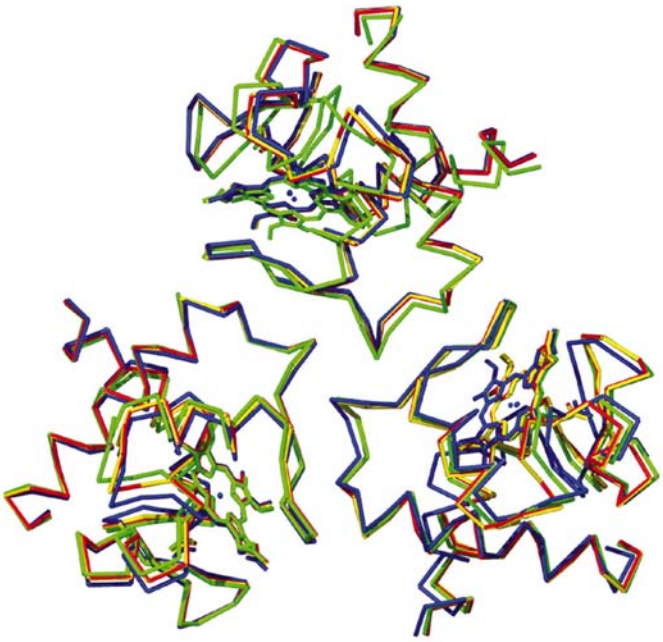

Figure 3
Overlap of the four crystallographically distinct *A. maxima* cytochrome c_6 trimers found in the 24-molecule assembly.

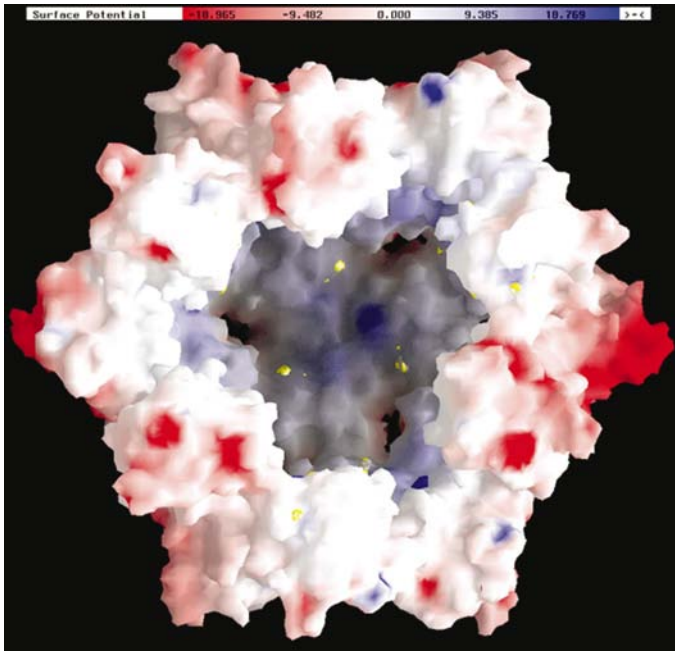

Figure 4
Electrostatic surface potential of the 24-molecule assembly. One of the trimers has been removed to reveal the inner surface of the cluster. Polar atoms are colored in red (negatively charged) and blue (positively charged) and the heme atoms are shown in yellow. This figure and Figs. 5(*b*) and 5(*c*) were drawn with *GRASP* (Nicholls *et al.*, 1991). Dielectric values of 80 and 2 (program default values) were used for the solvent and protein interior, respectively. Screening effects of ionic strength were ignored.

trimer. A trimeric arrangement of molecules was also observed in the structure of cytochrome c_6 from the green alga *Monoraphidium braunii* (Frazao *et al.*, 1995) and in one crystal form of *C. reinhardtii* cytochrome c_6 (Kerfeld *et al.*, 1995; Fig. 5). In each trimer the intermolecular contacts are predominantly hydrophobic. They bury comparable amounts of surface area: 260, 316 and 458 Å^2 per monomer in the *A. maxima*, *C. reinhardtii* and *M. braunii* cytochrome c_6 trimers, respectively. However, the *A. maxima*, *C. reinhardtii* and *M. braunii* cytochrome c_6 trimers are not superimposable. They also differ in their interfacial residues (Fig. 6) and present very different electrostatic profiles (Figs. 5*b* and 5*c*).

Other oligomeric forms of cytochrome c_6 that have been described in various crystal structures include dimeric forms of cytochrome c_6 from *Anacystis nidulans* (Ludwig *et al.*, 1982) and the oxidized cytochrome c_6 from *Scenedesmus obliquus* (Schnackenberg *et al.*, 1999). In addition to the trimeric form described above, *C. reinhardtii* cytochrome c_6 also crystallized

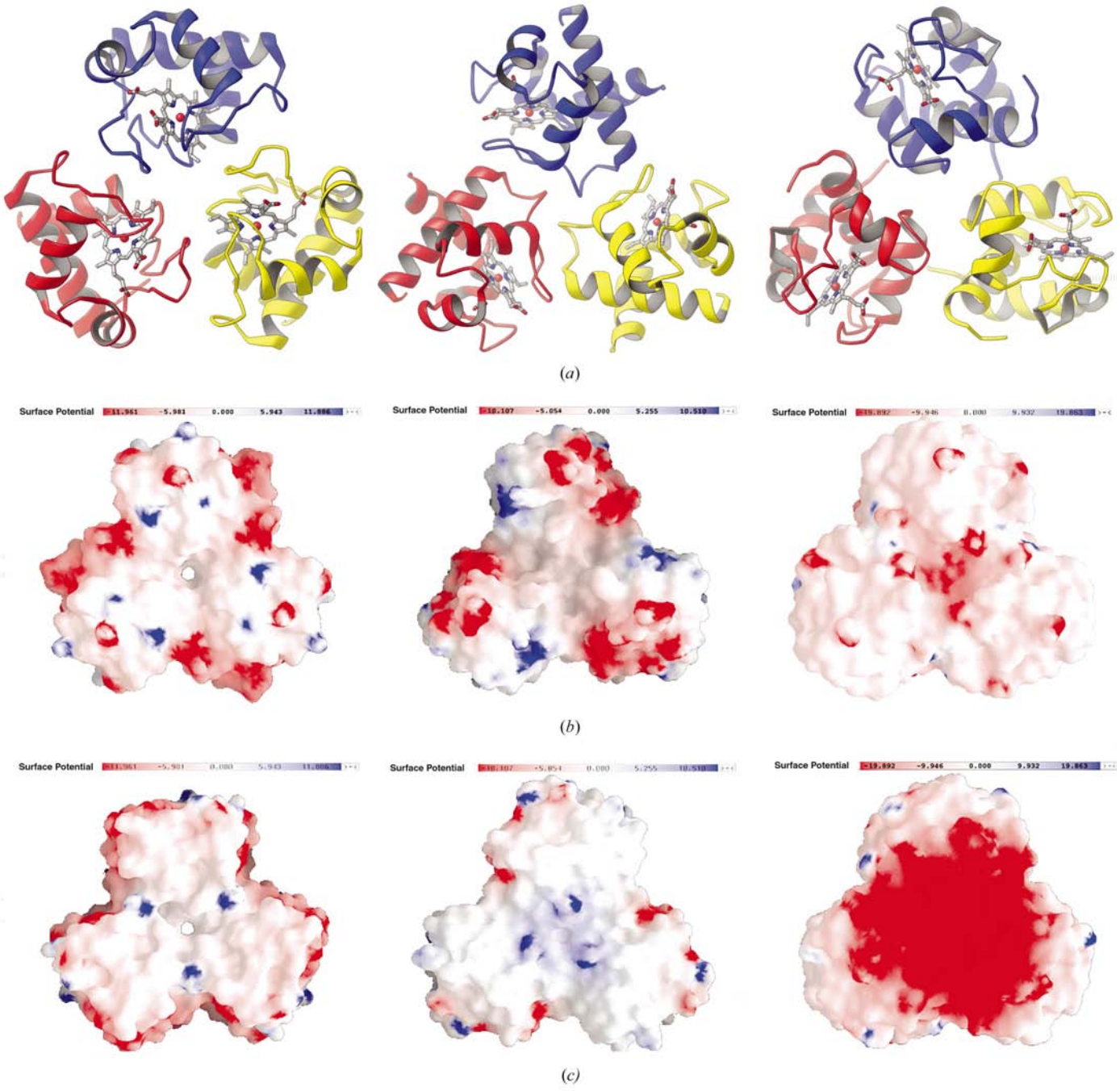

Figure 5
(*a*) Comparison of the *A. maxima* cytochrome c_6 trimer to trimers observed in the crystal structures of *C. reinhardtii* (Kerfeld *et al.*, 1995) and *M. braunii* (Frazao *et al.*, 1995) cytochrome c_6. (*b*) and (*c*) Comparison of the electrostatic surface potential of the trimers. The view shown in (*b*) is the same as in (*a*); the view shown in (*c*) shows the opposite face.

as a dimer; the different crystal forms were attributed to different levels of methionine oxidation of the protein (Kerfeld *et al.*, 1995). As noted above, *A. maxima* form 3 cytochrome c_6 is post-translationally modified. If the additional 16 Da present in the form 3 form of the protein corresponds to the oxidation of Met19 (the only Met residue in addition to the sixth axial ligand), this may explain the formation of the different crystal forms. In the 24-molecule cluster, the Met19 side chains from four cytochrome c_6 molecules meet at the pore (Fig. 3). The S atoms of adjacent Met19 residues are 7.7–8.2 Å apart. Intermolecular steric effects resulting from Met19 oxidation in form 3 could prevent formation of the cluster, consistent with this form of the protein adopting an alternative crystal packing (Sawaya *et al.*, 2001).

The large complex of 24 cytochrome c_6 molecules found here appears to represent yet another of the varied oligomeric arrangements favored by cytochrome c_6. The relative amount of surface area buried upon oligomerization is one criterion for evaluating the relevance of oligomeric forms in crystal structures (Janin & Rodier, 1995). The range of surface area buried per monomer owing to its assembly into a cluster varies between 870 and 1200 Å^2. This corresponds to 23.0–31.7% of the cytochrome c_6 monomer surface.

Cytochrome c_6 isolated from *S. obliquus* (Schnackenberg *et al.*, 1999), *Chlorella fusca* (Inda *et al.*, 1997) and *Anabena* PCC 7119 (Medina *et al.*, 1997) have been characterized biochemically as oligomers. However, evidence for oligomerization of *A. maxima* form 4 cytochrome c_6 in solution is inconclusive. The 23 kDa form visible in SDS–PAGE may correspond to a trimer (Fig. 1*b*). A form of *A. maxima* cytochrome c_6 that is retained by a 30 kDa ultrafiltration membrane can also be isolated (Krogmann, unpublished work; Fig. 1*c*). Ultracentrifugation experiments carried out under a variety of pH and ionic strength conditions provided no conclusive evidence of oligomerization of form 4 cytochrome c_6. In these experiments the protein concentration also varied, but with an upper limit of 2 mg ml^{-1}. Native gel electrophoresis experiments are carried out at protein concentrations similar to that of crystallization experiments and these results suggest that there are multiple forms of the protein in solution, some larger than 30 kDa (Fig. 1*c*). Thus, it may be that the oligomerization requires high protein concentrations (∼8 mg ml^{-1}). Larger forms of form 4 cytochrome c_6 appear when the gel is overloaded or when form 4 is incubated in the crystallization mother liquor or in an alkaline buffer prior to loading (data not shown). The redox state of form 4 has a slight effect on the migration in native gels: the second most abundant band disappears with addition of potassium ferricyanide, but no change in migration is apparent with addition of reductant (data not shown).

The oligomerization observed in other cytochrome c_6 structures (Kerfeld *et al.*, 1995; Schnackenberg *et al.*, 1999) has been suggested to be important in recognizing different oligomeric membrane-bound electron-transfer partners (cytochrome b_6f complex, PSI, cytochrome *c* oxidase) or in facilitating the transfer of multiple redox equivalents. Recent evidence suggest that there are two distinct routes of electron transfer from the Rieske iron–sulfur protein to P700; one through the cytochrome b_6f complex and one bypassing it (Fernandez-Valesco *et al.*, 2001). The different routes could be mediated by different forms of cytochrome c_6. Alternatively, oligomerization could affect the redox potential of cytochrome c_6 (*e.g.* by affecting the accessibility of the heme), thereby altering the driving force of electron transfer between cytochrome c_6 and its membrane-bound redox partners.

Large oligomeric assemblies of 24 molecules similar to that formed by *A. maxima* cytochrome c_6 have been described for a few other proteins. In most cases, the observed shell-like arrangements can be related to the known function of the protein. For example, the dihydrolipoamide succinyltransferase 24-subunit complex (Knapp *et al.*, 1998) provides a scaffold for a multienzyme complex. Ferritin (Harrison & Arosio, 1996) and Dps (Grant *et al.*, 1998) both form complexes of 24 subunits that sequester iron. Similarly, a small heat-shock protein also forms a 24-subunit complex with octahedral symmetry (Kim *et al.*, 1998). To date, no comparable role for cytochrome c_6 has been suggested.

The cubic assembly described here is radically different from cytochrome c_6 oligomers observed thus far and could be related to some of the distinctive characteristics of *A. maxima*, such as its preference for an alkaline-pH growth medium or its formation of multicellular trichomes. Alternatively, it may be that the various cytochrome c_6 oligomers observed in crystallographic studies represent storage forms of the protein. This would be analogous to the hexameric form of insulin (Badger *et al.*, 1991), which corresponds to the natural storage form of the protein (Blundell *et al.*, 1972). This explanation is also consistent with the lack of a conserved trimer interface among

```
A. maxima       GDVAAGASV FSANCAACHM GGRNVIVANK TLSKSDLAKY LKGFDDDAVAAVA
M. braunii     EADLALGKAV FDGNCAACHA GGGNNVIPDH TLQKAAIEQF LDGGFNIEAIV
C. reinhardtii ADLALGAQV FNGNCAACHM GGRNSVMPEK TLDKAALEQY LDGGFKVESII
S. obliquus     SADLALGKQT FEANCAACHA GGNNSVIPDH TLRKKAAMEQ FLQGGFNLEAIT

A. maxima          YQVTNGKN AMPGFNGRLS PKQIEDVAAY VVDQAEKGW
M. braunii         YQIENGKG AMPAWDGRLD EDEIAGVAAY VYDQAAGNKW...
C. reinhardtii     YQVENGKG AMPAWADRLS EEEIQAVAEY VFKQATDAAWKY
S. obliquus        YQVENGKG AMPAWSGTLD DDEIAAVAAY VYDQASGDKW
```

Figure 6
Primary structures of cytochromes c_6 observed as crystalline oligomers. Residues involved in trimerization are underlined.

cytochromes of different species (Fig. 5); presumably cytochrome c_6 storage would not impose any strict geometric requirements on how the trimers are assembled. Crowding or confinement of cytochrome c_6 by other solutes in the relatively restricted space of the thylakoid lumen could also promote oligomer assembly. Further experiments are required to investigate the possible biological significance of the highly unusual shell structure reported here.

CAK acknowledges the US Department of Agriculture for support of this research (USDA 1999-01759). MRS and TOY acknowledge the support of the National Institutes of Health (grant 31299). We thank Dr Martin Phillips of the UCLA-DOE Biochemistry Instrumentation Facility for making analytical ultracentrifugation measurements and for helpful discussions. We also thank Gilda Tossorian, Vishnu Brahmandam and Cheryl Chan for their assistance in protein characterization. Mass spectra were collected at the UCLA Center for Molecular and Medical Sciences Spectrometry.

References

Badger, J., Harris, M. R., Reynolds, C. D., Evans, A. C., Dodson, E. J., Dodson, G. G. & North, A. C. T. (1991). *Acta Cryst.* B**47**, 127–136.
Blundell, T. L., Dodson, G. G., Hodgkin, D. C. & Mercola, D. A. (1972). *Adv. Protein Chem.* **26**, 279–402.
Brünger, A. T., Adams, P. D., Clore, G. M., DeLano, W. L., Gros, P., Grosse-Kunstleve, R. W., Jiang, J. S., Kuszewski, J., Nilges, M., Pannu, N. S., Read, R. J., Rice, L. M., Simonson, T. & Warren, G. L. (1998). *Acta Cryst.* D**54**, 905–21.
Carson, M. (1997). *Methods Enzymol.* **277**, 493–505.
Fernandez-Valesco, J. G., Jamshidi, A., Gong, X.-S., Zhou, J. & Yeng, R. Y. (2001). *J. Biol. Chem.* **276**, 30598–30607.
Frazao, C., Soares, C. M., Carrondo, M. A., Pohl, E., Dauter, Z., Wilson, K. S., Hervas, M., Navarro, J. A., De la Rosa, M. A. & Sheldrick, G. M. (1995). *Structure*, **3**, 1159–1169.
Golbeck, J. H. (1994). *The Molecular Biology of Cyanobacteria*, edited by D. A. Bryant, pp. 319–360. Dordrecht: Kluwer Academic.
Grant, R. A., Filman, D. J., Finkel, S. E., Kolter, R. & Hogle, J. M. (1998). *Nature Struct. Biol.* **5**, 294–303.
Harrison, P. M. & Arosio, P. (1996). *Biochim. Biophys. Acta*, **1275**, 161–203.
Inda, L. A., Medina, M., Saraiva, L. M., Gomez-Moreno, C., Teixeira, M. & Peleato, M. L. (1997). *Photosynth. Res.* **54**, 107–114.
Janin, J. & Rodier, F. (1995). *Proteins Struct. Funct. Genet.* **23**, 580–587.
Jones, T. A., Zou, J. Y., Cowan, S. W. & Kjeldgaard, M. (1991). *Acta Cryst.* A**47**, 110–119.
Kallas, T. (1994). *The Molecular Biology of Cyanobacteria*, edited by D. A. Bryant, pp. 259–317. Dordrecht: Kluwer Academic.
Kerfeld, C. A., Anwar, H. P., Interrante, R., Merchant, S. & Yeates, T. O. (1995). *J. Mol. Biol.* **250**, 627–647.
Kerfeld, C. A. & Krogmann, D. W. (1998). *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **49**, 397–425.
Kim, K. K., Kim, R. & Kim, S.-H. (1998). *Nature (London)*, **394**, 595–599.
Kissinger, C. R., Gehlhaar, D. K. & Fogel, D. B. (1999). *Acta Cryst.* D**55**, 484–491.
Kleywegt, G. J. & Jones, T. A. (1994). *Proceedings of the CCP4 Study Weekend. From First Map to Final Model*, edited by S. Bailey, R. Hubbard & D. Waller, pp. 59–66. Warrington: Daresbury Laboratory.
Knapp, J. E., Mitchell, D. T., Yazdi, M. A., Ernst, S. R., Reed, L. J. & Hackert, M. L. (1998). *J. Mol. Biol.* **280**, 655–668.
Levine, R. L., Mosoni, L., Berlett, B. S. & Stadtman, E. R. (1996) *Proc. Natl Acad. Sci. USA*, **93**, 15036–15040.
Ludwig, M. L., Pattridge, K. A., Powers, T. B., Dickerson, R. E. & Takano, T. (1982). *Electron Transport and Oxygen Utilization*, edited by C. Ho, pp. 27–32. North Holland: Elsevier.
Medina, M., Louro, R., Gagnon, J., Peleato, M. L., Gomez-Moreno, C., Xavier, A. & Teixeira, M. (1997). *J. Biol. Inorg. Chem.* **2**, 225–234.
Molina-Heredia, F. P., Hervas, M., Navarro, J. A. & De la Rosa, M. (2001). *J. Biol. Chem.* **276**, 601–605.
Morand, L. Z., Cheng, R. H., Ho, K. K. & Krogmann, D. W. (1994). *The Molecular Biology of Cyanobacteria*, edited by D. A. Bryant, pp. 394–396. Dortrecht: Kluwer Academic.
Navaza, J. (1994). *Acta Cryst.* A**50**, 157–163.
Nicholls, A., Sharp, K. A. & Honig, B. (1991). *Proteins*, **11**, 281–296.
Page, C. C., Moser, C. C., Chen, X. X. & Dutton, P. L. (1999). *Nature (London)*, **402**, 47–52.
Sawaya, M. R., Krogmann, D. W., Serag, A., Ho, K. K., Yeates, T. O. & Kerfeld, C. A. (2001). *Biochemistry*, **40**, 9215–9225.
Schnackenberg, J., Than, M. E., Mann, K., Wiegand, G., Huber, R. & Reuter, W. (1999). *J. Mol. Biol.* **290**, 1019–1030.
Sutter, M. (1997). Personal communication.
Thomas, P. E., Ryan, D. & Levin, W. (1976). *Anal. Biochem.* **75**, 168–176.
Tong, L. & Rossmann, M. G. (1997). *Methods Enzymol.* **276**, 594–611.
Yamada, S., Park, S.-Y., Shimizu, H., Koshizuka, Y., Kadokura, K., Satoh, T., Suruga, K., Ogawa, M., Isogai, Y., Nishio, T., Shiro, Y. & Oku, T. (2000). *Acta Cryst.* D**56**, 1577–1582.