The structure of the cytochrome \( b_{6f} \) complex has been investigated by electron microscopy and image analysis of thin three-dimensional crystals. Electron micrographs of negatively stained specimens were recorded and showed optical diffraction peaks to 10 Å resolution. A projection map was calculated at 8 Å resolution and showed the presence of cytochrome \( b_{6f} \) dimers. The extramembrane part of each monomer featured a C shape, with mean external diameter of 53 Å and an internal groove of 14 Å long and 9 Å wide. Within each monomer, strong features were clearly resolved and tentatively attributed to some of the subunits of the cytochrome \( b_{6f} \) complex. The data are consistent with the Rieske iron-sulfur protein lying close to the monomer-monomer interface and the heme-bearing domain of cytochrome \( f \) far from it.

Cytochrome \( b_{6f} \) (plastoquinol:plastocyanin oxidoreductase) is an integral membrane protein complex that participates in electron transfer and generation of an electrochemical proton gradient in oxygenic photosynthesis. It is homologous to the cytochrome \( bc_1 \) complex (ubiquinolcytochrome \( c \) oxidoreductase) of the respiratory chains of the mitochondrion and many bacteria. The \( b_{6f} \) complexes from higher plants (1) and from the unicellular green alga \textit{Chlamydomonas reinhardtii} (2) are highly similar and comprise four subunits with a molecular mass of >17 kDa. Three of them, cytochrome \( b_{6f} \), cytochrome \( f \), and the Rieske protein, contain redox prosthetic groups. The fourth, subunit IV, is involved together with cytochrome \( b_{6f} \) and the Rieske protein in forming the oxidizing plastoquinol-binding site \( Q_{b} \) (1). There are at least three additional small hydrophobic polypeptides (4 kDa). The number of transmembrane helices in the seven-subunit monomeric complex (105 kDa) is probably 11 (3, 4). A dimeric form is believed to be the native state both in higher plants (5) and in \textit{C. reinhardtii} (6).

Spectroscopic, biochemical, genetic, and electron microscopy studies of cytochrome \( b_{6f} \) (reviewed in Ref. 1) have yielded only sparse information about the three-dimensional structure of this complex. Huang \textit{et al.} (5) reported that negatively stained monomers and dimers of the \( b_{6f} \) complex from spinach both appeared as round particles with clefts with diameters of 77 ± 10 and 91 ± 9 Å, respectively. Boekema \textit{et al.} (7) have reported, also from single particle analysis, an elongated shape of the complex from \textit{Synechocystis PCC 6803} with dimensions of 83 × 44 × 60 Å, which they interpreted as monomers. Freeze-fractured vesicles reconstituted with purified spinach \( b_{6f} \) featured particles 83 Å in diameter and 110 Å in height, which Mörschel and Staehelin interpreted as dimers (8), whereas reconstituted monomers and dimers from \textit{C. reinhardtii} appeared as particles with diameters of ~80 and ~100–110 Å, respectively (6).

Finally, Mosser \textit{et al.} (9) obtained tubular crystals and two types of thin three-dimensional crystals of spinach \( b_{6f} \). The projection map that was calculated did not give unambiguous limits of the molecule. Thus, these studies have not led so far to a clear description of the size and shape of the complex. Most interestingly, the structure of the cleaved extramembrane domain of turnip cytochrome \( f \) (10) and that of the catalytic domain of the mitochondrial Rieske protein (11) have both been solved to high resolution by x-ray crystallography. On the basis of these data, Link and Iwata have proposed a model for the association of cytochrome \( f \) with the photosynthetic Rieske protein (12). X-ray crystallography is expected to lead rapidly to a detailed structural model of the entire mitochondrial \( bc_1 \) complex (13).

However, due to the important differences between the two complexes (different subunit compositions, low sequence similarities of homologous subunits, presence of photosynthetic pigments in \( b_{6f} \), sensitivity to antimycin limited to \( bc_1 \), and possible functional differences), high resolution structural data on one complex might not be easily transposable to the other.

Understanding the functional mechanism of cytochrome \( b_{6f} \) hangs on the knowledge of its detailed structure. Growing well-ordered two-dimensional crystals suitable for analysis by electron crystallography is one approach toward determining the structure of proteins at high resolution (14, 15). The yield, purity, and stability of the \( b_{6f} \) preparations from \textit{C. reinhardtii} (2, 6) fulfill the prerequisites for crystallization attempts, whereas enzymatic activity, the presence of the Rieske protein, the spectral properties, and the dimeric state provide various checks on the native state of the complex (6, 16).

In the present article, we describe the crystallization of the \( b_{6f} \) complex from \textit{C. reinhardtii} in very thin three-dimensional crystals. For this purpose, we have adapted and improved a reconstitution strategy that uses Bio-Beads as a detergent-removing agent and has been demonstrated successful for two-dimensional crystallization of different membrane proteins (17). Optimizing the pre-reconstitution conditions and combining the use of Bio-Beads with freeze-thaw cycles led to the formation of large and highly ordered thin three-dimensional crystals that diffract to better than 10 Å resolution in negative stain. After correction of lattice distortions, crystallographic analysis has yielded a projection map of cytochrome \( b_{6f} \) at 8 Å resolution. The map is discussed in the light of available evidence on the organization of the subunits in the complex.

**EXPERIMENTAL PROCEDURES**

**Materials**—SM2 Bio-Beads were obtained from Bio-Rad and di-C\(_{18}\)-phosphatidylglycerol from Avanti Polar Lipids Inc. Sources for the other chemicals have been described in Ref. 2.
Purification of Cytochrome b₆f—Cytochrome b₆f complex was purified from C. reinhardtii thylakoid membranes in the presence of 6-O-(N-heptylcarbamoyl)-methyl-α-D-glucopyranoside (Hecameg) as described previously (2). Briefly, the purification protocol comprises three steps: selective solubilization from thylakoid membranes, sucrose gradient sedimentation, and hydroxylapatite chromatography. Following solubilization, all media are supplemented with egg phosphatidylincholine to prevent the loss of the Rieske protein from the complex that follows delipidation (2, 6).

Crystalization—Purified cytochrome b₆f complex was resuspended in 20 mM Hecameg, 2 mM CaCl₂, 0.3% glycerol, 0.3 mM NaN₃, 5.6 mM n-octyl fluoride, 245 mM ammonium phosphate, 6.8 mM Tricine, pH 8.0, and supplemented with a mixture of egg phosphatidylincholine and di-C₄₀₇₋ phosphatidylglycerol (1:1 to 1:4.1, w/w). The protein concentration in the final reconstitution mixture was adjusted to 0.5 g/liter and that of the lipid to a lipid/protein ratio of 0.2 w/w. The samples were preincubated overnight in the cold room under gentle stirring and subsequently treated with 200 g/liter SM2 Bio-Beads according to the batch procedure previously described (17). After 6–12 h of incubation with beads, the reconstituted material was pipetted off and kept for 24 h at 4 °C before three cycles of freezing (−190 °C) and thawing (37 °C). Aliquots were taken daily and examined by electron microscopy.

Electron Microscopy and Image Processing—Samples, negatively stained with 1% uranyl acetate, were observed on a Philips CM120 transmission electron microscope operating at 120 kV. Low dose electron micrographs were recorded at magnifications of 45,000 and 60,000×. The best images were selected by optical diffraction, and areas exhibiting strong coherent diffraction spots were digitized on a Leafscan 45 CCD-array microdensitometer with a 5 μm scan spot size. Areas ranging up to 3200 × 3200 pixels in size, corresponding to 0.34 × 0.34 μm at the specimen level, were subjected to analysis using the Spectra program package (18) and the MRC image processing system (19). A total of 14 crystalline areas from nine images were analyzed with unbending methods. The underfocus level was determined by multiple measurements on the Thou-rings from the Fourier transform of the raw image, and the reflections corrected for the overall transfer function. The reflections from the processed images were centered and averaged, utilizing spots up to IQ 7. An image scale factor and the reflection peak height were used as a weighing factor during averaging. A refinement step on the positions of the zeroes in the contrast transfer function was performed, using the preliminary average as a reference. An optimal position of the lattice extraction, centered around the best crystalline area of each image, was achieved by extracting a few sets of reflections and testing for phase quality. The data set was phase-minimized with a small step size and merged, in several rounds, to yield a new average. A cut-off selection of maximally 45 ° phase deviation from the real values, in addition to IQ 7 for the amplitudes, was applied for acceptance of a reflection prior to calculation of the map. An error weighing factor was included by multiplying the amplitudes by the cosine of the phase deviation. The symmetry was imposed, and the projection map was displayed using histogram equalization and the plot program Pluto. Equidistant line levels were generally employed to the maximum positive density (i.e. stain-excluding region) in the map.

RESULTS

Crystalization—Thin three-dimensional crystallization of cytochrome b₆f was achieved by reconstituting the solubilized complex into phospholipid bilayers containing egg phosphatidylcholine and di-octanoyl-phosphatidylglycerol, in the presence of calcium ions and protease inhibitors. Reconstitution was performed by removing detergent from the lipid-Hecameg-protein micellar solution by adsorption onto Bio-Beads SM2. Previous systematic studies have determined precisely the amount of beads to be added to remove the detergent initially present in about 6 h at 4 °C while avoiding protein adsorption and limiting lipid adsorption (17). Provided the amount of beads was carefully controlled, as well as the lipid to protein ratio, crystallization of cytochrome b₆f was reproducible. Another important parameter was the duration of the preincubation period before detergent removal, which had to be sufficiently long. Although the reason for this requirement is not definitely identified, it may be related to the time needed for full equilibration of the initial populations of detergent-lipid and detergent-lipid-protein micelles. Finally, the presence of calcium was found to be essential for crystal formation.

Following detergent removal, proteoliposomes with densely packed proteins were observed by electron microscopy. They tended to aggregate upon further incubation at 4 °C with concomitant formation of crystalline areas. Growth of large crystals from these aggregates was improved by treating the samples through freeze-thaw cycles. Possible explanations would be that such a treatment not only induces fusion of proteoliposomes but also creates some defects in the bilayer or some protein aggregation, which would favor crystal growth (20).

Samples were periodically checked by electron microscopy to monitor the growth of the crystals. The crystals presented a smooth homogeneous and continuous gray appearance (Fig. 1A) and were always composed of a stack of lamellae. The best time to collect them was generally between the third and the seventh day after three freeze-thaw cycles. After that, the crystals increased inhomogeneously in thickness and sometimes started to deteriorate.

Our belief that the crystals observed in the reconstituted preparations are formed by the b₆f in its native state is based on several lines of evidence. Neither visible absorption spectra nor SDS-polyacrylamide gel electrophoresis patterns changed during the 3-day to 1-week period required for crystallization. Two good indices of the native state of the complex are its dimeric nature and the stability of the spectrum of the b₆f-associated chlorophyll a; indeed, loss of the Rieske protein, which is the first step in the degradation of the complex (6), is always accompanied by a red shift of the visible absorption peak of the chlorophyll (16) and almost invariably followed by monomerization (6).

Electron Microscopy and Image Processing—The crystals (Fig. 1A) generally grew up to 5 μm in one direction (along the b direction) and up to 1.5 μm in the other (along the a direction). In some cases, they reached up to 10 μm 3 μm. At high magnification, the array appeared clearly and spread over the whole area (Fig. 1B). Although the crystals presented in this study are actually stacks of lamellae, we were selected such that they presented a small number of layers and were of extremely good crystallinity. The coherence of the lattice was confirmed by optical diffraction analysis of low dose negatives, which revealed sharp, coherent peaks out to 10 Å over the whole electron micrographs. This also strongly suggests that
the different layers are in perfect register, making the stacks equivalent to very thin three-dimensional crystals. The diffraction pattern corresponded to a rectangular lattice with parameters \( a = 175 \, \text{Å}, b = 68 \, \text{Å}, \gamma = 90^\circ \). Systematic extinctions were observed for \([h(\text{odd}),0]\) and \([0,k(\text{odd})]\).

Fourteen areas were selected by optical diffraction from nine of the best images and digitized, and the lattice distortions were corrected. Calculated phases indicated that the crystals belonged to plane group \( p22_1^2 \). In this space group, rows of molecules alternatively face up and down with respect to the membrane plane. The results of the \( R \) value test for this symmetry are shown in Table I for four resolution ranges, indicating the high quality of the phases to 8 Å resolution.

Fig. 2A shows the Fourier transform of the best distortion-corrected images. Reflections with \( IQ \) values of 4 are visible out to 8 Å resolution. Fig. 2B shows the phase deviations of reflections from the expected values of zero or \( \pi \) for all images. Excluding the phases of reflections having \( IQ \) values larger than 5, the root mean square phase error is about 16° at 8 Å resolution. Because plane group \( p22_1^2 \) gave the best phase residual, it was used to calculate projection maps to 20 and 8 Å resolution (Fig. 3, A and B).

The 20 Å density map reveals the dimeric organization of the \( b_{6f} \) complex. The dimer features an elongated \( S \) shape, \( \sim 88 \) Å long and \( \sim 53 \) Å wide. The monomer has a C-like shape with two major domains denoted X and Y. The 8 Å resolution map reveals within the extramembrane part of each monomer a ring of densities surrounding a deep groove (G). The external diameter of the monomer is \( \sim 53 \) Å, whereas the internal groove is \( \sim 14 \) Å long and \( \sim 9 \) Å wide.

### DISCUSSION

The main goal of this study was to generate a projection map of cytochrome \( b_{6f} \) to provide information about the localization of its different subunits. The purity and reproducibility of the \( b_{6f} \) preparations from \( C. \) reinhardtii facilitated the identification of conditions favoring the growth of large and coherent thin three-dimensional crystals suitable for structural analysis by electron microscopy. In addition to the usual parameters that affect the formation and quality of crystals, we believe that two key factors in the success of our procedure are the rapid and total removal of detergent by Bio-Beads (see also Ref. 17) and the use of freeze-thaw cycles to increase the size and ordering of the crystals (see also Ref. 20).

The excellent quality of the crystals allowed us to record low dose images of negatively stained specimens that diffracted out to 10 Å resolution prior to the correction of lattice distortions and to better than 8 Å following it. The IQ plot of reflection intensities (Fig. 2A) and the crowding of calculated phases around the values of 0° and 180° expected from the \( p22_1^2 \) lattice symmetry (Fig. 2B) are indicative of the quality of the data. Such a high resolution has never been reported before for negatively stained crystals of any membrane protein. The multi-layered nature of the crystals cannot by itself account for this result, because similar resolutions have already been observed with negatively stained single-layered crystals such as those of annexin V (21) and of subunit B of cholera toxin. Thus, our observations suggest that the resolution attainable following negative staining may be higher than usually accepted (\( \sim 15 \) Å).

The projection maps show a dimeric organization of cytochrome \( b_{6f} \), in keeping with biochemical determinations on the solubilized complex (6). Each monomer presents a C-like shape, \( \sim 53 \) Å in diameter, covering a total area of about 2,000 Å². By comparison with the dimensions of the transmembrane regions in bacteriorhodopsin (14) or cytochrome c oxidase (22), this area significantly exceeds that needed to accommodate 11 transmembrane \( \alpha \)-helices per monomer. In the 20 Å projection map, each monomer features two main domains (labeled X and Y in Fig. 3), surrounding a deep central groove (G in Fig. 3). Domain X, which is near the 2-fold axis of symmetry, is less bulky than domain Y, suggesting that much of the extramembrane mass of the complex lies away from this axis. The dimensions and overall appearance of cytochrome \( b_{6f} \) at 20 Å resolution are not dissimilar to those of the subcomplex of Neurospora crassa cytochromes b and c₁ (lacking the Rieske and core proteins), except that in the latter case domain X appeared stronger than domain Y (23), at variance with the \( b_{6f} \) map. A three-dimensional reconstruction of bovine heart mitochondrial bc₁ at 16 Å resolution has recently been calculated from electron micrographs of frozen tubes (24). It shows, protruding into the intermembrane space, four proteic masses per dimer, reaching into the solvent and delineating a relatively empty space around the 2-fold axis of symmetry. A depression about the C2 axis is also apparent in the preliminary x-ray map of beef heart bc₁ (13).

The projection of the monomer obtained does not resemble any of the three possible projections (Y, Z, and L shapes) previously observed with spinach \( b_{6f} \) (9). Differences in the conditions of crystal formation may explain these dissimilarities. Whereas low calcium concentration (0.5 mM), Hecameg, and Bio-Beads were used to crystallize the complex from \( C. \) reinhardtii, comparatively high calcium concentration (10 mM), octylglucoside, and dialysis were employed for the crystallization of the spinach complex. These differences may have led to the monomerization of the complex from spinach, explaining why no tight dimers were observed.

The location of the stain in the case of membrane proteins is not perfectly understood. It seems to vary from one protein to another, but it is usually accepted that the hydrophobic membrane core is largely stain-excluding. Therefore, we made the assumption that the structural information obtained, including the presence of the groove, primarily concerns the extramembrane parts of the \( b_{6f} \) complex. Positive densities should be due mainly to the cytochrome f extramembrane domain (28 kDa; residues 1–250), the Rieske protein (19 kDa), the main loops of cytochrome b₁ (11 kDa; residues 1–33, 58–82, and 140–181) and subunit IV (8 kDa; residues 215–249 and 272–308), and the extramembrane extensions of the three 4-kDa subunits (\( \sim 1 \) kDa each). However, because of the smallness of 4-kDa subunits’ extramembrane extensions, their contribution to the pro-

---

**TABLE I**

**Crystallographic data**

| Resolution range | Two-fold residual IQ 5 (45 degrees random) | Completeness |
|-----------------|------------------------------------------|--------------|
| Å               | Unweighted | Amplitude-weighted | IQ 5 | IQ 6 |
| 175–20          | 9.6        | 4.2               | 100.0 | 100.0 |
| 20–15           | 14.7       | 11.9              | 94.1  | 94.1  |
| 15–11           | 18.3       | 15.4              | 77.8  | 100.1 |
| 11–8            | 17.9       | 15.8              | 39.1  | 84.1  |
| Total range     | 15.4       | 7.8               | 65.1  | 91.8  |
projection map will not be discussed in the following.

The projection map at 8 Å resolution (Fig. 3A) confirms the C shape of the monomer. Each monomer features four domains of variable importance (II > I >> III > IV), which can be tentatively allocated to some of the \( b_6f \) subunits. The two major stain-excluding regions, I and II, are well individualized, indicating that they correspond to two independent proteic masses. Taking into account their respective importance (II > I) and the fact that the extramembrane part of cytochrome \( f \) and the Rieske protein fold into autonomous domains, one may tentatively assign density II to the largest of these subunits, the cytochrome \( f \), whereas domain I would correspond to the Rieske
The extramembrane part of the cytochrome I to monomer-monomer interface would imply that site Qo, which is almost never observed. The effects of mutations on the functionality and sensitivity to inhibitors of site Qo (reviewed in Ref. 27) and on the strength of the Rieske protein's association with the complex (27, 28) suggest that at least part of the monomer-monomer interface suggests a possible origin for the frequent correlation between these two events: destabilization could result from the loss of interactions between the Rieske protein of one monomer and neighboring subunit(s) of its partner.

In conclusion, our results provide the first crystallographic information on the structure of the cytochrome b_{6}f complex in its intact form. The 8 Å projection map of the enzyme has permitted proposals to be made regarding the location cytochrome f and the Rieske protein in the b_{6}f dimer. In future work, subunit assignment will be further examined using the same approach to crystallization associated with labeling methods or with the removal of specific subunits (cf. Ref. 6). Obtaining highly ordered crystals opens the way to the determination of a three-dimensional model of cytochrome b_{6}f using cryo-electron microscopy. Establishing a three-dimensional model of the complex, although facing the problem of analyzing multilayered crystals, can be attempted by appropriate treatment of the crystallographic data and/or by modifications of the crystallization protocol favoring the growth of monolayered crystals. It should be stressed that even a moderate resolution three-dimensional map would be extremely useful in helping to delineate structural differences between the bc_{1} and b_{6}f complexes.

Acknowledgments—We are extremely grateful to D. Picot (Institut de Biologie Physico Chimique, Paris) for useful discussions.

REFERENCES
1. Cramer, W. A., Soriano, G. M., Ponomarev, M., Huang, D., Zhang, H., Martinez, S. E. & Smith, J. L. (1996) Annu. Rev. Plant Physiol. Plant Mol. Biol. 47, 477–508
2. Pierre, Y., Breyton, C., Kramer, D. & Popot, J.-L. (1995) J. Biol. Chem. 270, 29342–29349
3. Breyton, C., de Vitry, C. & Popot, J.-L. (1994) J. Biol. Chem. 269, 7597–7602
4. Ppopet, J.-L., Pierre, Y., Breyton, C., Lemoine, Y., Takahashi, Y. & Rochais, J.-D. (1995) in Photosynthesis: From Light to Biosphere. Proceedings of the Xth International Congress on Photosynthesis (Mathis, P., ed.) Vol. II, pp. 507–512. Kluwer Academic Publishers, Dordrecht, the Netherlands
5. Huang, D., Evert, R. M., Cheng, B. H., Hetmann, J. B., Schagger, H., Sed, V., Ohnishi, T., Baker, T. S. & Cramer, W. A. (1994) Biochemistry 33, 4401–4409
6. Breyton, C., Tribet, C., Olive, J., Dubacq, J.-P. & Popot, J.-L. (1997) J. Biol. Chem. 272, in press
7. Boekema, E. J., Bonsenstra, A. F., Dekker, J. P. & Rogné, M. (1994) J. Bioenerg. Biomembr. 26, 17–29
8. Maresch, E. & Staehelin, L. A. (1983) J. Cell Biol. 97, 301–310
9. Møller, G., Dorr, C., Hausing, G. & Kühbrandt, W. (1994) International Congress on Electron Microscopy, Electron Microscopy 1994, Les Éditions de Physique 3, 609–610
10. Martinez, S. E., Huang, D., Szczepaniak, A., Cramer, W. A. & Smith, J. L. (1994) Structure 2, 95–105
11. Iwata, S., Saynowits, M., Link, T. A. & Michel, H. (1996) Structure 4, 567–579
12. Link, T. A. & Iwata, S. (1996) Biochim. Biophys. Acta 1275, 54–60
13. Xu, C.-A., Xia, J.-Z., Keiswetter, M., Yu, L., Xin, D., Kim, H. & Deisenhofer, J. (1996) Biochim. Biophys. Acta 1275, 47–53
14. Unwin, P. N. T. & Henderson, R. (1975) J. Mol. Biol. 94, 425–440
15. Kühbrandt, W. (1992) Quart. Rev. Biophys. 25, 1–49
16. Pierre, Y., Breyton, C., Lemoine, Y., Robert, B., Vernotte, C. & Popot, J.-L. (1997) J. Biol. Chem. 272, in press
17. Rigaud, J.-L., Møller, G., Lacapère, J.-J., Ofodun, A., Levy, D. & Ranck, J.-L. (1990) J. Struct. Biol. 108, 226–255
18. Schmidt, M. F., Dargahi, R. & Tam, M. W. (1993) Ultramicroscopy 48, 251–264
19. Crowther, R. A., Henderson, R. & Smith, J. M. (1996) J. Struct. Biol. 116, 9–16
20. Young, H. S., Rigaud, J.-L., Lacapère, J.-J. & Stokes, D. L. (1997) Biophys. J. 72, 2545–2558

FIG. 4. Electron microscopy projection map at 8 Å resolution. The main densities of the cytochrome b_{6}f monomer are numbered from I to IV; the central groove is indicated by G. The presumed positions of the extramembrane part of the cytochrome f and the Rieske protein are indicated.

The allocation of the Rieske protein to density I and of parts of the bc_{1} extramembrane loops of cytochrome b_{6} to less intense features near the large extramembrane domain is rather flat, and it is reasonable to assume that the domain IV appears to connect more closely to domain I. This suggests that the two densities IV of the dimer suggests that the subunits while contributing in a negligible way to domain I.

The main densities of the cytochrome I to monomer-monomer interface would imply that site Qo, which is almost never observed. The effects of mutations on the functionality and sensitivity to inhibitors of site Qo (reviewed in Ref. 27) and on the strength of the Rieske protein's association with the complex (27, 28) suggest that at least part of the second (c-d) extramembrane loop of cytochrome b_{6} and of the first loop of subunit IV is located close to the Rieske protein.

The allocation of the Rieske protein to density I and of parts of cytochrome b_{6} and subunit IV to less intense features near the monomer-monomer interface would imply that site Qo, which is formed by these three subunits, might lie relatively close to the 2-fold axis of the dimer. Such a location would be consistent with the close apposition of the two b_{6} hemes in the bc_{1} dimer, as revealed by x-ray diffraction (13). The relative proximity of the two Q sites and the two b_{6} hemes within the bc_{1} and b_{6}f dimers opens up interesting vistas regarding the possibility of a functional cooperation between the two monomers (cf. Ref. 13). It is also worth noting that removal of the Rieske protein is generally, even though not always, accompanied by the monomerization of the complex and vice versa (6). The proposed positioning of the Rieske protein close to the monomer-monomer interface suggests a possible origin for the frequent correlation between these two events: destabilization could result from the loss of interactions between the Rieske protein of one monomer and neighboring subunit(s) of its partner.

In conclusion, our results provide the first crystallographic information on the structure of the cytochrome b_{6}f complex in its intact form. The 8 Å projection map of the enzyme has permitted proposals to be made regarding the location cytochrome f and the Rieske protein in the b_{6}f dimer. In future work, subunit assignment will be further examined using the same approach to crystallization associated with labeling methods or with the removal of specific subunits (cf. Ref. 6). Obtaining highly ordered crystals opens the way to the determination of a three-dimensional model of cytochrome b_{6}f using cryo-electron microscopy. Establishing a three-dimensional model of the complex, although facing the problem of analyzing multilayered crystals, can be attempted by appropriate treatment of the crystallographic data and/or by modifications of the crystallization protocol favoring the growth of monolayered crystals. It should be stressed that even a moderate resolution three-dimensional map would be extremely useful in helping to delineate structural differences between the bc_{1} and b_{6}f complexes.
21. Olofsson, A., Mallouh, V. & Brisson, A. (1994) *J. Struct. Biol.* **113**, 199–205
22. Iwata, S., Ostermeier, C., Ludwig, B. & Michel, H. (1995) *Nature* **376**, 660–669
23. Hovmoller, S., Leonard, K. & Weiss, H. (1993) *FEBS Lett.* **315**, 118–122
24. Akiba, T., Toyoshima, C., Matsunaga, T., Kawamoto, M., Kubota, T., Fukuyama, K., Namba, K. & Matsuhara, H. (1996) *Nat. Struct. Biol.* **3**, 553–561
25. Chain, R. K. & Malkin, R. (1991) *Photosynth. Res.* **28**, 59–68
26. Vater, J., Heinze, K., Friedrich, B., Kablitz, B., Blokesch, A., Irrgang, K.-D., Thirde, B. & Salnikow, J. (1996) *Ber. Bunsenges. Phys. Chem.* **100**, 2107–2111
27. Brasseur, G., Saribas, G. S. & Daldal, F. (1996) *Biochim. Biophys. Acta* **1275**, 61–69
28. Finazzi, G., Büschlen, S., de Vitry, C., Rappaport, F., Juliot, P. & Wollman, F.A. (1997) *Biochemistry* **36**, 2867–2874