The Location of the Mobile Electron Carrier Ferredoxin in Vascular Plant Photosystem I*

Received for publication, July 21, 2000, and in revised form, August 16, 2000
Published, JBC Papers in Press, August 17, 2000, DOI 10.1074/jbc.M006549200

Stuart V. Ruffle‡, Aziz O. Mustafa‡, Ashraf Kitmitto‡, Andreas Holzenburg§§, and Robert C. Ford§§

From the ‡Department of Biomolecular Sciences, University of Manchester Institute of Science and Technology (UMIST), Manchester, M60 1QD and the §School of Biochemistry and Molecular Biology, University of Leeds, Leeds, LS2 9JT, United Kingdom

In this study, we present the location of the ferredoxin-binding site in photosystem I from spinach. Image analysis of negatively stained two-dimensional crystals indicates that the addition of ferredoxin and chemical cross-linkers do not significantly alter the unit cell parameters (for untreated photosystem I, \(a = 26.4 \text{ nm}, b = 27.6 \text{ nm}, \text{ and } \gamma = 90^\circ\), space group \(p22_121\), and for ferredoxin cross-linked photosystem I, \(a = 26.2 \text{ nm}, b = 27.2 \text{ nm}, \text{ and } \gamma = 90^\circ\), space group \(p22_121\)). Fourier difference analysis reveals that ferredoxin is bound on top of the stromal ridge principally interacting with the extrinsic subunits PsaC and PsaE. This location would be accessible to the stroma, thereby promoting efficient electron transfer away from photosystem I. This observation is significantly different from that of the ferredoxin binding site proposed for cyanobacteria. A model for the binding of ferredoxin in vascular plants is proposed and is discussed relative to observations in cyanobacteria.

The light reactions of oxygenic photosynthesis are found in specialized membranes and are catalyzed by pigment protein complexes. PS-I provides a location at the edges of the granal stacks and in the stromal lamellae in plants (1, 2). It is that part of the light reactions of photosynthesis that is responsible for the absorption of light energy and the generation of reduced Fd via a series of electron carriers across the thylakoid membrane. The mobile electron carrier Fd has been shown to be involved in a wide variety of redox reactions in plants. These include mediating the transfer between PS-I and NADP⁺ in linear electron transfer and playing a key role in cyclic photophosphorylation (reviewed in Refs. 3 and 4). Fd also plays a significant role in the redox reactions of glutamate synthase, sulfate reductase, nitrite reductase, and Fd-thioredoxin oxidoreductase (5). The interaction of Fd with PS-I is well described in both the cyanobacterium, *Synechocystis* sp. PCC6803 (6) and the vascular plant, spinach (7). Interaction between Fd and PS-I has been proposed to be mediated by the subunit PsaD. A three-dimensional structure of a PS-I reaction center trimer has been described from *Synechococcus* (8, 9, 10) to 4 Å resolution. This structure does not show the location of the mobile electron carriers because they are eliminated during the preparation of the crystals. The model of the stromal surface generated by the x-ray data led to the proposal of a Fd binding site (11) approximately 14 Å from the putative PsaC-bound iron-sulfur center, FA/B2. Recently, this iron-sulfur center has been determined to be Fd by site-directed mutagenesis and functional analysis in *Chlamydomonas reinhardtii* (12).

Nearest neighbor analysis of the polypeptides of PS-I indicates that the stromal subunits PsaC, PsaD, and PsaE are all in contact with each other, revealed by cross-linking and two-dimensional gel electrophoresis (13). PsaD has been closely implicated in forming part of the Fd binding site that is required for forward electron transfer to Fd (14). It is also required for the stable binding of PsaC to the PS-I complex (15), as well as for the formation of a binding site for the PsaE subunit (16). The loss of PsaE from the complex has been shown to reduce the rate of forward electron transfer to Fd by a factor of 25 (16).

The Fd binding site has been characterized in the cyanobacterium *Synechocystis* with cross-linking studies and electron microscopy followed by single particle image analysis of PS-I (17). The electron carrier was shown to bind 77 Å away from the center of the trimer, toward the side of the stromal ridge. This location is similar to that determined by similar methods for the alternative electron carrier flavodoxin (18).

Well ordered two-dimensional crystals from spinach thylakoids have been shown to contain PS-I and have yielded two-dimensional data to 2.7 nm resolution (2) and refined three-dimensional data to 2.5 nm resolution (19). However, neither of these studies retained the mobile electron carrier Fd. Labeling with Fd-colloidal gold complexes showed that the PS-I was located in the crystalline arrays and at the periphery of the grana (2). Significantly, the vascular plant PS-I does not appear to form trimers but does show an analogous stromal ridge (2), indicating that the structure in vascular plants may have some significant differences compared with that of cyanobacteria. In this study, we present the location of the vascular plant Fd binding site following chemical cross-linking of the subunit to two-dimensional crystalline arrays of PS-I.

**EXPERIMENTAL PROCEDURES**

PSI-enriched grana membranes were prepared from market spinach (*Spinacia oleracea*) as described previously (20). A 2-step incubation procedure was used to prepare crystal-containing grana membranes as described previously (2). Stacked thylakoid membranes, at a chlorophyll concentration of 2 mg/ml in buffer A (20 mM MES, NaOH, pH 6.3, 5 mM MgCl₂, 15 mM NaCl) were incubated in the dark for 20 min at 20 °C with Triton X-100 at a detergent/chlorophyll ratio of 13.75:1 (w/w). The membrane fragments were harvested by centrifugation at...
Location of Ferredoxin in Photosystem I

4 °C for 5 min at 13,000 × g (Eppendorf microcentrifuge 5415C). The resulting pellet was resuspended in cold buffer A. The chlorophyll concentration was then reset to 2 mg/ml and incubated in the dark at 20 °C for 20 min at a detergent/chlorophyll ratio of 5:1 (w/w). The sample was then centrifuged at 4 °C for 5 min at 13,000 × g, and the pellet was resuspended in cold buffer A. The sample was loaded onto glow-discharged carbon-coated copper electron microscopy grids at a chlorophyll concentration of 30–60 μg/ml. The loaded grids were negatively stained as described previously (2) and examined in a Philips CM10 or 400 transmission electron microscope. Micrographs were recorded on Kodak ESTAR 4489 or Agfa Scientia film at calibrated magnifications. Films were digitized on a LEAPSCAN 45 with a scan step of 20 μm. Crystalline areas were selected using CRISP (21) and then processed using the lattice-unbending procedures in the UNIX-MRC suite of programs (22). The projection map was assembled from multiple images by TRIMEERGE (21).

Fourier vector difference maps were calculated using the CCP4 suite of programs (23). The two-dimensional projection of spinach Fd was generated using SPIDER (24) from the Protein Data Bank 1A70 coordinates (25).

Chemical cross-linking between PS-I from crystals and spinach Fd was essentially an adaptation of the method described by Lelong et al. (26). Crystal-containing samples (4 mg/ml total chlorophyll) were incubated either in the presence of 5 μM Fd with 2 mM EDC, 2 mM NHS in 20 mM HEPES-NaOH, pH7.5, 5 mM MgCl₂ in a final volume of 50 μl for 30 min at room temperature. The reaction was stopped by the addition of ammonium acetate to a final concentration of 100 mM. The PS-I-Fd covalent complex-containing crystals were then washed twice with 20 mM tricine-NaOH, pH7.8 and then resuspended in 20 mM tricine-NaOH, pH8.0, 20% glycerol and loaded onto carbon-coated copper electron microscopy grids at a chlorophyll concentration of 30–60 μg/ml. Free oxidized Fd was determined spectrophotometrically at 423 nm (27).

The assay to monitor NADP⁺ photoreduction in cross-linked samples was adapted from the method of Shin (28). The reaction mixture consisted of cross-linked sample at a final amount of 75 μM Fd with 2 mM EDC, 2 mM NHS in 20 mM HEPES-NaOH, pH7.5, 5 mM MgCl₂ in a final volume of 50 μl for 30 min at room temperature. The reaction mixture contained either 5 mM Tris-HCl buffer, pH7.8 in a final volume of 2.5 ml. In the cuvette the reaction mixture was settled by centrifugation. A polypeptide profile of PS-I crystals was determined using a Bio-Rad 250SD spectrophotometer with side illumination. The sample was illuminated with non-saturating red light (Schott RG 665 filter), and a Schott BG18 filter protected the photomultiplier tube. The initial rate was recorded, and the sample was allowed to re-oxidize in the dark. 75 μg of Fd (excess) was added and stirred. Again the rate of NADPH generation at 340 nm was recorded.

RESULTS

Two-dimensional crystalline arrays of PS-I as seen in the preparations reported here adhere to the edges of PS-II-enriched grana membranes as seen in previous studies (2). The disordered particles within the bulk of the membrane are the non-ordered PS-II complexes. A polypeptide profile of PS-I crystall-containing preparations has been previously reported (2). These crystal-containing membrane fractions have been shown to contain PS-I as well as PS-II.

Image analysis of 12 crystalline arrays from the native untreated preparations resulted in unit cell dimensions of a = 26.4 nm, b = 27.6 nm, and γ = 90°, space group p22 21, with an average inter-image phase residual of 24.7° (Table I). These data are compatible with the values derived from earlier studies with an identical a/b axis ratio of 0.96 (2, 19). Each unit cell is composed of four PS-I complexes; two with the luminal side up and two with the stromal face up. The projection map for the untreated data (Fig. 1) shows a characteristic ridge of protein density on the projection where the stromal face is presented closest to the support film, as described previously (2). This feature appears to have an approximate “L-shape,” and its size and orientation match that of previous reports (2, 19). This stromal ridge has been proposed to be the location of the extrinsic proteins PsaC, PsaD, and PsaE, which are believed to contain the Fd binding site in vascular plant PS-I. The stromal ridge measures approximately 6.3 × 2.4 nm along its long axis.

A determination of the amount of Fd bound to PS-I before

and after EDC/NHS cross-linking yields a linear relationship from a double reciprocal plot with a binding constant for Fd in crystal-containing thylakoids of 1.1 μM (data not shown). This is in close agreement with that reported by Sétif and Bottin (7).

Table II clearly shows that the cross-linking of Fd to PS-I was functional. The conditions and amount of Fd used for the binding studies were determined from Lelong et al. (26) and Sétif and Bottin (7). The rate of photoreduction of NADP⁺ decreases as the amount of Fd added prior to incubation with EDC and NHS increases. It should be noted however, that these values were only obtained when an excess of Fd was present in the reaction mixture prior to illumination and recording of the absorption change at 340 nm. In the absence of Fd in the reaction mixture, there was no evidence of NADP⁺ photoreduction (data not shown).

For the crystalline areas cross-linked with Fd, 14 areas were subjected to image analysis, which yielded data with unit cell dimensions of a = 26.2 nm, b = 27.2 nm, and γ = 90°, space group p22 21, with an average inter-image phase residual of 28.3° (Table I). These data are in good agreement with the untreated data described above and have an a/b axis ratio of 0.96. This indicates that the treatment of the crystalline arrays with Fd, the cross-linker, and the subsequent washing steps does not significantly alter the overall structure of the complex. Initial inspection of the projection map for the Fd cross-linked crystals (Fig. 1B) shows the characteristic stromal ridge to be further accentuated with an increase in protein density over the control data.

Fourier vector difference analysis indicates a major large increase in density that is significantly above the background variations between the two projections. Fig. 1C shows the difference map for the whole unit cell. It is clear that the major difference between the Fd cross-linked and untreated projec-

| Table I | Crystallographic and image processing data for the PS-I untreated and PS-I-Fd cross-linked two-dimensional projection map |
|---------|---------------------------------------------------------------------------------|
| PS-I untreated | PS-I-Fd cross-linked |
| Number of processed images | 12 | 14 |
| Unit cell dimensions: | | |
| a (nm) | 26.4 ± 0.96 | 26.2 ± 0.50 |
| b (nm) | 27.6 ± 0.88 | 27.2 ± 0.58 |
| γ (°) | 90 | 90 |
| Two-dimensional space group | p22 21 | p22 21 |
| Average inter-image phase residual (°) | 24.7 | 28.3 |
| No. of structure factors | 467 | 469 |
| Approximate resolution (nm) | 1.8 | 1.8 |
| a/b axis ratio | 0.96 | 0.96 |

Fig. 1. Projection maps of the stromal projection of untreated PS-I (A) and ferredoxin cross-linked PS-I unit cell (B) in space group p2 displayed using gray scales. The stromal projection view is upper left and lower right in the unit cell. The luminal projection view is upper right and lower left in the unit cell. The Fourier vector difference map is shown for the whole unit cell (C). Scale bar = 5 nm.
**DISCUSSION**

To date, the structures presented of the higher plant PS-I complex do not include the extrinsic electron carrier Fd. Fd has been shown to be cross-linked to subunits PsaC, PsaD, PsaE, and PsaH by gel electrophoresis (26, 29, 30). Fd has also been shown to have a key functional relationship with PsaC with site-directed mutants indicating that it is the iron-sulfur center Fe₅ that interacts with Fd (12).

Data presented by Andersen et al. (30) indicate that the addition of the cross-linker EDC in the presence of Fd had a negligible effect on NADP⁺ photoreduction activity. These data conflict with observations by both Merati and Zanetti (31) and Zanetti and Merati (29) who reported a reduction of the control rate of NADP⁺ photoreduction by 50% when the thylakoids were cross-linked with EDC in the presence of Fd.

The data presented in Table II show that the rate of NADP⁺ photoreduction does decrease with an increase in the amount of preincubated Fd prior to cross-linking. When PS-I crystal-containing preparations were preincubated with Fd and then treated with EDC and NHS, we observed a significant reduction in the NADP⁺ photoreduction activity. Additionally, we note that 5 mM NHS did not have a greater effect on denaturation or a greater effect upon the NADP⁺ photoreduction activity than 5 mM EDC alone (data not shown). Therefore, it can be concluded that because thylakoids cross-linked with Fd have less NADP⁺ reduction activity, the Fd is irreversibly bound to its binding site on PS-I by the action of cross-linkers and cannot reduce NADP⁺.

This assumption is further verified by the binding constant for the Fd cross-linking reaction in thylakoid membranes (1.1 μM), which is in good agreement with that reported for solubilized spinach reaction centers by Sétif and Bottin (7). Additionally, the fact that higher initial concentrations of Fd prior to cross-linking lowers the rate of NADP⁺ photoreduction indicates that more binding sites are occupied by Fd and are therefore blocked when cross-linked. This relationship, however, does not saturate as expected from the binding constant (described above). This is because of several factors. First, to relate the functional assay to conditions used for the structural analysis, it was necessary to perform the cross-linking reaction at high chlorophyll concentrations and then dilute the samples back to one suitable for optical spectroscopy. Second, the cross-linking reactions were performed on crystal-containing thylakoid membrane preparations and previous studies report on Fd binding in purified reaction centers (7). It is to be expected that there will be some nonspecific binding in thylakoid membrane preparations. Random nonspecific binding of Fd to the crystalline-containing thylakoid membranes would have no effect on the structural determination of the Fd binding sites. The nonspecific sites are likely to be either associated with other proteins or lipids in the thylakoids or randomly distributed across the crystals and therefore will be averaged into the background during image processing.

Several models for the location of the Fd binding site have been presented. The structure of the PS-I reaction center has been examined by x-ray crystallography and a model was presented for data to 4 Å (32). Included in that work was a proposal for the Fd binding site that was previously presented (11), which modeled a fit between Fd from *Spirulina platensis* to the PS-I complex from *Synechococcus elongatus*. These studies suggested that the binding site for Fd was on the side of the stromal ridge, with an Fd–FB iron-sulfur center distance of approximately 14 Å. This distance is in good agreement with that proposed from spectroscopic determination of the rate of electron transfer from the terminal electron acceptor in PS-I to Fd (6, 7). These workers estimated that PS-I to Fd electron transfer occurred with a center to center distance of 11–15 Å.

Results of cross-linking studies and single particle analysis in the cyanobacterium *Synechocystis* PCC 6803, indicate that Fd binds 77 Å from the center of the trimer of PS-I complexes (17). The Fd was proposed to be in close contact with the

**TABLE II**

| Amount of Fd added prior to cross-linking (μM) | Average rates of non-saturating NADP⁺ photoreduction of EDC/NHS cross-linked membranes in the presence of excess Fd in the reaction mixture (μmoles NADPH produced/mg chl. • hr⁻¹) |
|---------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 0.0                                         | 6.0 ± 0.4                                                                                                                                  |
| 6.0                                         | 4.1 ± 2.2                                                                                                                                  |
| 10.0                                        | 3.5 ± 0.1                                                                                                                                  |
| 20.0                                        | 2.3 ± 0.3                                                                                                                                  |

*Mean ± S.E.*
stromal ridge; however, the binding site was proposed to be on the side of the ridge close to that suggested by Fromme et al. (11).

Our results with cross-linked Fd in spinach show that the Fd binding site is on top of the stromal ridge (Figs. 3 and 4). The Fourier vector difference map shows one major peak; the remaining contours in the difference map are small and fall outside of the protein envelope. These are most likely attributed to small variations in the negative stain between preparations as discussed in detail by Ford et al. (33). Fd is bound toward the end of the ridge directly above the PsaC and PsaE subunits and partly in contact with PsaD. Fig. 4 contrasts the previous model (Ref. 32, derived from Ref. 11) with the data presented here. Whereas the cyanobacterial model places the Fd on one side of the stromal ridge in contact with PsaC, PsaD, and PsaE, our data place Fd on top of the stromal ridge and primarily in contact with PsaE and PsaC. Remaining uncertainties about the exact extent of the Fd binding site are demonstrated in Fig. 3. A two-dimensional projection of spinach Fd has been generated from the three-dimensional Protein Data Bank coordinates (1A70) and modeled onto the stromal ridge of the untreated PS-I projection map. Although there is a very good agreement between the x-ray data and the Fourier vector difference map as far as the overall shape is concerned, it is clear that Fd is somewhat smaller in the projection from the x-ray data than that seen by negative staining.

A model for the binding of Fd to the PS-I reaction center is presented in Fig. 5. The Protein Data Bank coordinates (1A70) of the Fd from spinach (25) were modeled by real-space alignment onto the structural data for the α-carbon trace for the cyanobacterial PS-I reaction center, Protein Data Bank accession number 2PPS (9). The locations of the outlines of PsaD and PsaE were estimated from figures given in Klukas et al. (32) as the coordinates for these subunits do not appear in 2PPS. It was possible to site the spinach Fd on top of the stromal ridge in a location equivalent to the Fourier vector difference map in Fig. 2 and the spinach Fd two-dimensional projection in Fig. 3. This position brought the Fd iron-sulfur center and the Fe center in PsaC to a center-to-center distance of approximately 11 Å, which is in good agreement with the kinetic spectroscopic data (7). This distance was the closest approach of the two subunits when accounting for the limited structural data for 2PPS by allowing some extra distance between the subunits for side chains from PsaC. In conclusion, Fig. 5 shows that Fd can

FIG. 3. Projection map (stromal view) of the untreated PS-I unit cell (p2) displayed using gray scales with the outline of the complex and the stromal ridge highlighted. A two-dimensional projection contour map of spinach Fd, generated by SPIDER from the three-dimensional coordinates of Protein Data Bank accession number 1A70, is superimposed over this image. Scale bar = 2.5 nm.

FIG. 4. Schematic representation of the ferredoxin binding sites of vascular plant (above) and cyanobacterial (below) PS-I (modified from Ref. 32). The cyanobacterial outline is flipped and rotated relative to its original presentation (32) to be consistent with the projections presented in Figs. 2 and 3. The locations of the extrinsic stromal subunits follow the assignment of Schubert et al. (10) and Kitmitto et al. (19). Scale bar = 2.5 nm.
be modeled to interact principally with PsaC and PsaE in agreement with our electron microscopy data.

The results presented here do not preclude an interaction between Fd and PsaD, which has been implicated in Fd binding (14). Fig. 5 appears to suggest that Fd is not in contact with PsaD; however the location of PsaD, (derived from Ref. 32) is only presented in two dimensions to the side of PsaC. In reality PsaD is likely to extend around PsaC making interactions with Fd more likely. The accuracy of the modeling resides within the accuracy of the Fourier vector difference map (see above). This interpretation is supported by the Fourier vector difference map (Figs. 2 and 4). As discussed above, the area of stain exclusion associated with Fd binding extends over an area of the stromal ridge predicted to be occupied by PsaD.

It is interesting to note that the data presented from single particle analysis (17) for the cross-linked Fd-PS-I complex from *Synechocystis* sp. PCC6803 is in close agreement with the model for Fd binding in *Synechococcus* (32). This leads to an interesting dichotomy between the cyanobacterial system and the vascular plant system. One of the major structural differences between the photosynthetic systems is the presence of phycobilisomes on the cyanobacterial photosynthetic membrane. Models for the interaction between the phycobilisome and PS-I propose that the mobile light harvesting complex can reside on the stromal surface of PS-I (34). It is interesting to speculate on whether the mobility of the electron carrier, i.e. Fd, may be hindered in cyanobacteria with a phycobilisome attached if the Fd binding site was on top of the stromal ridge as it is in the vascular plant system. In plants there is no phycobilisome and the PS-I complexes are distributed on the edges of the granal stacks and in the stromal lamellae (1) where Fd is presumably freely accessible to the stroma. It is well established that PS-I is not found in the appressed regions of the granal stacks. It is possible that the 2–3-nm high stromal ridge cannot be accommodated between the membrane layers. A further refinement of the location of the Fd in PS-I must await a complete three-dimensional reconstruction using cryo-electron microscopy.

REFERENCES
1. Andersson, B., and Anderson, J. M. (1980) *Biochim. Biophys. Acta* 593, 427–440
2. Kitmitto, A., Holzenburg, A., and Ford, R. C. (1997) *J. Biol. Chem.* 272, 19497–19501
3. Golbeck, J. H. (1993) *Proc. Natl. Acad. Sci. U. S. A.* 90, 1642–1646
4. De Pascalis, A. R., Schurmann, P., and Bossard, H. R. (1994) *FEBS Lett.* 337, 217–220
5. Knaff, D. B. and Hirose, K. (1991) *Biochim. Biophys. Acta* 1056, 93–125
6. Sétié, P. Q., and Bottin, H. (1994) *Biochemistry* 33, 8495–8504
7. Sétié, P. Q., and Bottin, H. (1995) *Biochemistry* 34, 9059–9070
8. Krauss, N., Hinrichs, W., Witt, I., Fromme, P., Pritzkow, W., Dauter, Z., Betzel, C., Wilson, K. S., Witt, H. T., and Saenger, W. (1993) *Nature* 361, 326–331
9. Krauss, N., Schubert, W.-D., Klukas, O., Fromme, P., Witt, H. T., and Saenger, W. (1996) *Nat. Struct. Biol.* 3, 965–973
10. Schubert, W.-D., Klukas, O., Krauss, N., Saenger, W., Fromme, P., and Witt, H. T. (1997) *J. Mol. Biol.* 272, 741–769
11. Fromme, P., Schubert, W.-D., and Krauss, N. (1994) *Biochim. Biophys. Acta* 1187, 99–105
12. Fischer, N., Sétié, P., and Rochaix, J.-D. (1999) *J. Biol. Chem.* 274, 23335–23340
13. Jansson, S., Andersen, B., and Scheller, H. V. (1996) *Plant Physiol.* 112, 409–420
14. Chitnis, P. R., Reilly, P. A., and Nelson, N. (1989) *J. Biol. Chem.* 264, 18381–18385
15. Li, N., Zhao, J. D., Warren, P. V., Warden, J. T., Bryant, D. A., and Golbeck, J. H. (1991) *Biochemistry* 30, 7863–7872
16. Rousseau, F., Sétié, P., and Lauter, B. (1993) *EMBO J.* 12, 1755–1765
17. Lelong, C., Boekema, E. J., Krup, J., Bottin, H., Roby, M., and Sétié, P. (1998) *EMBO J.* 17, 2169–2176
18. Mühlenhoff, U., Krup, J., Bryant, D. A., Röger, M., Sétié, P., and Boekema, E. (1996) *EMBO J.* 15, 488–497
19. Kitmitto, A., Mustafa, A. O., Holzenburg, A., and Ford, R. C. (1998) *J. Biol. Chem.* 273, 29592–29599
20. Berthold, D. A., Babcock, G. T., and Yocum, C. F. (1981) FEBS Lett. 134, 231–234
21. Hermöller, S. (1992) Ultramicroscopy 36, 275–276
22. Amos, L., Henderson, R., and Urwin, P. N. T. (1982) Prog. Biophys. Mol. Biol. 39, 183–231
23. Bailey, S. (1994) Acta Crystallogr. Ser. D 50, 760–763
24. Frank, J., Shimkin, B., and Dowse, H. (1981) Ultramicroscopy 6, 343–358
25. Binda, C., Coda, A., Aliverti, A., Zanetti, G., and Mattevi, A. (1998) Acta Crystallogr. Ser. D. 54, 1353–1358
26. Lelong, C., Sérif, P., Lagoutte, B., and Bottin, H. (1994) J. Biol. Chem. 269, 10034–10039
27. Tagawa, K., and Arnon, D. I. (1968) Biochim. Biophys. Acta 153, 602–613
28. Shin, M. (1971) Methods Enzymol. 33, 440–446
29. Zanetti, G., and Merati, G. (1987) Eur. J. Biochem. 169, 143–146
30. Andersen, B., Koch, B., and Scheller, H. V. (1992) Physiol. Plantarum 84, 154–161
31. Merati, G., and Zanetti, G. (1987) FEBS Lett. 215, 37–40
32. Klukas, O., Schubert, W. D., Jordan, P., Krauss, N., Fromme, P., Witt, H. T., and Saenger, W. (1999) J. Biol. Chem. 274, 7351–7360
33. Ford, R. C., Rosenberg, M. F., Shepherd, F. H., McPhie, P., and Holzenburg. A. (1995) Micron 26, 133–140
34. Mullineaux, C. W. (1999) Aust. J. Plant Physiol. 26, 671–677