Residue Glu-91 of *Chlamydomonas reinhardtii* ferredoxin is essential for electron transfer to ferredoxin-thioredoxin reductase

Jean-Pierre Jacquot[a,*], Mariana Stein[a], Akira Suzuki[b], Sandrine Liottet[a], Gabriel Sandoz[a], Myroslawa Miginiac-Maslow[a]

[a] *URA 1128 CNRS, Université de Paris-Sud, Institut de Biotechnologie des Plantes, Bâtiment 630, 91405 Orsay-Cedex, France*
[b] *Laboratoire d'étude du métabolisme, INRA, route de Saint-Cyr, 78000 Versailles Cedex, France*

Received 10 October 1996; revised version received 18 November 1996

Abstract The [2Fe-2S] soluble ferredoxin from *Chlamydomonas reinhardtii* was mutated by site directed mutagenesis, using PCR and the expression plasmid pET-Fd as a template. The recombinant mutated proteins were purified to homogeneity and tested in the activation of NADP-malate dehydrogenase, a light dependent reaction in which ferredoxin thioredoxin reductase (FTR) and thioredoxin are involved. The mutation of residue Glu-91 (E92 in spinach, E94 in *Anabaena*) alone, either to Gln (E91Q) or to Lys (E91K), was found to completely abolish the reaction of the enzyme light activation. On the other hand, the mutants (E92Q) or (E92K) were as efficient as the wild type ferredoxin in this reaction whereas the double mutants (E91Q/E92Q) or (E91K/E92K) had no activity. In addition, a triple mutant (D25A/E28Q/E29Q) was also found to be inactive for this redox dependent light activation. All these mutations had much weaker effects on the ferredoxin/ferredoxin NADP reductase interaction as measured by the cytochrome *c* reduction assay. These results indicate that there is a recognition site for FTR in the C terminus part of ferredoxin, but also that a core of negatively charged residues in the α1 helix of ferredoxin might be important in the general process of light activation.

Key words: *Chlamydomonas reinhardtii*; Ferredoxin; Mutagenesis

1. Introduction

Chloroplastic soluble ferredoxins are small proteins containing a [2Fe-2S] iron sulfur center of low redox potential (ca. −420 mV). They play a key role, linking the photosynthetic electron transfer chain to various pathways, including fatty acid synthesis, nitrogen and sulfur assimilation, chloroplastic enzyme light regulation and NADP photoreduction necessary for the operation of the CO_2 fixation cycle [1]. The amino acid sequence identity between the various soluble ferredoxins is high (generally ca. 70%) for proteins ranging from cyanobacteria to higher plants [2,3]. At the molecular level, it is interesting to note that although soluble chloroplastic ferredoxin is small in size (93–100 amino acids), it is able to interact with a great variety of enzymes and to form strong protein-protein complexes [4,5]. The affinities are such that the interacting enzymes can be purified by affinity chromatography on ferredoxin sepharose [6]. So far, the most studied interaction of ferredoxin is with its associated enzyme ferredoxin NADP oxidoreductase (FNR), a protein involved in the NADP reduction in the chloroplast stroma. Contrasting results have been obtained by site directed mutagenesis, Hurley et al. [7] reporting a 20 000-fold decrease in the second order rate constant for electron transfer to FNR for the mutant (E94K) in *Anabaena*, and Piubelli et al. [8] finding only moderate effects of the equivalent mutation (E92K) in spinach. However, the two papers did not measure the same reaction, one utilizing flash laser spectroscopy, and the other cytochrome *c* reduction, which makes the results more difficult to compare. A report by de Pascalis et al. [9] based on experiments of chemical derivatization/protection indicates that the recognition sites for ferredoxin thioredoxin reductase (FTR, an enzyme involved in thiol dependent light regulation) are not identical to the ones for FNR. In that study, residues D34, D65, E92, E93, E94 and A97 were proposed as the binding site of FTR on the spinach ferredoxin molecule. Conversely, residues D26, E29, E30 and D65 constitute the binding site for FNR [10]. Based on these results, we investigated in this study the effects of two sets of mutations of *Chlamydomonas reinhardtii* ferredoxin, one triple mutant (D25A/E28Q/E29Q) in the helix α1 which was supposed to affect the reactivity with FNR and another set of mutants (involving residues E91 and E92) at the C terminus, designed to hamper the reactivity with FTR.

2. Materials and methods

2.1. Materials

Purified oligonucleotides, EuroTaq polymerase and restriction enzymes *Nco*I and *Bam*HI were obtained from Eurogentech. T4 DNA ligase was from Appligene and benzonase from Merck. The DNA sequencing kit was from Pharmacia. Chromatographic matrixes were from Pharmacia. All spectrophotometric measurements were carried out with a Uvikon 860 Kontron spectrophotometer.

2.2. Mutagenesis

The strategy and oligonucleotides used for the mutations are described in Fig. 1. Plasmid pET-Fd [11] was used as a template and in order to facilitate the subsequent cloning of the fragments, two 'cloning primers' (pETup and pETdo) homologous to plasmid sequences 334 bp upstream of the *Nco*I site and 217 bp downstream of the *Bam*HI site were constructed. C terminus single or double mutants were produced in a one step PCR reaction with the mutagenic oligonucleotide and primer pETup. The triple mutant (D25A/E28Q/E29Q) was generated in a two step method. First two overlapping mutated fragments of respectively ca. 400 bp were produced (primers pETup and oligo D25A, E28Q, E29Qdo and primers pETdo and oligo D25A, E28Q, E29Qup). The two mutated fragments were used in turn as templates together with oligos pETup and pETdo to generate a 850 bp cDNA fragment. The mutated fragments (850 bp for the triple mutant and 660 bp for all the other mutations) were purified by

*Corresponding author. Fax: (33) (1) 69336423.
E-mail: jacquot@ibp.u-psud.fr

Abbreviations: FNR, ferredoxin NADP oxidoreductase; FTR, ferredoxin thioredoxin-reductase; NADP-MDH, NADP malate dehydrogenase; WT, wild type

PII S0014-5793(96)01407-X

agarose gel electrophoresis and digested with *Bam*HI and *Nco*I. The resulting 300 bp fragments which contained the ferredoxin sequence were then ligated into pET-3d. *E. coli* XL 1 cells were then transformed and analyzed for the recombinant plasmid. Midipreps of plasmids of the positive colonies were then sequenced. All clones were found to be mutants and no other mutation was detected. All the work involving plasmid preparation, cloning and sequencing was carried out as in [12] or as described by the suppliers.

2.3. Expression and purification of WT and mutated ferredoxins

E. coli BL 21(DE3) cells were transformed by the recombinant mutagenic plasmids. A single colony was then used to inoculate 3 ml of LB medium supplemented with 50 μg/ml ampicillin. The culture was in turn amplified at 37°C to 400 ml (for ca. 9 h) in the presence of ampicillin, transferred into 5 liters of LB with no ampicillin and left shaking for 24 h at 28°C. Cells were then harvested by centrifugation at 5000×*g* for 5 min, resuspended in a minimal volume of Tris-HCl 30 mM pH 7.9 and stored frozen if necessary. This protocol was based on the following rationale. Firstly, we and others have observed that maintaining the cultures for a long time in stationary phase alleviates the need for induction by IPTG. This is interesting in terms of economic cost since IPTG is a very expensive product, and moreover the protein yields are enhanced in these conditions (this was also observed for fructose-1,6-bisphosphatase and thioredoxin *h*, unpublished). It is believed that in a complex medium such as LB, lactose can be an alternative inducer [13]. Secondly, since ferredoxin is a protein which contains an iron sulfur center, it is beneficiary to reduce the incubation temperature during the late stages of the production, so that the synthesis of the polypeptide which is extremely fast with the pET system [14] would not exceed the rate of iron sulfur assembly, resulting in the formation of insoluble aggregates (P. Schürmann, personal communication).

After thawing, the cells were broken by 4 passages through a French press cell at a pressure of 18000 psi and the ferredoxin fraction was collected by ammonium sulfate fractionation (it precipitated between 50–80% of the saturation). The proteins were collected by centrifugation and the pellet dissolved and dialysed overnight against 5 liters of Tris-HCl 30 mM pH 7.9 at 4°C. The sample was then applied on a DEAE Sephacel column (2.5×25 cm) washed with excess buffer and eluted with a linear gradient 250 ml–250 ml, 0–400 mM NaCl. The red fractions were pooled, concentrated on an Amicon cell equipped with a YM 10 membrane and treated with benzonase (an enzyme which degrades DNA and RNA) for ca. 1 h at 37°C. The sample was then centrifuged (30 min, 50000×*g*) and laid atop a Sephadex G50 column (5×80 cm). The column was developed by gravity and only the fractions with a A420/A273 > 0.38 were kept and concentrated by ultrafiltration as above. The samples were kept at −20°C at a concentration of 2–10 mg/ml.

2.4. Activity measurements

The ferredoxin-FTR linked activation of NADP malate dehydrogenase was followed as in [15]. The proteins used in the reconstituted light activation system were spinach thioredoxin *m* purified as in [16], spinach FTR purified as in [6] and recombinant sorghum NADP malate dehydrogenase (NADP-MDH) obtained as in [17]. The ferredoxin-FNR interaction was followed by the cytochrome *c* reduction assay [18].

3. Results and discussion

Fig. 2 shows partial sequence comparisons of three 2Fe-2S plant type soluble ferredoxins (the *Chlamydomonas reinhardtii* sequence used in this study, and the spinach and *Anabaena* sequences used in previous mutagenesis studies). As indicated in this figure, the similarity between the proteins around the putative interaction sites is very high. Noteworthy is the absence of a third negatively charged amino acid residue in the C terminus end of the *Chlamydomonas* sequence. Overall, the spinach and *Anabaena* proteins display ca. 70% similarity towards *Chlamydomonas reinhardtii* ferredoxin. As a consequence, the latter protein is certainly very similar to its counterparts from higher plants or cyanobacteria. This is further supported by earlier experiments where *Chlamydomonas reinhardtii* ferredoxin was found to be an excellent substitute for spinach ferredoxin as an electron donor to either FNR or FTR ([19] and unpublished results], and for the *Synechocystis* 6803 ferredoxin in the reduction process by the photosystem I [20]. Despite all the similarities, *Chlamydomonas reinhardtii* ferredoxin also presents some notable differences when compared to spinach ferredoxin, the most important being that it is readily precipitated with ammonium sulfate [19] while the spinach protein remains soluble even at 90% ammonium sulfate. This suggests that they have different hydrophobic properties, the *Chlamydomonas reinhardtii* molecule having a global deficit of four negative charges and being thus less hydrophilic.

In this study, seven different mutations of *Chlamydomonas reinhardtii* ferredoxin have been constructed. The recombinant plasmids were sequenced to verify that the right mutants had been generated and that no other mutation had occurred (see Section 2). As earlier experiments had shown that the yield of ferredoxin production was low, even when using the plasmid pET, we checked several expression conditions. The best production yields were obtained by growing the production strain BL 21(DE3) for a long period (ca. 24 h) at 28°C with no induction by IPTG (see Section 2).

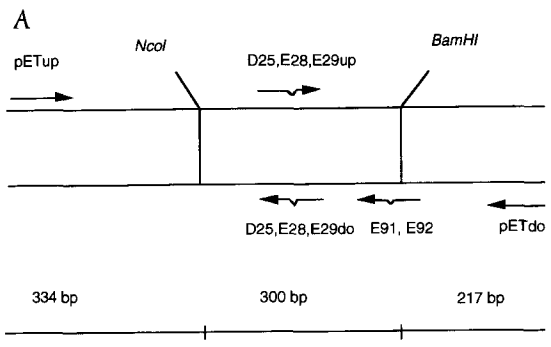
All mutated proteins were purified to homogeneity as described in Section 2. Special care was taken to ensure that all protein preparations had a very low nucleic acid content, since the FTR-dependent NADP-MDH light activation reaction is inhibited by these compounds for reasons that are not yet understood (unpublished results). The removal of nucleic acids was achieved through the use of the enzyme benzonase which degrades both RNA and DNA molecules. All ferredoxin fractions used in this study had a nucleic acid content less than 2% (w/w protein). Table 1 shows the final yields and A420/A273 absorbance ratios of all the proteins. Depending on the nature of the mutation, the yields were either similar to those obtained with the wild type protein or substantially lower (especially for the double mutant (E91K/E92K)). It is possible that in this case the double mutation somehow destabilizes the protein, resulting in the final poor yield. On the other hand, the triple mutant (D25A/E28Q/E29Q) could be recovered with reasonably good yields suggesting that its structure is not substantially altered.

The effects of the various mutations on the reactivity either with FNR or with FTR are listed in Tables 2 and 3 and Fig. 3. The ability of ferredoxin to donate electrons to FTR is measured through a reconstituted light activation system where photochemically reduced ferredoxin serves as an elec-

Table 1
Absorbance ratios and yield of the different ferredoxins

Mutation	$A_{420/273}$	Yield
WT	0.52	48.5
E91Q	0.52	30
E91K	0.41	23.5
E92Q	0.45	38
E92K	0.39	12
E91Q, E92Q	0.65	3.5
E91K, E92K	0.39	1.5
D25A, E28Q, E29Q	0.59	10

The yield is expressed as mg purified protein per 5 liters culture. $A_{420/273}$ indicates the relative absorbances of the prurified preparations at 420 and 273 nm.



B

Mutation	Synthetic primer	Codon change
D25A, E28Q, E29Qup	5' TACATCCTGGCCGCCTGCTCAGCAGGCCGGCCTG 3'	GAC → GCC
D25A, E28Q, E29Qdo	5' CAGGCCGGCCTGCTGAGCAGCGGCCAGGATGTA 3'	GAG → CAG
E91Q	5' CAGCCGGATCCTTAGTACAGGGCCTCCTGCTGGT 3'	GAG → CAG
E91K	5' CAGCCGGATCCTTAGTACAGGGCCTCCTTCTGGT 3'	GAG → AAG
E92Q	5' CAGCCGGATCCTTAGTACAGGGCCTGCTCCTGGT 3'	GAG → CAG
E92K	5' CAGCCGGATCCTTAGTACAGGGCCTTCTCCTGGT 3'	GAG → AAG
E91Q, E92Q	5' CAGCCGGATCCTTAGTACAGGGCCTGCTGCTGGT 3'	
E91K, E92K	5' CAGCCGGATCCTTAGTACAGGGCCTTCTTCTGGT 3'	
pETup	5' AGTAGGTTGAGGCCGTTGAGCACCGCCGCCGC 3'	
pETdo	5' GCCGCCGCCCAGTCCTGCTCGCTTCGCTAC 3'	

Fig. 1. Strategy for mutagenesis and oligonucleotides used. A: The shaded area is the coding sequence for ferredoxin. Open area: pET sequence. Mutagenic oligonucleotides are indicated by a broken arrow. B: *Bam*HI sites are underlined and mutagenic bases are in bold characters.

tron donor to finally activate NADP-MDH via the FTR and thioredoxin. Interaction with FNR is measured in a reverse reaction where NADPH is the electron donor and ferredoxin serves in the reduction of cytochrome *c*. None of the mutants tested was found to be completely inactive in the cytochrome *c* reduction assay, indicating that they are all still able to serve as a substrate for FNR. Two mutations ((E91Q) and (E91Q/E92Q)) actually decrease the K_m by a factor of 2–3 and one (D25A/E28Q/E29Q) increases the K_m value by a factor of 4. All the other mutations had little effect in the cytochrome *c* assay. The effect of the mutation (D25A/E28Q/E29Q) on the interaction with FNR agrees well with the results described in [10].

Table 2
Reactivity of FNR with *Chlamydomonas reinhardtii* ferredoxin mutants

Mutation	K_m (μM)	kcat (s^{-1})
WT	0.40 ± 0.02	37
E91Q	0.10 ± 0.02	34
E91K	0.38 ± 0.02	38
E92Q	0.92 ± 0.2	39
E92K	0.35 ± 0.15	35
E91Q, E92Q	0.17 ± 0.2	37
E91K, E92K	0.57 ± 0.2	42
D25A, E28Q, E29Q	1.70 ± 0.2	37

Cytochrome *c* reduction was measured at 30°C and followed spectrophotometrically at 550 nm. The reaction mix (1 ml) contained 1 μg FNR, 0.2 μmol NADPH and 500 μg cytochrome *c* in Tris-HCl 30 mM pH 7.9. The ferredoxin concentration varied between 0 and 3 μM.

```
                30                          90
1 ---YILDAAEEAGL----        ----QTHQEEALY
                31                          91
2 ---YILDAAEEEGI----        ----ETHKEEELTA
                33                          93
3 ---YILDAAEEQGY----        ----QTHKEEDLY
```

Fig. 2. Partial sequence comparison of *Chlamydomonas reinhardtii*, *Spinacia oleracea* and *Anabaena* ferredoxins. 1: *Chlamydomonas reinhardtii*, 95 amino acid residues [11]; 2: *Spinacia oleracea*, (ferredoxin I), 96 amino acid residues [25]; 3: *Anabaena* (vegetative cells), 98 amino acid residues [26,27]. The amino acids mutated in this study are underlined.

When the same mutants were tested in the assay of NADP-MDH light activation, strikingly different results were obtained (Table 3). All mutants on the residue Glu-91 were completely inactive as substrates of FTR (the activity values are equivalent to the dark control; not shown) regardless of whether the negative charge was replaced by a null charge (E91Q mutation) or by a positive charge (E91K mutation). Equally interesting was the observation that the single mutants of the adjacent Glu-92 residue behaved exactly as the wild type protein. The double mutants (E91Q/E92Q) and (E91K/E92K) were totally ineffective, indicating that the Glu-91 mutation was dominant. Finally, (D25A/E28Q/E29Q) was also totally inactive with FTR. These results agree to some extent with the results of de Pascalis et al. [9] who showed that there is indeed a recognition site for FTR in the C terminus of *Chlamydomonas reinhardtii* ferredoxin. But our results are also more precise since the chemical derivatization studies showed that residues E92, E93 and E94 of spinach ferredoxin were shielded when the protein was incubated with FTR. Our results strongly suggest that in the C terminus, only residue Glu-91 (E92 in spinach) is essential for this interaction. The results obtained in the present study also fit very well with the data of [8] where mutations of the E92 residue of spinach ferredoxin was found to increase its reactivity with FNR in the cytochrome *c* reduction assay. On the other hand, the triple mutant in the α1 helix is completely inactive in the NADP-MDH light activation assay, a feature which was not expected from the results in [10]. Then, either it is not reduced by PSI or it is unable to interact with FTR. We still do not have enough information to decide which of the

Table 3
Light dependent activation of sorghum NADP-MDH

Mutation	NADP-MDH activity
WT	0.90
E91Q	0.04
E91K	0.05
E92Q	1.06
E92K	1.08
E91Q, E92Q	0.02
E91K, E92K	0.01
D25A, E28Q, E29Q	0.06

The activation mix (50 μl) contained freshly isolated pea thylakoids (30 μg), spinach ferredoxin thioredoxin reductase (2 μM), spinach thioredoxin *m* (20 μM), recombinant sorghum NADP-MDH (1 μM) in Tris-HCl 30 mM pH 7.9. After 5–8 min incubation in the light, 20 μl aliquots were used for activity determination. The ferredoxin concentration was ca. 10 μM. The results are expressed in ΔA_{340}/min.

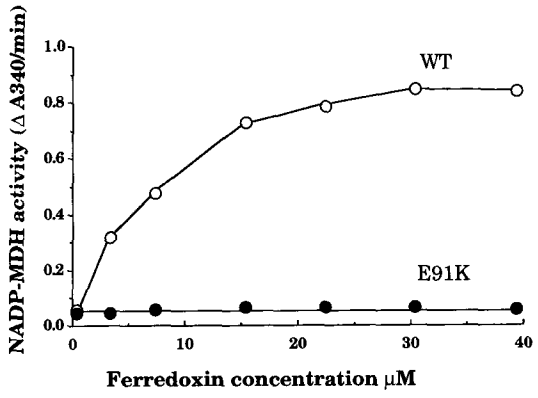


Fig. 3. Activation rates of NADP-MDH as a function of ferredoxin concentration. Experimental conditions are as in Table 3. The Glu-91 ferredoxin mutants were tested at several concentrations, up to 200 μM, and were totally inactive through the whole range.

possibilities is the right one. On the other hand, the Glu-91 mutants of *Chlamydomonas reinhardtii* are indeed reduced by PSI since the (E91K) mutant supports NADP photoreduction, albeit at a reduced rate (ca. 50%, data not shown), in a similar manner to the corresponding (E92K) mutant of spinach [8]. It is highly unlikely that the incapacity of the Glu-91 mutants to promote the NADP-MDH activation results from the poorer interaction with PSI since the process of enzyme light activation requires a much lower electron flow than NADP photoreduction [21]. In addition the neighboring Glu-92 residue in *Chlamydomonas reinhardtii* is the residue which cross-links to PSI in Synechocystis [22]. As a consequence its mutation is very likely to severely impair the light dependent reduction of ferredoxin. Nevertheless, as noted above, the Glu-92 mutants are perfectly active, an observation which agrees very well with the low electron flow requirement for enzyme photoactivation. All available evidence thus indicates that the Glu-91 mutation affects the complex formation with FTR as proposed in [9]. The *Chlamydomonas reinhardtii* E91K mutant was also found to be less effective as a substrate of GOGAT and nitrite reductase, but it could nevertheless still be used as a substrate by these enzymes (data not shown). In conclusion, these data suggest that the mutation of a single Glu residue in the C terminus part of ferredoxin is able to selectively impair the light dependent thiol/disulfide regulation of chloroplast enzymes. Thus, the use of such a mutant could be a very powerful tool to evaluate the effect of this process in vivo.

In this paper, all the experiments involving FNR were effected with a *Chlamydomonas reinhardtii* recombinant unmethylated protein produced in *Escherichia coli* cells (Decottignies et al., in preparation). This enzyme is thus different from the protein isolated from algal cells where the protein is methylated at three different positions (K83, K89 and K135) [23]. K89 is the structural equivalent of spinach K88 which is one of the positive charges of FNR involved in the interaction with ferredoxin [24]. Further experiments aimed at evaluating the effect of FNR methylation on the interaction with ferredoxin are under way, using both methylated and unmethylated FNR and the various ferredoxin mutants.

Acknowledgements: The authors would like to thank the students Karine David, Gaëlle Henry, Nemo Peeters, Nicolas Sicard and Lyne Bricka for participation in the experiments and Paulette Decottignies and Stephane Lemaire for critical reading of the manuscript.

References

[1] Arnon, D.I. (1988) Trends Biochem. Sci. 13, 30–33.
[2] Tsukihara, T., Fukuyama, K., Mizushima, M., Harioka, T., Kusunoki, M., Katsube, Y., Hase, T. and Matsubara, H. (1990) J. Mol. Biol. 216, 399–410.
[3] Rypniewski, W.R., Breiter, D.R., Benning, M.M., Wesenberg, G., Byung-Ha, O., Markley, J.L., Rayment, I. and Holden, H.M. (1991) Biochemistry 30, 4126–4131.
[4] Ricard, J., Nari, J. and Diamantidis, G. (1980) Eur. J. Biochem. 108, 55–66.
[5] Hirasawa, M., Chang, K.T. and Knaff, D.B. (1991) Arch. Biochem. Biophys. 286, 171–177.
[6] Droux, M., Jacquot, J.P., Miginiac-Maslow, M., Gadal, P., Huet, J.-C., Crawford, N.A., Yee, B.C. and Buchanan, B.B. (1987) Arch. Biochem. Biophys. 252, 426–439.
[7] Hurley, J.K., Salamon, T.E., Meyer, J.C., Fitch, J.C., Cusanovich, M.A., Markley, J.L., Cheng, H., Xia, B., Chae, Y.K., Medina, M., Gomez-Moreno, C. and Tollin G. (1993) Biochemistry 32, 9346–9354.
[8] Piubelli, L., Aliverti, A., Bellintani, F. and Zanetti, G. (1996) Eur. J. Biochem. 236, 465–469.
[9] de Pascalis, A.R., Schürmann, P. and Bosshard, H.R. (1993) FEBS Lett. 337, 217–220.
[10] de Pascalis, A.R., Jelesarov, I., Ackermann, F., Koppenol, W.H., Hirasawa, M. and Bosshardt, H. (1993) Protein Sci. 2, 1126–1135.
[11] Rogers, W.J., Hodges, M., Decottignies, P., Schmitter, J.M., Gadal, P. and Jacquot, J.P. (1992) FEBS lett. 310, 240–245.
[12] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
[13] Hofman, B.J., Broadwater, J.A., Johnson, P., Harper, J., Fox, B.G. and Kenealy, W.R. (1995) Protein Exp. Purif. 6, 646–654.
[14] Studier, F.W., Rosenberg, A.H., Dunn, J.J. and Dubendorff, J.W. (1990) Methods Enzymol. 185, 60–89.
[15] Jacquot, J.P., Issakidis, E., Decottignies, P., Lemaire, M. and Miginiac-Maslow, M. (1995) Methods Enzymol 252, 240–252.
[16] Schürmann, P. and Jacquot, J.P. (1979) Biochim. Biophys. Acta 569, 309–312.
[17] Issakidis, E., Decottignies, P. and Miginiac-Maslow, M. (1993) FEBS Lett. 321, 55–58.
[18] Jacquot, J.P., Suzuki, A., Peyre, J.B., Peyronnet, R., Miginiac-Maslow, M. and Gadal, P. (1988) Eur. J. Biochem. 174, 629–635.
[19] Sétif, P.Q.Y. and Bottin, H. (1994) Biochemistry 33, 8495–8504.
[20] Schmitter, J.M., Jacquot, J.P., de Lamotte-Guéry, F., Beauvallet, C., Dutka, S., Gadal, P. and Decottignies, P. (1988) Eur. J. Biochem. 172, 405–412.
[21] Miginiac-Maslow, M., Jacquot, J.P. and Droux, M. (1985) Phot. Res. 6, 201–213.
[22] Lelong, C., Sétif, P., Lagoutte, B. and Bottin, H. (1994) J. Biol. Chem. 269, 10034–10039.
[23] Decottignies, P., Le Maréchal, P., Jacquot, J.P., Schmitter, J.M. and Gadal, P. (1995) Arch. Biochem. Biophys. 316, 249–259.
[24] Aliverti, A., Corrado, M.E. and Zanetti, G. (1994) FEBS Lett. 343, 247–250.
[25] Wedel, N., Bartling, D. and Herrmann, R.G. (1988) Bot. Acta 101, 295–300.
[26] van der Plas, J., de Groot, R.P., Weisbeek, P.J. and van Arkel, G.A. (1986) Nucleic Acids Res. 14, 7803.
[27] Alam, J., Whitaker, R.A., Krogmann, D.W. and Curtis, S.E. (1986) J. Bacteriol. 168, 1265–1271.