Characterization of the NifS-like Domain of ABA3 from Arabidopsis thaliana Provides Insight into the Mechanism of Molybdenum Cofactor Sulfuration*

Received for publication, September 30, 2004, and in revised form, November 10, 2004
Published, JBC Papers in Press, November 22, 2004, DOI 10.1074/jbc.M411195200

Torsten Heidenreich, Silke Wollers, Ralf R. Mendel‡, and Florian Bittner
From the Department of Plant Biology, Technical University of Braunschweig, 38023 Braunschweig, Germany

The molybdenum cofactor sulfurate ABA3 from Arabidopsis thaliana specifically regulates the activity of the molybdenum enzymes aldehyde oxidase and xanthine dehydrogenase by converting their molybdenum cofactor from the desulfo-form into the sulfo-form. ABA3 is a two-domain protein with an NH2-terminal domain cofactor from the desulfo-form into the sulfo-form. ABA3 is a two-domain protein with an NH2-terminal domain sharing significant similarities to NifS proteins that catalyze the decomposition of L-cysteine to L-alanine and elemental sulfur for iron-sulfur cluster synthesis. Although different in its physiological function, the mechanism of ABA3 for sulfur mobilization was found to be similar to NifS proteins. The protein binds a pyridoxal phosphate cofactor and a substrate-derived persulfide intermediate, and site-directed mutagenesis of strictly conserved binding sites for the cofactor and the persulfide demonstrated that they are essential for molybdenum cofactor sulfurate activity. In vitro, the NifS-like domain of ABA3 activates aldehyde oxidase and xanthine dehydrogenase in the absence of the C-terminal domain, but in vivo, the C-terminal domain is required for proper activation of both target enzymes. In addition to its cysteine desulfurase activity, ABA3-NifS also exhibits selenocysteine lyase activity. Although L-selenocysteine is unlikely to be a natural substrate for ABA3, it is decomposed more efficiently than L-cysteine. Besides mitochondrial AtNFS1 and plastidial AtNFS2, which are both proposed to be involved in iron-sulfur cluster formation, ABA3 is proposed to be a third and cytosolic NifS-like cysteine desulfurase in Arabidopsis thaliana. However, the sulfur transferase activity of ABA3 is used for post-translational activation of molybdenum enzymes rather than for iron-sulfur cluster assembly.

NifS and NifS-like enzymes are present in almost all organisms and fulfill their main functions during iron-sulfur ([Fe-S]) cluster synthesis. Accordingly, they have a cysteine desulfurase activity that is required for the mobilization of sulfur from L-cysteine by simultaneous release of L-alanine. In all NifS-like enzymes, the sulfide is bound as a persulfide to a conserved cysteine residue of the protein, from which it is subsequently transferred to other target proteins such as the scaffold proteins NilU and/or IscU in bacteria to finally assemble [Fe-S] clusters. A pyridoxal phosphate (PLP) cofactor bound to a conserved lysine residue is essential for this cysteine desulfurase activity (1). However, besides [Fe-S] cluster formation, other functions for NifS-like proteins are described as well. The Escherichia coli IscS protein also was found to be involved in the biosynthesis of thiamin and NAD+ and to be able to transfer a sulfur atom to uridine to produce a 4-thio-uridine tRNA (2). In E. coli, another NifS-like protein, SufS (CsdB), is able to catalyze the elimination of selenium from L-selenocysteine more efficiently than the elimination of sulfur from L-cysteine (3). However, its physiological function is connected to its cysteine desulfurase activity, which is up to 50× higher when SufS forms a complex with SufE (4). The only NifS-like protein in yeast, Nis1p, is essential not only for [Fe-S] cluster formation in mitochondria but also for tRNA splicing (5). For cell viability, this protein also needs to be localized in the cytosol and to some extent in the nucleus (6).

Similar to bacteria, Arabidopsis thaliana also possesses more than one NifS-like protein: AtNFS1, which is located in mitochondria (7), and AtNFS2, which is located in chloroplasts (8,9). However, cysteine desulfurase activity was also shown for ABA3 (10). ABA3 is a molybdenum cofactor (Moco) sulfurate consisting of an N-terminal NifS-like domain that is fused to a C-terminal domain with an as yet unknown function, thereby being distinguished from all other NifS-like proteins described thus far. Although its physiological function is unrelated to [Fe-S] cluster assembly, cysteine desulfurase activity and sulfur transferase activity of ABA3 were shown to be essential for the activation of the two Moco-containing enzymes aldehyde oxidase (AO; EC 1.2.3.1) and xanthine dehydrogenase (XDH; EC 1.1.1.204) that are involved in abscisic acid biosynthesis and degradation of purines, respectively (for review, see Refs. 11–13). After having incorporated the Moco, AO and XDH remain inactive unless one of the two oxygen ligands at the molybdenum center is replaced by a sulfur atom that is delivered by ABA3. This sulfuration step catalyzes the final maturation of AO and XDH enzymes that occur in both the sulfide and the desulfo-form in the living cell. By this mechanism, the plant is able to rapidly increase the activities of AO and XDH, e.g. for adapting to altering environmental conditions without de novo synthesis of AO and XDH apoproteins.

In this work, we examined the mechanism of sulfur mobilization as catalyzed by the NifS-like domain of ABA3 from Arabidopsis thaliana. We identified the nature of the cofactor and the sulfur bound by ABA3-NifS and characterized the sub-activities of the protein. Finally, we examined the function of the highly conserved lysine 271 and cysteine 430, and we discussed the role of the NifS-like domain during Moco sulfuration.
NifS-like Domain of Molybdenum Cofactor Sulfurase ABA3

EXPERIMENTAL PROCEDURES

Construction of Expression Vectors—During purification of the N-terminally His6-tagged protein, it became obvious that ABA3 is not only present in full-length with a molecular mass of about 90 kDa but also as a C-terminally truncated cleavage product. The molecular mass of this protein was subcloned into pET32a(+) and expressed in E. coli BL21 (DE3). The C-terminal region of 1518 bp of the aba3 gene was amplified and subcloned into the EcoRI and HindIII sites of pMAL-c2X (New England Biolabs). The resulting protein was expressed in E. coli DH5α and purified using amylose resin. The protein was subsequently dialyzed against 20 mM Tris/HCl, pH 8.0, 2 mM DTT, and 0.5 mM EDTA and applied to a nickel-nitrilotriacetic acid superflow matrix (Qiagen) under native conditions at 4 °C according to the manufacturer’s instructions. Proteins were eluted using 0.2 mM isopropyl β-D-thiogalactopyranoside and 5 mM lactose/liter expression culture. After dialysis, proteins were subjected to anion exchange chromatography with 20 mM Tris/HCl, pH 8.0, 2 mM DTT, and 0.5 mM EDTA.

Enzyme Assays—L-Cysteine desulfurase activity of recombinant ABA3-NifS, ABA3-NifS-like domain, and ABA3-C was assayed using L-cysteine as substrate as described previously (10). For expression of ABA3-NifS, cells were grown at 37 °C to an A600 of 0.5 before a combined induction with 0.2 mM isopropyl β-D-thiogalactopyranoside and 5 mM lactose/liter expression culture. After induction, cells were cultured for an additional 22 h at 22 °C. Hyperproduction of the C-terminal domain of ABA3 was performed by growing cells at 37 °C to an A600 of 0.5 before induction with 0.2 mM isopropyl β-D-thiogalactopyranoside and additional culturing for 20 h at 30 °C. Cells were harvested by centrifugation and stored at −70 °C until use.

Expression of ABA3 in Yeast—Oversynthesis of recombinant His6-tagged Arabidopsis AOA in the yeast Pichia pastoris (kindly provided by Tomokazu Koshiba, Tokyo, Japan) was performed as described previously (14).

Purification of His6-tagged Proteins—Purification of recombinant ABA3, its separately expressed domains, and AOA was performed on a nickel-nitrilotriacetic acid superflow matrix (Qiagen) under native conditions following the manufacturer’s instructions. The resulting proteins were rebuffered to 20 mM Tris/HCl, pH 8.0, containing 2 mM diethytheritol (DTT) and stored at 4 °C, except for the C-terminal domain of ABA3 that was found to be unstable in low-salt buffers. For further purification, ABA3 proteins were subject to anion exchange chromatography using a 10-ml Source Q-15 column (Amersham Biosciences) equilibrated with 20 mM Tris/HCl, pH 8.0, 2 mM DTT, and 0.5 mM EDTA (buffer A). Protein samples were applied to the column and eluted with buffer A followed by a linear gradient of 0–1 M NaCl in buffer A. Final purification and size determination were achieved by chromatography on a preparative 100-ml Superose 12 size exclusion column (Amersham Biosciences) equilibrated with 20 mM Tris/HCl, pH 8.0, containing 300 mM NaCl and 2 mM DTT.

Enzyme Assays—L-Cysteine desulfurase activity of recombinant ABA3 was measured by monitoring either as the amount of L-alanine or as the amount of hydrogen sulfide released from L-cysteine as described previously (Refs. 1 and 15, respectively). L-Cysteine lyase activity was measured by determination of H2Se with lead acetate as described previously (14). L-Selenocysteine lyase activity always was freshly prepared from its oxidized form, L-selenocystine, and measured by following the production of H2S. Specific activities were determined with 20 μg of ABA3-NifS using 0.5 mM of each substrate during 10 min at 37 °C. Inactivation of recombinant AOA by cyanide was performed in 20 mM Tris/HCl, pH 8.0, in the presence of 50 mM KCN for 20 h at 37 °C. After this incubation period, the generated thiocyanate was removed by rebuffering the protein to 20 mM Tris/HCl, pH 8.0, before subsequent reconstitution assays. In vitro reconstitution of recombinant AOA by ABA3 was performed aerobically in a total volume of 150 μl of 20 mM Tris/HCl, pH 8.0. Three μg of AOA were incubated with the respective ABA3 protein (10 μl ABA3 protein/mmol AOA) in the presence of 0.5 μM L-cysteine for 1 h at 37 °C, followed by native PAGE with 0.33 volume of the reaction mixture and activity staining with indole-3-carboxaldehyde as substrate as described in Ref. 14.

Determination of Protein Concentrations—Concentrations of total soluble protein were determined by use of Roti Quant solution (Roth, Karlsruhe, Germany) as described in Ref. 17.

Wavelength of ABA3 Proteins—Absorption spectroscopy was carried out using an Ultrascop 3000® spectrophotometer (Amersham Biosciences).

Identification of Pyridoxal Phosphate as the ABA3-bound Chromophore—PLP was identified by the specific fluorescence of a PLP-cyanide product that is formed during coinoculation of free PLP and cyanide (18). PLP therefore has to be released from the protein by combined treatment with trichloroacetic acid and heating, by which the protein is denatured. Subsequent addition of potassium cyanide and adjustment of pH to 3.8 allows the detection of the PLP-cyanide product by following the fluorescence at 425 nm after excitation at 325 nm. Comparison of PLP-cyanide-specific fluorescence with a PLP standard curve allows the determination of absolute PLP amounts within a given protein sample of known concentration. As known from other NiFS-like enzymes and indicated by the presence of one putative PLP-binding site within the primary sequence of ABA3, binding of one PLP molecule per monomer was assumed. The concentration of the protein in each sample therefore was adjusted to 1 ± 1 nmol to fit into the standard curve range (0–1 nmol). Because the molecular mass of the NiFS-like domain of ABA3 is 55 kDa, a maximum amount of 55 μg of purified ABA3-NifS (1 μg = 0.01818 nmol) per sample can be used. Finally, fluorescence of the protein samples was measured, and excitation and emission spectra were detected and compared with those of pure PLP.

Identification of the ABA3-bound Persulfide Intermediate—To prove the nature of the transferable sulfur bound by ABA3-NifS, we followed a procedure described previously (19). Initially, 250 μg of ABA3-NifS were coincubated with 1 mM L-cysteine for 30 min in a total volume of 500 μl to yield maximum saturation of the protein with transferable sulfur. Excess substrate in the sample was removed by buffer exchange using a microcon concentrator with a 30-kDa molecular weight cutoff (Millipore). ABA3-NifS then was coincubated with 0.2 mM 1,5-AEDANS for 1 min at 25 °C. The excess of unbound 1,5-AEDANS was removed by buffer exchange as described above to avoid nonspecific fluorescence. Subsequently, addition of 1 mM DTT released only those 1,5-AEDANS molecules from the protein that were bound via a disulfide bridge. The amount of the enzyme-bound cysteinyl persulfide. After separating low mass molecules from the protein sample, the low mass molecule fraction was analyzed for 1,5-I-AEDANS-specific fluorescence under UV light. A protein sample that was incubated in the absence of L-cysteine but treated in the same way at all other steps served as a control.

RESULTS

Identification of the ABA3-bound Chromophore as PLP—A specific motif with an invariant lysine residue, conserved among NiFS-like enzymes (1), argued for PLP as a chromophoric cofactor bound to ABA3-NifS. Spectra of protein samples that were treated as described under “Experimental Procedures” were compared with those of pure PLP and found to be very similar, thus indicating that PLP is in fact PLP (Fig. 1). Furthermore, comparison of the protein-derived fluorescence with a PLP standard curve showed that the calculated number of PLP molecules bound by one monomer of ABA3-NifS is 0.91 in protein samples that were obtained when culturing E. coli in the presence of 5 μM pyridoxine (Table 1). Lowering the pyridoxine concentrations in the E. coli growth medium also lowered the values for the PLP/monomer ratio, indicating that supplementation with pyridoxine is required when hyperproducing PLP-binding ABA3-NifS in E. coli and that each monomer of ABA3-NifS binds one PLP molecule.
Identification of the ABA3-bound Sulfur as Persulfide—For specific proof of a bound persulfide intermediate, we used the fluorescent alkylating reagent 1,5-I-AEDANS, which binds to exposed thiol groups of protein cysteinyl residues, thereby forming either thio-esters at free SH groups or disulfide bonds in the case of prebound persulfides. Once having bound to free SH groups, 1,5-I-AEDANS cannot be released from the protein by reducing agents such as DTT, whereas 1,5-I-AEDANS linked to the protein by disulfide bonds can be cleaved off efficiently in the presence of DTT by reducing both thiol groups of the disulfide bond. 1,5-I-AEDANS released from the protein by DTT treatment can be identified by its specific fluorescence. Our results show that a significantly higher fluorescence was detected in samples that contained ABA3-NSiS pre-incubated with L-cysteine in comparison with samples that were not pre-incubated with L-cysteine (Table II). Thus, it can be concluded that during the incubation of ABA3-NSiS with L-cysteine, a persulfide intermediate was formed that was later released by DTT treatment. Furthermore, significantly less 1,5-I-AEDANS-specific fluorescence was detected when using an altered protein carrying an alanine instead of a cysteine residue at position 430 (ABA3-NSiS/C430A), indicating that the motif responsible for binding the persulfide intermediate contains cysteine 430 as an essential core residue.

Cysteine Desulfurase Activity of ABA3-NSiS—To determine whether cysteine desulfurase activity of ABA3 is located within the NiS-like domain, we used ABA3-NSiS to convert L-cysteine into L-alanine and elemental sulfur according to Ref. 1 and as described previously for full-length ABA3 (10). ABA3-NSiS converted L-cysteine even more efficiently within a given time than equimolar amounts of full-length ABA3 (Fig. 2A). Addition of the C-terminal domain to the NiS-like domain reduced rather than enhanced cysteine desulfurase activity. These results were also reflected by the in gel AO activity assay, in which the sulfur as mobilized from L-cysteine and bound to ABA3 or ABA3-NSiS, respectively, is transferred to the Moco of recombinant AOa. The NiS-like domain of ABA3 alone was found to have the ability to donate sulfur for activation of AOa (Fig. 2B). Furthermore, as seen for cysteine desulfurization, ABA3-NSiS was more efficient than full-length ABA3 in activation of AOa, whereas the presence of the C-terminal domain again had no enhancing effect.

Additionally, we quantified the amount of sulfur that was released as hydrogen sulfide in the presence of the reductant DTT to analyze the conversion of sulfur substrates other than L-cysteine that might not release L-alanine. Of all putative sulfur substrates tested, only L-cysteine and L-cysteine methylster were converted by ABA3-NSiS. However, the amount of H$_2$S detected when using L-cysteine methylster as substrate was only 37.9% relative to L-cysteine. No release of H$_2$S was detected from the other tested substrates (L-cysteine methylster, glutathione, $\beta$-mercaptopyruvate, L-cysteamine, and L-cystine).

Identification of Residues Essential for Catalysis—Substitution of the invariant ABA3-NSiS lysine 271, which corresponds to Azotobacter vinelandii cysteine 271, with a serine (ABA3-NSiS/R271S) resulted in complete loss of the yellow color that is typical for purified PLP-containing ABA3-NSiS protein. This change in color is accompanied by a change of the visible spectrum of the altered protein. Whereas the spectrum of ABA3-NSiS showed a maximum at 420 nm, no maximum but just a shoulder could be observed in the ABA3-NSiS/K271S spectrum (Fig. 3A). On the activity level, this mutation caused a complete loss of cysteine desulfurase activity and Moco sulfurrease activity (Fig. 3, B and C).

Substituting the cysteinyl residue, which corresponds to A. vinelandii cysteine 325 and is proposed to bind the persulfide (19), with an alanine (ABA3-NSiS/C430A) did not affect the spectroscopic properties of ABA3 (data not shown) but strongly...
reduced the two sub-activities of the protein. A residual cysteine desulfurase activity of 14% was found when comparing ABA3-NifS/C430A with ABA3-NifS (Fig. 3B). This decreased ability to mobilize sulfur from L-cysteine is reflected by the reduced ability of ABA3-NifS/C430A to activate recombinant AOα (Fig. 3C).

Selenocysteine Lyase Activity of ABA3-NifS—Most NifS-like proteins catalyze not only the formation of elemental sulfur and alanine from L-cysteine but also the formation of elemental selenium and alanine from L-selenocysteine. It was found that ABA3-NifS decomposed the selenium substrate even more efficiently than the sulfur substrate L-cysteine. Using 0.5 mM L-cysteine as substrate, the specific activity of the purified enzyme was determined to be 3.7 mol product/min/mol protein, as measured by the production of H2S (Table III). In contrast, the specific activity with L-selenocysteine as substrate was 21.4 mol product/min/mol protein, as measured by the formation of L-alanine. Whereas the apparent \( K_m \) of ABA3-NifS for L-cysteine is 50 \( \mu \)M, the \( K_m \) for L-selenocysteine is 200 \( \mu \)M, indicating that ABA3-NifS prefers L-cysteine over L-selenocysteine. However, it should be noted that deviation from standard Michaelis-Menten kinetics was observed at concentrations above 1.0 mM for either substrate, thereby precluding the determination of true \( K_m \) values for ABA3-NifS. Furthermore, because activity of ABA3-NifS is very sensitive to pH and temperature, the \( K_m \) value might also depend on the conditions used.

ABA3-NifS/K271S, which was shown to be unable to bind the PLP cofactor and therefore lost its cysteine desulfurase activity, was found to be blocked in selenocysteine lyase activity as well (Fig. 4). Furthermore, like the capacity to desulfurate L-cysteine, the ability to deselenate L-selenocysteine also was reduced to a residual activity of about 20% in the ABA3-NifS/C430A protein that was found earlier to be affected in persulfide binding.

![Image](http://www.jbc.org/Downloaded from)

**TABLE III**

| Substrate        | \( K_m \) (\( \mu \)M) | Specific Activity (mol product/min/mol protein) |
|------------------|------------------------|-----------------------------------------------|
| L-Cysteine       | 50                     | 1.8                                           |
| L-Selenocysteine | 200                    | 21.4                                          |

**FIG. 2.** Cysteine desulfurization and Moco sulfuration by ABA3 and its separately expressed domains. A, relative cysteine desulfurase activity of full-length ABA3 (ABA3-FL), ABA3-NifS, and the C-terminal domain of ABA3 (ABA3-CT). Activity was determined by quantifying the amount of H2S released from 0.5 mM L-cysteine in the presence of 1 mM DTT by equimolar amounts (1 nmol) of the respective protein within 30 min at 37 °C. B, reconstitution of AOα activity by full-length ABA3 (FL), ABA3-NifS (NifS), and the C-terminal domain of ABA3 (CT) as visualized by in situ staining for AO activity after native PAGE. Each lane contains 1 \( \mu \)g of KCN-inactivated AOα coincubated with a 10× molar excess of the respective ABA3 protein for 1 h at 37 °C in the presence of 0.5 mM L-cysteine (control, KCN-inactivated AOα alone).

**FIG. 3.** Relevance of ABA3-NifS residues lysine 271 and cysteine 430 on PLP and persulfide binding. A, visible spectra of ABA3-NifS (solid line) and ABA3-NifS/K271S (dotted line). Concentrations of proteins were 2 mg/ml. B, relative cysteine desulfurase activity of ABA3-NifS, ABA3-NifS/C430, and ABA3-NifS/K271S. Activity was determined by quantifying the amount of H2S released from 0.5 mM L-cysteine in the presence of 1 mM DTT by equimolar amounts (1 nmol) of the respective protein within 30 min at 37 °C. C, reconstitution of AOα activity by ABA3-NifS (NifS), ABA3-NifS/C430 (NifS C430A) and ABA3-NifS/K271S (NifS K271S) as visualized by in situ staining for AO activity after native PAGE. Each lane contains 1 \( \mu \)g of KCN-inactivated AOα coincubated with a 10× molar excess of the respective ABA3 protein for 1 h at 37 °C in the presence of 0.5 mM L-cysteine (control, KCN-inactivated AOα alone).
is reduced in the presence of the C-terminal domain, an interaction of ABA3-NifS and the C-terminal domain during catalysis is not unlikely, although such an has not been able to be shown to date. Both full-length ABA3 and ABA3-NifS form homodimers with an apparent molecular mass of 200 ± 10 and 105 kDa, respectively, whereas the C-terminal domain is monomeric with a mass of 31 kDa. This implies that dimerization of full-length ABA3 is mediated by its NifS-like domain and that one ABA3 dimer mobilizes two sulfur atoms as required by AO and XDH, which are dimers of two independently acting subunits as well. Thus, it might well be that the C-terminal domain coordinates the sulfur transfer from one subunit of ABA3-NifS to one subunit of AO or XDH, thereby also affecting the efficiency of ABA3-NifS during sulfur mobilization.

Like most of the NifS-like proteins, ABA3-NifS also was found to act on l-cysteine and l-selenocysteine. Decomposition of l-selenocysteine by ABA3-NifS was found to be more efficient than cysteine desulfurization, which is true for other NifS-like enzymes as well (24). However, no specific physiological function is obvious for the selenocysteine lyase activity of ABA3 because no selenium-containing enzymes were described for higher plants to this end. It is still questionable whether higher plants even possess the protein environment for the formation of l-selenocysteine and thus whether a mechanism for the decomposition of selenocysteine is required at all.

When comparing ABA3-NifS with other NifS-like enzymes that were described to function in [Fe-S] cluster formation, it became obvious that ABA3-NifS comprises all properties and features that are typical for “classical” NifS-enzymes: (i) ABA3-NifS is a homodimer and mediates dimerization of the full-length ABA3 protein. (ii) It binds a PLP cofactor that is essential for cysteine desulfurase activity and consequently for sulfurtransferase activity, as was shown by proving binding of one mole PLP per mole of ABA3-NifS and by characterization of the ABA3-NifS/K271S protein. (iii) It catalyzes the formation of L-alanine and elemental sulfur by using L-cysteine as substrate to generate an enzyme-bound cysteinyl persulfide, which was shown by treatment of the ABA3-NifS protein with the alkylating agent 1,5-I-AEDANS and by site-directed mutagenesis of the proposed persulfide binding site at cysteine 430. (iv) ABA3-NifS possesses sulfurtransferase activity, which is required for transferring the persulfide sulfur to the Moco of AO and XDH, and (v) it harbors selenocysteine lyase activity, which also depends on the presence of PLP and is profoundly dependent on cysteine 430. Although the physiological function of ABA3 is very distinct from the function of all NifS proteins described thus far, our findings indicate that ABA3 can be referred to as a NifS-like protein. Hence, it can be concluded that besides AtNFS1 in mitochondria and AtNFS2 in chloroplasts, ABA3 represents a third and putative cytosolic NifS-like protein in A. thaliana.

Acknowledgments—We thank Tomokazu Koshiya for kindly providing the AOa-expressing P. pastoris strain and Saskia Helmsing for technical assistance.

REFERENCES

1. Zheng, L., White, R. H., Cash, V. L., Jack, R. F., and Dean, D. R. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 2754–2758
2. Lauhon, C. T., and Kambampati, R. (2000) J. Biol. Chem. 275, 20096–20110
3. Mihara, H., Maeda, M., Fujii, T., Kuribara, T., Hata, Y., and Esaki, N. (1999) J. Biol. Chem. 274, 14768–14772
4. Loiseau, L., Ollagnier-de-Choudens, S., Nachin, L., Fontecave, M., and Barras, F. (2003) J. Biol. Chem. 278, 38352–38359
5. Kolman, C., and Soll, D. (1993) J. Bacteriol. 175, 1433–1442
6. Nakai, Y., Nakai, M., Hayashi, H., and Kagaqyama, H. (2001) J. Biol. Chem. 276, 8314–8320
7. Kushnir, S., Babiyshchuk, E., Storozhenko, S., Davey, M. W., Papenbrook, J., De

[2] T. Heidenreich and S. Wollers, unpublished data.
[3] T. Heidenreich, S. Wollers, and M. Oreb, unpublished data.
Rycke, R., Engler, G., Stephan, U. W., Lange, H., Kispal, G., Lill, R., and Van Montagu, M. (2001) Plant Cell 13, 89–100
8. Léon, S., Touraine, B., Briat, J. F., and Lobreaux, S. (2002) Biochem. J. 366, 557–564
9. Pilon-Smits, E. A., Garifullina, G. F., Abdel-Ghany, S., Kato, S., Mihara, H., Hale, K. L., Burkhedt, J. L., Esaki, N., Kurihara, T., and Pilon, M. (2002) Plant Physiol. 130, 1309–1318
10. Bittner, F., Oreb, M., and Mendel, R. R. (2001) J. Biol. Chem. 276, 40381–40384
11. Mendel, R. R., and Haensch, R. (2002) J. Exp. Bot. 53, 1689–1698
12. Mendel, R. R., and Schwarz, G. (2002) Met. Ions Biol. Syst. 39, 317–368
13. Hesberg, C., Haensch, R., Mendel, R. R., and Bittner, F. (2004) J. Biol. Chem. 279, 13547–13554
14. Koiwai, H., Akaba, S., Seo, M., Komano, T., and Koshiha, T. (2000) J. Biochem. (Tokyo) 127, 659–664
15. Fogo, J. K., and Popowsky, M. (1949) Anal. Chem. 21, 732–734
16. Esaki, N., Nakamura, T., Tanaka, H., and Soda, K. (1982) J. Biol. Chem. 257, 4386–4391
17. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
18. Adams, R. (1969) Anal. Biochem. 31, 118–122
19. Zheng, L., White, R. H., Cash, V. L., and Dean, D. R. (1994) Biochemistry 33, 4714–4720
20. Schwartz, S. H., Leon-Kloosterziel, K. M., Koornneef, M., and Zeevaart, J. A. (1997) Plant Physiol. 114, 161–166
21. Xiong, L., Ishitani, M., Lee, H., and Zhu, J. K. (2001) Plant Cell 13, 2083–2083
22. Cutler, A. J., and Krochko, J. E. (1999) Trends Plant Sci. 4, 472–478
23. Sagi, M., Scanzocchio, C., and Flahr, R. (2002) Plant J. 31, 305–317
24. Mihara, H., Kurihara, T., Yoshimura, T., and Esaki, N. (2000) J. Biochem. (Tokyo) 127, 559–567
Characterization of the NifS-like Domain of ABA3 from *Arabidopsis thaliana*
Provides Insight into the Mechanism of Molybdenum Cofactor Sulfuration
Torsten Heidenreich, Silke Wollers, Ralf R. Mendel and Florian Bittner

*J. Biol. Chem.* 2005, 280:4213-4218.
doi: 10.1074/jbc.M411195200 originally published online November 22, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M411195200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 24 references, 13 of which can be accessed free at
http://www.jbc.org/content/280/6/4213.full.html#ref-list-1