Bacillus subtilis YxaG is a novel Fe-containing quercetin 2,3-dioxygenase

Laura Bowater, Shirley A. Fairhurst, Victoria J. Just, Stephen Bornemann*

Biological Chemistry Department, John Innes Centre, Norwich Research Park, Colney, Norwich NR4 7UH, UK

Received 4 November 2003; revised 2 December 2003; accepted 2 December 2003

First published online 15 December 2003

Edited by Hans Eklund

Abstract The *Bacillus subtilis* genome contains genes for three hypothetical proteins belonging to the bicupin family, two of which we have previously shown to be Mn(II)-dependent oxalate decarboxylases. We have now shown that the third, YxaG, exhibits quercetin 2,3-dioxygenase activity and that it contains Fe ions. This contrasts with the eukaryotic enzyme which contains a Cu ion. YxaG is the first prokaryotic carbon monoxide-forming enzyme that utilises a flavonol to be characterised and is only the second example of a prokaryotic dioxygenolytic carbon monoxide-forming enzyme known to contain a cofactor. It is proposed to rename the *B. subtilis* gene *qdoI*.

Key words: Quercetin 2,3-dioxygenase; Prokaryote; Flavonol; Carbon monoxide production; Iron; *Bacillus subtilis*

1. Introduction

An analysis of the *Bacillus subtilis* genome revealed the presence of three genes that encode hypothetical proteins that belong to the bicupin family of proteins [1–3]. The bicupins are a subset of the cupin superfamily, which are defined by a β-barrel protein domain structure and a well but not strictly conserved motif; $G(X)_5HXH(X)_{3-4}E(X)_6G$ followed by $G(X)_5PXG(X)_2H(X)_3N$ [3]. What sets the bicupins apart from the rest of the superfamily is the duplication of the protein domain and cupin motif within the polypeptide sequence. Two of the *B. subtilis* bicupin proteins (OxdC *aka* YvrK and OxdD *aka* YoaN) were predicted from phylogenetic analyses to be oxalate decarboxylases [1,2] and this was recently shown to be correct [4]. Both of these enzymes were shown to be Mn(II)-containing enzymes. The third hypothetical protein, YxaG, was an outlier in the phylogenetic analyses and did not possess oxalate decarboxylase activity.

A clue as to the function of YxaG came from a recent BLAST search. The most closely related protein sequence was from a hypothetical protein from *Corynebacterium glutamicum* ATCC 13032 (GenBank™ accession no. 19552121; *E* value = $5e^{-31}$). More informative was the sequence with the next highest score that was from the recently structurally characterised quercetin 2,3-dioxygenase from *Aspergillus japonicus* (GenBank™ accession no. 21465744; *E* value = $4e^{-13}$ [5]). The N-terminal domain of this enzyme contains a Cu(II) ion that is coordinated by the one Glu and three His of its cupin motif (Fig. 1). Although the sequence of YxaG is only 17% identical and 39% similar to the *A. japonicus* copper-quercetin 2,3-dioxygenase sequence, their N-terminal domains share the greatest similarity, particularly in the vicinity of their cupin motifs.

Quercetin 2,3-dioxygenases (EC 1.13.11.24; *aka* quercetinase or flavonol 2,4-dioxygenase) have to date only been isolated from the eukaryotes *A. japonicus* [5], *Aspergillus flavus* [6–8] and *Aspergillus niger* DSM 821 [9] and all have been shown to contain a Cu(II) ion. These dioxygenase enzymes catalyse the cleavage of the *O*-heteroaromatic ring of the flavonol quercetin to the depside 2-protocatechuoyl-phloroglucinol carboxylic acid and carbon monoxide (Fig. 2). This involves the remarkable dioxygenolytic cleavage of two carbon–carbon bonds [10–13]. This paper addresses the properties of *B. subtilis* YxaG and whether it also exhibits quercetin 2,3-dioxygenase activity.

2. Materials and methods

2.1. Bacterial strains and growth conditions

B. subtilis 168, *Escherichia coli* DH5α and *E. coli* BL21(DE3)pLysS cells were grown in Luria–Bertani medium at 37°C and 200 rpm. Carbenicillin (125 μg ml^{-1}) or chloramphenicol (100 μg ml^{-1}) was added as required.

2.2. Cloning

The gene *yxaG* (GenBank™ accession no. 2280496) was amplified by polymerase chain reaction (PCR) using KOD DNA polymerase (Novagen), *B. subtilis* genomic DNA [14] as the template and two primers: yxagfor1 5′-CGATCATATGAAAACATTATG (inserts an *Nde*I site at the start codon) and yxagrev1 5′-CGCTCGAGTGG-

```
                    * *    *                  *
YxaG N  56 GDAFPLHVHKDTHEGILVLDG 15 GDYANIPAGTPHSYRM
YxaG C 228 GDRIVDHYHEYHTETFYCLEG 15 GDFLHVPANTVHSYRL
CQDO N  58 ALGVLPHIHQKHYENFYCNKG 20 GDYGSVPRNVTHTFQI
HGDO C 327 FRPPYYHRNC-MSEFMGLIRG 11 GGGSLHSTMTPHGPDA
OxdC N  89 GAIRELHWHK-EAEWAYMIYG 20 GDLWYFPSGLPHSIQA
OxdC C 267 GAMRELHWHPNTHEWQYYISG 20 GDVGYVPFAMGHYVEN
OxOx    82 GGTNPPHIHPRATEIGMVMKG 23 GETFVIPRGLMHFQFN
```

Fig. 1. An alignment of the cupin motifs of *B. subtilis* YxaG (GenBank™ accession no. 2280496), *A. japonicus* Cu(II)-quercetin 2,3-dioxygenase (CQDO; 21465744), human Fe(II)-homogentisate dioxygenase (HGDO; 2498441), *B. subtilis* Mn(II)-oxalate decarboxylase (OxdC; 21362729) and *Hordeum vulgare* Mn(II)-oxalate oxidase (OxOx; 539054). The letters N and C indicate N- and C-terminal domains each motif is in. The number of amino acids between the two parts of the cupin motifs is shown. Amino acids known to ligate active site metal ions are underlined and their positions are indicated with asterisks.

*Corresponding author. Fax: (44)-1603-450018.
E-mail address: stephen.bornemann@bbsrc.ac.uk (S. Bornemann).

Abbreviations: PCR, polymerase chain reaction; PAGE, polyacrylamide gel electrophoresis; Tris, tris(hydroxymethyl)aminomethane; MALDI-TOF, matrix-assisted laser desorption/ionisation time of flight

0014-5793/03/$30.00
doi:10.1016/S0014-5793(03)01439-X

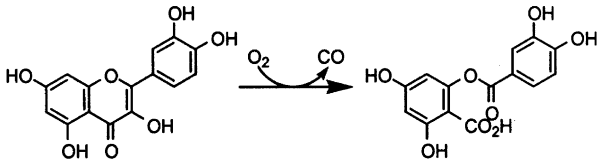

Fig. 2. The quercetin 2,3-dioxygenase reaction.

TTTCATCACC (inserts an *Xho*I site at the stop codon) (Sigma-Genosys). The PCR product was inserted into pET-32a (Novagen) to give pLByxag enabling the expression of the protein with a C-terminal His-tag. The DNA sequence of the open reading frame of this construct and of independent clones was identical to that in the database except for the codon for amino acid 160 that was GAC rather than ACA giving Asp instead of Thr. All sequence analyses were on this corrected sequence.

2.3. Protein expression and purification

Expression of YxaG in *E. coli* BL21(DE3)pLysS pLByxag was induced with isopropyl-β-D-thiogalactopyranoside. Cells were disrupted with three passes through a French pressure cell in the presence of 50 mM tris(hydroxymethyl)aminomethane (Tris)–HCl buffer, pH 8.0, and DNase I. The extract was centrifuged (20 000 × *g*, 30 min) and the resulting supernatant was filtered (0.2 μm) and loaded onto a HiTrap Chelating HP column (Amersham Biosciences) which was previously charged with Ni^{2+} ions and equilibrated with Tris buffer containing 500 mM NaCl and 20 mM imidazole. The bound protein was eluted with a linear gradient of imidazole (0.02–1 M) in the same buffer. The purity of the protein was determined by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (PAGE) with Simply Blue™ Safe Stain (Invitrogen) Coomassie staining. Protein concentration was determined with the Micro bicinchoninic acid protein assay (Pierce) using bovine serum albumin as the standard. Protein assays using the Coomassie Plus-200 reagent (Pierce) and the folin phenol reagent [15] gave relative values of 125 and 88%, respectively.

2.4. Enzyme assay and characterisation

Quercetin 2,3-dioxygenase activity was determined spectrophotometrically using a method described previously [9]. One unit of enzyme activity is defined as the amount of enzyme required to convert 1 μmol of quercetin per min at 25°C in 50 mM 2-[*N*-morpholino]-ethanesulfonic acid buffer, pH 6.0. The decrease in absorption was followed at 367 nm, which corresponds to the absorption maximum of quercetin (ε_{367} 20 000 M^{-1} cm^{-1}). UV–visible spectra and time courses were obtained using a Lambda 18 UV/Vis spectrophotometer (Perkin Elmer) with a path length of 1 cm. Michaelis–Menten constants were estimated by fitting the curve directly. Oxygen consumption was monitored using a Clarke-type oxygen electrode. Carbon monoxide production was detected using a Dräger MicroPac detector housed within a gas-tight vessel along with a reaction mixture (10 ml) essentially identical to that used for the spectrophotometric assay. The enzyme was added last to the reaction mixture, just before closing the vessel, and the maximum carbon monoxide level was recorded after the yellow colour of the substrate had disappeared. The detector was calibrated by injecting known volumes of carbon monoxide into the vessel through a rubber seal. Reaction mixtures were acidified with ice-cold 50 mM HCl to about pH 2 before extraction with ice-cold ethyl acetate. The solvent was dried over Na_2SO_4 before being evaporated under vacuum. Nuclear magnetic resonance (NMR) spectra were obtained using a JEOL JNM LA400 spectrometer. The molecular mass of the YxaG oligomer in Tris buffer containing 150 mM NaCl was determined by gel filtration using a Superdex 200 HR 10/30 column (Pharmacia) calibrated with a Sigma Molecular Weight Marker Kit (12–200 kDa). It was also determined by dynamic light scattering using a Proteinsolutions DynaPro 99 instrument. Matrix-assisted laser desorption/ionisation time of flight (MALDI-TOF) mass spectra were obtained with a Reflex III MALDI-TOF mass spectrometer (Bruker UK Ltd., Coventry, UK). A dried-droplet preparation (0.5 μl) consisting of 3,5-dimethoxy-4-hydroxycinnamic acid (Fluka, Dorset, UK) and YxaG (25 pmol μl^{-1}) was spotted directly onto a stainless steel target plate. Mass spectra were acquired in positive ion linear mode. Accurate calibration was achieved with reference to the average isotopic $[M+H]^+$ (16 952.5) and $[2M+H]^+$ (33 904.0) ions of horse heart myoglobin (Sigma), spotted adjacent to the sample.

2.5. Metal analysis and electron paramagnetic resonance (EPR) spectroscopy

Metal analysis was provided by Southern Science Laboratories (Lewes, Sussex, UK) using inductively coupled plasma emission spectroscopy of acid digested samples. The metal content of dialysed protein is quoted after the subtraction of control values obtained with dialysis buffer alone. X-band EPR spectra were recorded using a Bruker ELEXYS 500 spectrometer fitted with an ER049X SuperX microwave bridge, a shq cavity, an Oxford Instruments ESR-900 cryostat and an ITC3 temperature controller. Conditions were adjusted to ensure non-saturation of signals. Spectra were obtained between 4 and 80 K with a microwave frequency of 9.49 GHz, a power of 1.6 mW and a modulation amplitude of 4 G.

3. Results

B. subtilis YxaG with a C-terminal His-tag was expressed in *E. coli*. It was purified to >95% purity according to denaturing PAGE with Coomassie staining (not shown). The purified recombinant protein was found to consume quercetin according to a decrease in absorbance at 367 nm. It adhered to normal Michaelis–Menten kinetics when assayed over a range of substrate concentrations from 0.12 to 72 μM in air-saturated buffer. It had a K_m for quercetin of 3.8 μM and a V_{max} of 1.2 U mg^{-1} giving a k_{cat} of 0.8 s^{-1}. Using an electrode to monitor oxygen consumption, the V_{max} was determined to be approximately 2 U mg^{-1}; essentially the same value obtained above. The yield of carbon monoxide from a reaction that had proceeded to completion was essentially stoichiometric with the substrate (107%). Both ^1H and ^{13}C NMR spectra of the depside product of the reaction were identical to those previously reported [16,17] with no indication of any side products. These results conclusively show that YxaG possesses quercetin 2,3-dioxygenase activity. The value for the K_m was slightly lower than for the copper-quercetin 2,3-dioxygenases from *A. niger* DSM 821 (6.6 μM [9]) and *A. flavus* (5.2 μM [7]). The V_{max} was several orders of magnitude lower than that reported for the *A. flavus* enzyme (180 U mg^{-1} [7]).

Using MALDI-TOF mass spectrometry, the His-tagged YxaG gave an $[M+2H]^{2+}$ ion with a mass of 19 330.0 Da from which M was calculated to be 38 658 Da. This is very close to the theoretical average isotopic mass of the protein (38 665 Da, which includes the C-terminal tag) and is well within the expected error. The mobility of the protein in denaturing PAGE was anomalous because it had an apparent

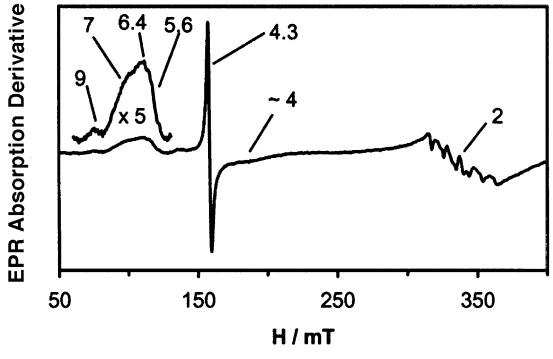

Fig. 3. X-band EPR spectrum of *B. subtilis* YxaG (0.62 mM) in 50 mM Tris buffer, pH 8.0, at 12 K with a microwave power of 1.6 mW.

mass of ~44 kDa. The native protein was found to be a dimer in solution according to both gel filtration (~69 kDa) and dynamic light scattering (~85 kDa; 99.1% monodisperse). Remarkably, it was also possible to detect a dimer by MALDI-TOF mass spectrometry ($[2M+H]^+$ ion; 77 kDa observed and expected). The *A. japonicus* copper-quercetin 2,3-dioxygenase was previously found to be a dimer of 38 kDa subunits (excluding glycan) [5], while the corresponding enzyme from *A. niger* DSM 821 appeared to be a heterotrimer [9].

Metal analysis showed YxaG to contain between 1.7 and 2.2 atoms of Fe per subunit. It contained little Zn, Ni, Cu and Mn (0.4, 0.1, <0.2 and <0.05 atoms per subunit, respectively).

The EPR spectrum of the as isolated enzyme showed several signals (Fig. 3). A dominant rhombic Fe(III) signal with a derivative line shape was present at $g=4.3$ ($S=5/2$ $|\pm 3/2\rangle$). Peaks at $g=6.4$ and 5.6 were attributed to an axial $S=5/2$ $|\pm 1/2\rangle$ E/D ~ 0 system. An associated peak expected at $g=2$ was lost under the six line signal at $g\sim 2$ that is attributable to Mn(II). A small peak at $g=9$, assigned to an axial $S=5/2$ $|\pm 5/2\rangle$ E/D ~ 0 system, was detected between 4 and 16 K and its intensity decreased with temperature (with Curie Law correction). This feature may be associated with the $g=6.4$ and 5.6 features. All of the main features are therefore generally attributable to $S=5/2$ species, apart from a peak at $g=7$ and a broad feature at $g\sim 4$, which have not been assigned. All signals except for the Fe(III) $g=4.3$ and Mn(II) $g\sim 2$ species were lost on anaerobic reduction with 3 mM sodium dithionite. Dialysis of the enzyme against 1 mM ethylene diamine tetraacetic acid resulted in only very small changes in the EPR spectrum; most notably a smaller Mn(II) signal. Apart from the six line Mn(II) signal, all of the others are consistent with Fe(III) ions. It appears that two (or possibly more) types of Fe(III) ions are present in the enzyme, only one of which is resistant to reduction by dithionite. Although the complexity of the Fe(III) signals precludes their accurate integration, their relative intensities show that there was roughly the same order of magnitude of Fe(III) as Mn(II) in the enzyme as isolated. Given that metal analysis showed that there was little Mn present (<0.05 atoms per subunit), it therefore appears that the enzyme is predominantly in the EPR-silent Fe(II) oxidation state.

The UV–visible spectrum of the as isolated enzyme showed a broad absorbance peak at about 350 nm with an extinction coefficient of 3400 M^{-1} cm^{-1}, which extended to about 500 nm (Fig. 4). The addition of dithionite to the enzyme solution resulted in the bleaching of this peak. Dialysis of the enzyme against 1 mM ethylene diamine tetraacetic acid did not bleach this absorbance. The 350 nm peak is consistent with the presence of mononuclear ferric iron ions similar to those observed with the iron superoxide dismutase from *E. coli* (ε_{350} 1675–1850 M^{-1} cm^{-1} [18]).

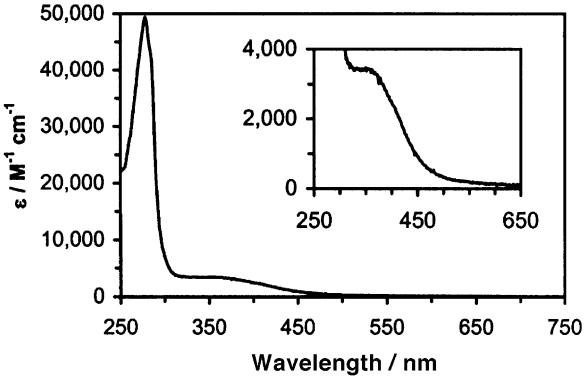

Fig. 4. UV–visible spectrum of *B. subtilis* YxaG (41 μM) in 50 mM Tris buffer, pH 8.0.

4. Discussion

We have conclusively shown *B. subtilis* YxaG to exhibit quercetin 2,3-dioxygenase activity. It is perhaps not surprising that *B. subtilis* possesses an enzyme that consumes this abundant plant product because it is a soil bacterium that grows on rotting vegetation. There has indeed been a report of a *Bacillus* sp. being able to convert rutin, a glycosylated form of quercetin, to the depside [16]. Unusually, YxaG is an Fe-containing quercetin 2,3-dioxygenase, unlike the eukaryotic enzymes which contain Cu [5,8,9]. Interestingly, it has been proposed that the copper-containing enzymes evolved from an ancestral iron-containing enzyme because copper has become biologically available relatively recently on the evolutionary time scale [11]. YxaG may well have evolved from such a prokaryotic ancestor but has retained its specificity for iron.

Recent structural and spectroscopic studies on the *A. japonicus* enzyme [10,11] are consistent with the proposal [19] that dioxygen initially attacks an electron-deficient radical at C2 of the substrate and that it is Cu(II) that transiently accepts the electron from the substrate. If the catalytic mechanisms of the Cu(II) and Fe-dependent enzymes were similar, one might expect the resting Fe-enzyme to be in the ferric oxidation state. The 350 nm absorbance of YxaG indicated the presence of Fe(III). However, the low intensity of the ferric EPR signals shows this is a minor component. The intensity of the 350 nm band would therefore seem to be unexpectedly high. If the Fe(II) form of the enzyme were the catalytically active form, this would suggest that the mechanism of the Fe-enzyme might be different from the Cu-enzyme. For example, the mechanism might involve dioxygen activation rather than substrate activation analogous to the difference between the Fe(II)-extra- and Fe(III)-intra-diol dioxygenases, respectively [20]. Further studies will be required to address these issues.

CO-forming enzymes are rare and, until now, none from prokaryotes that convert flavonols have been characterised. Only three prokaryotic enzymes are known to catalyse the dioxygenolytic release of CO analogous to the dioxygenation of the flavonol quercetin. *Arthrobacter* sp. Rü61a 1*H*-3-hydroxy-4-oxoquinaldine 2,4-dioxygenase [21,22] and *Pseudomonas putida* 33/1 1*H*-3-hydroxy-4-oxoquinoline 2,4-dioxygenase [22,23] cleave the ring of *N*-heteroaromatic compounds to release carbon monoxide. These two prokaryotic enzymes appear to be related to α/β-hydrolases and, intriguingly, do not seem to contain any metal ion or organic cofactor [24]. *Klebsiella pneumoniae* 1,2-dihydroxy-3-oxo-*S*-methylthiopentene anion 1,3-dioxygenase (acireductone dioxygenase) [25,26] has recently been shown to contain Ni(II) [27] and represents the first prokaryotic dioxygenolytic CO-forming enzyme known to contain a cofactor. The coordination of the active site metal ion of this enzyme is predicted to resemble that of other metalloproteins of the cupin superfamily. When the Ni(II) ion is substituted with an Fe(II) ion, this enzyme catalyses a 1,2-

dioxygenation reaction with acireductone, which does not lead to carbon monoxide formation [28,29]. YxaG therefore represents only the second prokaryotic enzyme in this category to possess a known cofactor.

There is a high degree of structural conservation in the metal binding sites of cupin proteins. For example, the amino acid residues of the cupin motifs that coordinate the metal ions in *A. japonicus* Cu(II)-quercetin 2,3-dioxygenase [5], human Fe(II)-homogentisate dioxygenase [30] *B. subtilis* Mn(II)-oxalate decarboxylase [31] and barley Mn(II)-oxalate oxidase [32] are well conserved in terms of primary sequence (Fig. 1) as well as structure. Given the conservation of the one Glu and three His in both of the cupin motifs of YxaG and the apparent homology between the two domains (28% identity and 32% similarity), it is likely that each domain of YxaG binds an Fe ion with these amino acid residues. Metal analysis indicates the presence of two Fe ions per subunit and the EPR experiments are consistent with two (or possibly more) independent Fe ion environments. Further studies will be required to define the metal ion binding sites and to establish whether one or both domains are catalytically active. Now that a function has been established for the hitherto hypothetical protein YxaG, we propose to rename its gene *qdoI*.

Acknowledgements: This work was supported by the Biotechnology and Biological Sciences Research Council. Mass spectrometry was carried out by Drs Andrew R. Bottrill and Mike J. Naldrett of the Joint IFR-JIC Proteomics Facility which is funded in part by BBSRC JREI grant numbers JRE10832, JE412701, JE412631 and grants from Syngenta and Unilever. We thank Dr Karl Fisher and Roger Harris for technical advice and assistance. This paper is dedicated to Professor David H.G. Crout on the occasion of his retirement.

References

[1] Dunwell, J.M., Khuri, S. and Gane, P.J. (2000) Microbiol. Mol. Biol. Rev. 64, 153–179.
[2] Tanner, A. and Bornemann, S. (2000) J. Bacteriol. 182, 5271–5273.
[3] Khuri, S., Bakker, F.T. and Dunwell, J.M. (2001) Mol. Biol. Evol. 18, 593–605.
[4] Tanner, A., Bowater, L., Fairhurst, S.A. and Bornemann, S. (2001) J. Biol. Chem. 276, 43627–43634.
[5] Fusetti, F. et al. (2002) Structure 10, 259–268.
[6] Krishnamurty, H.G. and Simpson, F.J. (1970) J. Biol. Chem. 245, 1467–1471.
[7] Oka, T., Simpson, F.J., Child, J.J. and Sister Mills, C. (1971) Can. J. Microbiol. 17, 111–118.
[8] Oka, T., Simpson, F.J. and Krishnamurty, H.G. (1972) Can. J. Microbiol. 18, 493–508.
[9] Hund, H.K., Breuer, J., Lingens, F., Huttermann, J., Kappl, R. and Fetzner, S. (1999) Eur. J. Biochem. 263, 871–878.
[10] Kooter, I.M., Steiner, R.A., Dijkstra, B.W., van Noort, P.I., Egmond, M.R. and Huber, M. (2002) Eur. J. Biochem. 269, 2971–2979.
[11] Steiner, R.A., Kalk, K.H. and Dijkstra, B.W. (2002) Proc. Natl. Acad. Sci. USA 99, 16625–16630.
[12] Steiner, R.A., Kooter, I.M. and Dijkstra, B.W. (2002) Biochemistry 41, 7955–7962.
[13] Steiner, R.A., Meyer-Klaucke, W. and Dijkstra, B.W. (2002) Biochemistry 41, 7963–7968.
[14] Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A. and Struhl, K. (1994) in: Current Protocols in Molecular Biology (Chanda, V.B., Ed.), Wiley, New York.
[15] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[16] Omori, T., Shiozawa, K., Sekiya, M. and Minoda, Y. (1986) Agric. Biol. Chem. 50, 779–780.
[17] Krishnamachari, V., Levine, L.H. and Pare, P.W. (2002) J. Agric. Food Chem. 50, 4357–4363.
[18] Averill, B.A. and Vincent, J.B. (1993) Methods Enzymol. 226, 33–51.
[19] Matsuura, T., Matsushima, H. and Sakamoto, H. (1967) J. Am. Chem. Soc. 89, 6370–6371.
[20] Bugg, T.D.H. (2001) Curr. Opin. Chem. Biol. 5, 550–555.
[21] Hund, H.K., Debeyer, A. and Lingens, F. (1990) Biol. Chem. Hoppe-Seyler 371, 1005–1008.
[22] Bauer, I., Max, N., Fetzner, S. and Lingens, F. (1996) Eur. J. Biochem. 240, 576–583.
[23] Bott, G., Schmidt, M., Rommel, T.O. and Lingens, F. (1990) Biol. Chem. Hoppe-Seyler 371, 999–1003.
[24] Fetzner, S. (2002) Appl. Microbiol. Biotechnol. 60, 243–257.
[25] Wray, J.W. and Abeles, R.H. (1993) J. Biol. Chem. 268, 21466–21469.
[26] Wray, J.W. and Abeles, R.H. (1995) J. Biol. Chem. 270, 3147–3153.
[27] Pochapsky, T.C., Pochapsky, S.S., Ju, T.T., Mo, H.P., Al-Mjeni, F. and Maroney, M.J. (2002) Nat. Struct. Biol. 9, 966–972.
[28] Dai, Y., Wensink, P.C. and Abeles, R.H. (1999) J. Biol. Chem. 274, 1193–1195.
[29] Dai, Y., Pochapsky, T.C. and Abeles, R.H. (2001) Biochemistry 40, 6379–6387.
[30] Titus, G.P., Mueller, H.A., Burgner, J., de Cordoba, S.R., Penalva, M.A. and Timm, D.E. (2000) Nat. Struct. Biol. 7, 542–546.
[31] Anand, R., Dorrestein, P.C., Kinsland, C., Begley, T.P. and Ealick, S.E. (2002) Biochemistry 41, 7659–7669.
[32] Woo, E.J., Dunwell, J.M., Goodenough, P.W., Marvier, A.C. and Pickersgill, R.W. (2000) Nat. Struct. Biol. 7, 1036–1040.