Plant Sulfite Oxidase as Novel Producer of H$_2$O$_2$

**COMBINATION OF ENZYME CATALYSIS WITH A SUBSEQUENT NON-ENZYMATIC REACTION STEP*\**

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Sulfite oxidase (EC 1.8.3.1) from the plant *Arabidopsis thaliana* is the smallest euakaryotic molybdenum enzyme consisting of a molybdenum cofactor-binding domain but lacking the heme domain that is known from vertebrate sulfite oxidase. While vertebrate sulfite oxidase is a mitochondrial enzyme with cytochrome c as the physiological electron acceptor, plant sulfite oxidase is localized in peroxisomes and does not react with cytochrome c. Here we describe results that identified oxygen as the terminal electron acceptor for plant sulfite oxidase and hydrogen peroxide as the product of this reaction in addition to sulfate. The latter finding might explain the peroxisomal localization of plant sulfite oxidase. $^{18}$O labeling experiments and the use of catalase provided evidence that plant sulfite oxidase combines its catalytic reaction with a subsequent non-enzymatic step where its reaction product hydrogen peroxide oxidizes another molecule of sulfite. *In vitro*, for each catalytic cycle plant SO will bring about the oxidation of two molecules of sulfite by one molecule of oxygen. In the plant, sulfite oxidase could be responsible for removing sulfite as a toxic metabolite, which might represent a means to protect the cell against excess of sulfite derived from SO$_3$ gas in the atmosphere (acid rain) or during the decomposition of sulfur-containing amino acids. Finally we present a model for the metabolic interaction between sulfite and catalase in the peroxisome.

Sulfite oxidases (SO)$^3$ from vertebrates (published as EC 1.8.3.1) play an essential role in sulfur detoxification by catalyzing the reaction

$$\text{SO}_4^{2-} + H_2O + 2\ (\text{cyt c}_{ox} \rightarrow SO_4^{2-} + 2H^+ + 2\ (\text{cyt c}_{red})},$$

which is the fourth molybdenum enzyme present in plants in addition to nitrate reductase, xanthine dehydrogenase, and aldehyde oxidase. Cloning and characterization of plant SO was possible by using sequence homologies to the mammalian counterpart. However, in contrast to the animal enzyme plant SO lacks the heme domain, which is evident from the amino acid sequence, its enzymological and spectral properties (7), and the atomic structure (8). Also its subcellular localization differs from that of animals, in plants we showed SO to be localized in peroxisomes (9). SO is wide spread and highly conserved within the plant kingdom; the SO gene is present in higher and lower plants, and the protein encoded seems to be highly conserved because antibodies directed against *Arabidopsis* SO detect proteins of the correct size in a wide range of herbaceous and also woody plants (7). Obviously, SO has an important function if it was conserved during evolution. The major function of plant SO seems to be different from the role it plays in animals where it oxidizes sulfite derived from the decomposition of sulfur-containing amino acids. In plants, sulfur metabolism is different because plants assimilate sulfur by reducing sulfate via sulfite to sulfide and the cell because sulfite is toxic. Now that plant SO is isolated and characterized (7–9), it is important to study the special properties that make SO a unique catalyst and that were responsible for conserving SO during evolution among plants. The clue to understand the role of SO seems to lie in its peroxisomal localization, either because only there the substrate is available or because in this organelle the reaction product could be further metabolized. SO$_2$ gas can readily penetrate through biomembranes (15), and sulfite is generated during sulfate assimilation in the chloroplasts. Hence substrate availability is no convincing argument for explaining peroxisomal localization of SO. However, peroxisomes are known as the compartment in which reactive oxygen is produced, and therefore, identifying both the electron acceptor and the reaction products could be the clue for describing the special properties that make plant SO unique.

Here we identified molecular oxygen as the terminal electron acceptor for plant SO and showed that it converts molecular oxygen into hydrogen peroxide. Furthermore we provide evidence that this hydrogen peroxide is able to non-enzymatically oxidize sulfite. Thus, under in
vitro conditions plant SO combines both an enzymatic and a non-enzymatic reaction.

EXPERIMENTAL PROCEDURES

Protein Expression and Purification—For overexpression and purification of recombinant A. thaliana SO the QIAexpress kit (Qiagen) for metal ion chelating chromatography of His-tagged proteins was used. The protein was expressed and purified from pQE80-At-sox in Escherichia coli strain TP1000 (genotype ΔmobAB) as described earlier (7). Purification of soluble rAt-SO was performed at 4 °C by using nickel-nitrilotriacetic acid superflow matrix (Qiagen) followed by anion exchange chromatography on a 10 ml SourceQ15 column (Amersham Biosciences). Moco-containing SO did not bind to the column, whereas the inactive Moco-free form of SO bound to the column and eluted between 250 and 300 mM NaCl (8). The protein preparation was stable at 4 °C for several weeks after dialyzing and concentrating to 2–10 mg/ml. Concentration of recombinant plant SO was determined by UV spectroscopy using the calculated extinction coefficient of ε = 69,820 M⁻¹ cm⁻¹.

Detection of Plant SO Protein—Protein of leaves and roots were extracted and analyzed as described earlier (7). For immuno-detection, protein was separated on a 10% SDS-PAGE, and the polyclonal anti-rAt-SO antibody (Eurogentec) was diluted 1:500 prior to use.

SO Assays—SO was routinely assayed for the activity to reduce ferricyanide as described previously (7). In parallel, oxygen measurements were performed with an inoLab OxiLevel2 electrode (Wissenschaftlich-Technische Werkstätten GmbH) in 2.2-ml volume by using 2 μg of recombinant plant SO, oxygen-saturated 0.1 M Tris acetate buffer. For quantification of sulfite and sulfate used and produced by recombinant plant SO, respectively, the reaction was stopped by heating at 95 °C for 5 min, and sulfite and sulfate were determined as described below. All data presented are replicates from at least three independent experiments and expressed as mean ± S.D.

Detection of Hydrogen Peroxide—Determination of H₂O₂ formation was performed according to Nag et al. (16) with TiOSO₄. SO reaction (2 μg in 900 μl) was stopped at different time points by adding 100 μl of the TiOSO₄ stock solution (0.2 g of TiOSO₄ in 5 ml of 95% H₂SO₄ and diluted to a final volume of 35 ml with water). Formation of the yellow/orange peroxodisulfatotitanat(IV) complex was measured at 405 nm. H₂O₂ formation was also determined using lucigenin as described by Rost et al. (17).

Determination of ¹⁸O-Labeled Sulfate—Incorporation of ¹⁸O⁻ into sulfite (0.65 mM; final concentration) to produce sulfate by recombinant plant SO was determined either by using labeled H₂¹⁸O₂ (95% ¹⁸O, Euriso-Top) or by using ¹⁸O₂ gas (99% purity, 95% ¹⁸O excess, Linde AG) according to the method described by Lindigkeit et al. (18). After 30 min of incubation, 2 μl of 37% HCl per ml of reaction mix was added, and sulfate was precipitated with BaCl₂ (Sigma) in a final concentration of 0.85 mM. BaSO₄ was collected by centrifugation (10 min, 15,000 rpm). Air-dried pellets of approximately 0.5 mg were transferred into silver capsules (ThermoFinnigan). Samples were injected into a high temperature conversion/elemental analyzer (ThermoFinnigan) for δ¹⁸O analysis, coupled by a Conflo II interface to an isotope ratio mass spectrometer (Delta Plus; ThermoFinnigan). Isotopic values are expressed in atom % δ¹⁸O.

RESULTS

Constitutive Expression of SO in Roots and Leaves—The expression of SO on plant level was analyzed. SO protein was detected in all plant organs of Arabidopsis including roots, leaves, and flowers (Fig. 1A). The overall protein concentration of SO in leaves was found to be in a range of 0.1% of the total leaf protein. This quantification was performed by comparing a linear dilution of recombinant SO with the total extracted SO protein, as detected with the purified peptide antibody against recombinant Arabidopsis SO (data not shown). Using this antibody, immunoblot analysis was employed to measure the diurnal variation of SO expression. With 20 μg of total crude proteins of Arabidopsis leaves and roots, respectively, only slight variations were detected over the day (Fig. 1B). Six to nine hours after light setting, SO expression was highest, while during the night and in the morning the protein amount was slightly decreased.

Molecular Oxygen as Terminal Electron Acceptor—Vertebrate SO is an intensively studied enzyme. Different electron acceptors were described to interact with purified animal SO including cytochrome c, oxygen, ferricyanide, methylene blue, and 2,6-dichlorophenol indophenol (2, 3). Electrons derived from sulfite are passed via the heme domain of the enzyme onto cytochrome c, the physiological electron acceptor (19). Plant SO, however, is unable to interact with cytochrome c because it lacks a heme domain. Furthermore, plant SO is not localized in mitochondria like animal SO but in peroxisomes. Therefore an electron acceptor other than cytochrome c has to be assumed. As animal SO was described to function with oxygen, although with much lower affinity, we tested the consumption of molecular oxygen by plant SO. The experiments showed that plant SO is able to use molecular oxygen as terminal

Sulfate and Sulfite Analysis—50-μl aliquots of samples were injected into an ion exchange chromatography system (DX 120; Dionex). Sulfate and sulfite were separated on an IonPac® column (AS9-Sc 250 × 4 mm; Dionex) and eluted with a solution containing 1.8 mM Na₂CO₃ plus 1.7 mM NaHCO₃ at a flow rate of 0.9 ml min⁻¹. Both compounds were detected by a conductivity detector module (Dionex) combined with an upstream-inserted micromembrane suppressor (ASRS-Ultra 4 mm, Dionex). The detection limit of this setup is 0.3 μmol liter⁻¹ for both ions.

FIGURE 1. Distribution and diurnal variation of SO protein in A. thaliana. Protein was extracted and SO was immunoblotted as described under “Experimental Procedures.” Equal amounts of protein were loaded on the gel. A, distribution of SO throughout different organs in A. thaliana. B, diurnal variation of SO protein in leaves and roots.
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The turnover number of plant SO was calculated on the basis of $V_{\text{max}}$ determined in the presence of catalase to measure only the enzymatic step.

$H_2O_2$ Can Oxidize Sulfite in a Non-enzymatic Reaction—In clouds and rain droplets, $H_2O_2$ is known as one of the most effective non-enzymatic oxidants for $HSO_3^-$ (20). Therefore we tested whether under the conditions of our assay sulfite could be oxidized non-enzymatically by $H_2O_2$. At first we checked the stability of sulfite in aqueous solution and whether it undergoes spontaneous oxidation in the absence of enzyme. Commercial sulfite salts always contain a certain amount of sulfate; in our case 5% sulfate were detected in an aqueous sulfite solution, and this percentage did not increase with time, for up to 12 h (data not shown). Under standard conditions (buffer composition and reaction temperature as described under "Experimental Procedures"), sulfite did not spontaneously oxidize to sulfate. However, the addition of physiological concentrations of $H_2O_2$ in the micromolar range led to the conversion of sulfite into sulfate (Fig. 2A), and this oxidation became remarkably quantitative at different pH. Thus, under standard assay conditions $H_2O_2$ is able to oxidize sulfite non-enzymatically to sulfate.

Plant Sulfite Oxidase Combines an Enzymatic with a Non-enzymatic Step—During sulfite oxidation by plant SO we showed that $H_2O_2$ is generated. Therefore, we determined the contribution of non-enzymatic $H_2O_2$-dependent sulfite oxidation to the net reaction catalyzed by plant SO. To delineate both steps we made use of catalase that converts $H_2O_2$ into water and molecular oxygen according to the equation $2H_2O_2 \rightarrow 2H_2O + O_2$. At first we studied the reaction kinetics of plant SO in the presence of varying amounts of catalase. Oxygen consumption of plant SO is decreasing with increasing catalase concentrations in the reaction mixture. For each molecule of oxygen consumed by plant SO one molecule of $H_2O_2$ is formed, and two molecules of $H_2O_2$ generate one molecule of oxygen in the catalase reaction, hence addition of catalase lowers the net oxygen consumption of plant SO by 50%. Exactly this point can be reached experimentally (data not shown). This observation is another indication for $H_2O_2$ being formed as a reaction product of plant SO. If plant SO combines catalysis with a subsequent non-enzymatic reaction of $H_2O_2$ with sulfite, the net production of sulfate should increase in comparison with the enzymatic step alone. This can be tested with catalase in the reaction mixture to prevent $H_2O_2$-dependent sulfite oxidation. Fig. 2B presents data from experiments with plant SO where the disappearance of sulfite and the production of sulfate are measured over time. In the absence of catalase, plant SO produces ~60% more sulfate at a given time point as compared with the reaction in the presence of catalase, or based on time, half of the substrate was oxidized to sulfate within 316 s, while in the mixed assay with 250 units of catalase, this time point was reached after 782 s.

On the basis of these findings we propose the following two-step reaction for plant SO. 1) An enzymatic SO-dependent reaction with oxygen as final electron acceptor leads to the production of $H_2O_2$: $SO_2^- + H_2O + O_2 \rightarrow SO_3^- + H_2O_2$. 2) In a non-enzymatic reaction, this $H_2O_2$ is used to oxidize a second molecule of sulfate to sulfite: $SO_3^- + H_2O_2 \rightarrow SO_2^2^- + H_2O$. The formation of sulfate and the disappearance of sulfite are followed over time. The reactions were stopped by heating (5 min at 95 °C). The detection of 27 and 40.0 $\mu$M sulfate at zero time go back to two reasons: (i) highly purified commercial sulfite preparations contain ~5% sulfate, and (ii) heat inactivation has a lag period of 45 s in the setup used.
reaction (without catalase $V_{\text{max}}$ is higher because of the contribution of the subsequent non-enzymatic reaction). The turnover number of plant SO was calculated to be 4,500 s$^{-1}$ and that for catalase is one of the highest known, namely 40,000,000 s$^{-1}$ (24). This ensures that at the catalase concentrations used in the experiments virtually all H$_2$O$_2$ generated by SO will be immediately decomposed. Furthermore, when determining the rate of sulfite oxidation by plant SO in the absence and in the presence of catalase, values of $0.15 \pm 0.015$ μM s$^{-1}$ and $0.064 \pm 0.009$ μM s$^{-1}$, respectively, were calculated. Thus the non-enzymatic reaction of sulfite with H$_2$O$_2$ has an apparent rate of 0.086 ± 0.012 μM s$^{-1}$.

Most remarkably, plant SO and catalase are found in the same cell organelle, namely in peroxisomes (7, 9, 25). These organelles harbor catabolic pathways that characteristically produce H$_2$O$_2$, and catalase is one of the enzymes detoxifying excess H$_2$O$_2$. Catalase is inhibited by sulfite with half-maximum inhibition below 500 μM sulfite (26). Therefore, we wanted to know whether catalase is inhibited by sulfite under our assay conditions. Fig. 3 shows that increasing amounts of sulfite inhibit catalase (half-maximal inhibition at 260 μM sulfite). In the combined SO + catalase assay (Fig. 3) the increase of sulfite concentration is accompanied by an initial increase in oxygen consumption because catalase is inhibited and does not contribute to the net oxygen consumption. This increase, however, is only transient because at higher sulfite concentrations of plant SO is also inhibited by its substrate.

**DISCUSSION**

From the data presented it is evident that plant SO is a housekeeping protein, expressed in every plant organ at any time with only low diurnal variation. Different from vertebrate SO that consists of a heme domain and a molybdenum cofactor domain, the heme domain is missing in the plant enzyme (7, 8). While vertebrate SO is a mitochondrial enzyme with cytochrome c as the physiological electron acceptor (19), plant SO is localized in peroxisomes and does not react with cytochrome c (7, 9). Therefore, plant SO should need an electron acceptor with a redox potential similar to a heme, but no evidence was found for the co-purification of plant SO with a heme-like protein (7), while in bacteria a separate heme domain was co-purified with the Moco domain of SO from *Thiobacillus novelius* (27). Our results identified oxygen as the terminal electron acceptor for plant SO that efficiently converts oxygen into H$_2$O$_2$. Thus, plant SO is the first eukaryotic member fully matching the EC nomenclature for sulfite:oxygen oxidoreductases (EC 1.8.3.1), while the animal enzymes with cytochrome c as the electron acceptor should fall into the class of sulfite:ferricytochrome c oxidoreductases (EC 1.8.2.1).

We found that *in vitro* the non-enzymatic oxidation of sulfite by H$_2$O$_2$ contributes to the net reaction of plant SO. Earlier, Miszalski and Ziegler (28) had speculated that oxidation of sulfite in plants might be initiated by superoxide anions formed on the reduction site of the electron transport system in chloroplasts, or by free radicals, or by H$_2$O$_2$. However, no experimental proof was presented. Later it was shown that in clouds and rain droplets H$_2$O$_2$ is one of the most effective oxidants for HSO$_3^-$. This reaction is the key step during the development of acid rain. H$_2$O$_2$ is highly soluble in water with ambient concentrations about 6 orders of magnitude higher than ozone. The reaction proceeds even at low temperatures (228 K) on the surface of ice clouds (20). The rate of

**TABLE 1**

Analysis of sulfite oxidation with H$_2$O$_2$ in the presence of catalase

| Reaction | Product 1 | Product 2 |
|----------|-----------|-----------|
| Plant SO: SO$_3^-$ + H$_2$O + O$_2$ → SO$_4^{2-}$ + H$_2$O | | |
| Non-enzymatic: SO$_3^-$ + H$_2$O$_2$ → SO$_4^{2-}$ + H$_2$O | | |
| Catalase: H$_2$O$_2$ + H$_2$O$_2$ → H$_2$O + H$_2$O + O$_2$ | | |

| 18O-Labeled sulfate | Plus catalase | Minus catalase |
|---------------------|--------------|---------------|
| H$_2$OsbO measured   | 19.6 ± 9.08   | 9.4 ± 2.88    |
| H$_2$OsbO measured   | 6.39 ± 0.92   | 4.32 ± 1.32   |

4 R. Hānsch, C. Lang, and R. R. Mendel, unpublished data.

**FIGURE 3.** Effect of increasing amounts of sulfite on the activities of plant SO and catalase. Oxygen consumption of 2 μg of plant SO in the presence of 94 units of catalase and increasing amounts of sulfite. SO activity was determined by measuring its oxygen consumption with an oxygen electrode; catalase activity was measured as H$_2$O$_2$ degradation using the titanium-(IV)-porphyrin reagent as described under “Experimental Procedures.” The influence of increasing amounts of sulfite on the activities of SO and of catalase is shown in the inset. As H$_2$O$_2$ reacts with sulfite in a non-enzymatic reaction, it was necessary to determine catalase activity in the following way: 1) determination of non-enzymatic H$_2$O$_2$ disappearance in the absence of catalase and 2) determination of H$_2$O$_2$ disappearance in the presence of catalase. The difference between 2) and 1) gives catalase activity.
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oxidation of SO₂ gas by H₂O₂ increases with increasing pH (preferably between pH 2 and 8) and tends to dominate other aqueous oxidative pathways at low pH (29). The proposed mechanism proceeds via equilibria with a peroxymonosulfurous acid ion (SO₂OOH⁻), where the velocity of the second step increases as pH decreases (29, 30).

Our labeling experiments and the use of catalase provide evidence that under in vitro conditions plant SO combines its enzymatic sulfite oxidation with a subsequent non-enzymatic step using its reaction product H₂O₂ as intermediate for oxidizing another molecule of sulfite. Under these in vitro conditions, the non-enzymatic step increases the production of sulfite and lowers the net consumption of oxygen. In planta, additional H₂O₂-generating reactions known to occur in peroxisomes may be involved in sulfite oxidation as well.

What could be the biological relevance of this process? Sulfite is a toxic metabolite that can break disulfide bridges, which is termed sulfotolysis; sulfite inhibits numerous enzymes, and it can attach to aldehydes forming hydroxysulfonates, which are metabolic inhibitors (14). Therefore, its fast removal by oxidation to non-toxic sulfate is a means to protect the cell against excess of sulfite derived from SO₂ gas in the atmosphere or during the decomposition of sulfur-containing amino acids. Prior to characterization of plant SO, this reaction was interpreted as the key step in protecting plants from acid rain that has been a major cause of forest damage (14, 31). Not only sulfite but also H₂O₂ is viewed as a toxic metabolite; however, in recent years it became clear that at lower concentrations H₂O₂ also plays an important role as signaling molecule-mediating cellular responses to different stresses (for review, see Neill et al. (32) and Laloi et al. (33)). The steady-state concentration of H₂O₂ for Arabidopsis has been reported to be in the range between 60 μM and 7 mM (34, 35). These variations may reflect technical difficulties in quantifying H₂O₂ concentrations. Furthermore, the production of H₂O₂ during sulfite oxidation also explains the peroxisomal localization (9) of plant SO because this organelle harbors catalase as one of the enzymes detoxifying excess H₂O₂.

To this end we have no direct proof for the existence of non-enzymatic sulfite oxidation in the living cell. For theoretical reasons, however, we want to propose a model (Fig. 4) summarizing the possible interaction between plant SO and catalase in the peroxisome. At low sulfite concentrations, H₂O₂ as a reaction product of plant SO is degraded by catalase. At higher sulfite concentrations, however, catalase becomes inhibited, and accumulating H₂O₂ derived from SO oxidation non-enzymatically a second sulfite molecule. Furthermore, it has to be taken into account that under catalase-inhibited conditions accumulating H₂O₂ derived from other peroxisomal processes could contribute to the non-enzymatic detoxification of sulfite as well. The detailed regulation of this process is not fully understood and needs further investigation.

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REFERENCES

1. Wilson, H. L., and Rajagopalan, K. V. (2004) J. Biol. Chem. 279, 15105–15113
2. MacLeod, R. M., Farkas, W., Fridovich, I., and Handler, P. (1961) J. Biol. Chem. 236, 1841–1846
3. Cohen, H. J., and Fridovich, I. (1971) J. Biol. Chem. 246, 359–366
4. Cohen, H. J., and Fridovich, I. (1971) J. Biol. Chem. 246, 367–373
5. Cohen, H. J., Betcher-Lange, S., Kessler, D. L., and Rajagopalan, K. V. (1972) J. Biol. Chem. 247, 7759–7766
6. Kisker, C., Schindelin, H., Pacheo, A., Webbi, W. A., Garrett, R. M., Rajagopalan, K. V., Ereman, J. H., and Rees. D. C. (1997) Cell 91, 973–983
7. Eilers, T., Schwarz, G., Brinkmann, H., Witt, C., Richter, T., Nieder, J., Koch, B., Hille R., Hänisch, R., and Mendel, R.-R. (2001) J. Biol. Chem. 276, 46989–46994
8. Schrader, N., Fischer, K., Theis, K., Mendel, R.-R., Schwarz, G., and Kisker, C. (2003) Structure (Camb.) 11, 1251–1263
9. Nowak, K., Luniak, N., Witt, C., Würstfeld, Y., Wachtler, A., Mendel R.-R., and Hänisch R. (2004) Plant Cell Physiol. 45, 1889–1894
10. Leustek, T. (2002) The Arabidopsis Book [Somerville, C. R., and Meyerowitz, E. M., eds] pp. 1–16, American Society of Plant Biologists, Rockville, MD
11. Saito, K. (2004) Plant Physiol. 136, 2443–2450
12. Garsed, S. G., and Read, D. J. (1977) New Phytol. 78, 111–119
13. Van der Kooji, T. A. W., de Kok, L. J., Haneklaus, S., and Schurg, E. (1997) New Phytool. 135, 101–107
14. Heber, U., and Hüve, K. (1998) Int. Rev. Cytol. 177, 255–286
15. Rennenberg, H., and Herschbach, C. (1996) (Yunus, M., and Iqbal, M., eds) pp. 285–294, John Wiley and Sons, Chichester, UK
16. Nag, S., Saha, K., and Choudhuri, M. A. (2000) Plant Sci. 157, 157–163
17. Rost, M., Karge, E., and Klungel, W. (1998) J. Biol. Chem. 13, 355–363
18. Lindigkeit, R., Biller, A., Buch, M., Schiebel, H. M., Boppre, M., and Hartmann, T. (1997) Eur J. Biochem. 245, 626–632
19. Garrett, R. M., Johnson, L. J., Graf, T. N., Feigenbaum, A., and Rajagopalan, K. V. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 6394–6398
20. Clegg, S. M., and Abbatt, J. P. D. (2001) Atmos. Chem. Phys. 1, 73–78
21. Hille, R. (1996) Chem. Rev. 96, 2757–2816
22. Mills, G. A. (1940) J. Am. Chem. Soc. 62, 2833–2838
23. Horner, D. A., and Connick, R. E. (2003) Inorg. Chem. 42, 1884–1894
24. Nagy, M., Cass, A. E. G., and Brown, K. A. (1997) J. Biol. Chem. 272, 31265–31271
25. Fredrick, S. E., and Newcomb, E. H. (1969) J. Cell Biol. 43, 343–353
26. Veljovic-Jovanovic, S., Onuki, T., and Takahama, U. (1998) Plant Cell Physiol. 39, 1203–1208
27. Kappler, U., Bennett, B., Rethmeier, J., Schwarz, G., Deutzmann, R., McEwan, A. G., and Dahl, C. (2000) J. Biol. Chem. 275, 13202–13212
28. Miszalski, Z., and Ziegler, H. (1992) Z. Naturforsch. 47, 360–364
29. Seinfeld, J. H., and Pandis, S. N. (1998) Atmospheric Chemistry and Physics: From Air Pollution to Climate Control, pp. 1326–1345, John Wiley and Sons, New York
30. McArdle, J. V., and Hoffmann, M. R. (1983) J. Phys. Chem. 87, 5425–5429
31. Winner, W. E., Goldstein, R. A., and Mooney, H. A. (1985) Sulfur Dioxide and Vegetation: Physiology, Ecology, and Policy Issues, pp. 593–601, Stanford University Press, Stanford, CA
32. Neill, S. J., Desikan, R., and Hancock, J. T. (2002) Curr. Opin. Plant Biol. 5, 388–395
33. Laloi, C., Apel, K., and Danon, A. (2004) Curr. Opin. Plant Biol. 7, 323–328
34. Veljovic-Jovanovic, S. D., Pignocchi, C., Noctor, G., and Foyer, C. H. (2001) Plant Physiol. 127, 426–435
35. Karpinski, S., Reynolds, H., Karpinka, B., Wingle, G., Creissen, G., and Mullineaux, P. (1999) Science 284, 654–657