Enhanced Formaldehyde Detoxification by Overexpression of Glutathione-Dependent Formaldehyde Dehydrogenase from Arabidopsis

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The ADH2 gene codes for the Arabidopsis glutathione-dependent formaldehyde dehydrogenase (FALDH), an enzyme involved in formaldehyde metabolism in eukaryotes. In the present work, we have investigated the potential role of FALDH in detoxification of exogenous formaldehyde. We have generated a yeast (Saccharomyces cerevisiae) mutant strain (sfa1/H9004) by in vivo deletion of the SFA1 gene that codes for the endogenous FALDH. Overexpression of Arabidopsis FALDH in this mutant confers high resistance to formaldehyde added exogenously, which demonstrates the functional conservation of the enzyme through evolution and supports its essential role in formaldehyde metabolism. To investigate the role of the enzyme in plants, we have generated Arabidopsis transgenic lines with modified levels of FALDH. Plants overexpressing the enzyme show a 25% increase in their efficiency to take up exogenous formaldehyde, whereas plants with reduced levels of FALDH (due to either a cosuppression phenotype or to the expression of an antisense construct) show a marked slower rate and reduced ability for formaldehyde detoxification as compared with the wild-type Arabidopsis. These results show that the capacity to take up and detoxify high concentrations of formaldehyde is proportionally related to the FALDH activity in the plant, revealing the essential role of this enzyme in formaldehyde detoxification.

Formaldehyde is a toxic compound produced during plant one-carbon (C1) metabolism. Most formaldehyde does not exist in vivo in a free state but is bound to endogenous nucleophiles, such as glutathione or tetrahydrofolate (Sardi and Tyihak, 1994; Chen et al., 1997). The main sources of formaldehyde in plants are dissociation of 5,10-methylene-tetrahydrofolate and oxidation of methanol, derived mainly from pectin demethylation (Fall and Benson, 1996). Formaldehyde can also arise from other oxidative demethylation reactions (Sardi and Tyihak, 1994), from glyoxylate decarboxylation (Prather and Sisler, 1972), and from P-450-dependent oxidation of herbicides (Clejan and Cederbaum, 1993). Furthermore, formaldehyde might have an exogenous origin from industrial waste, being a polluting agent of residual waters and of air (Wolferton et al., 1989; Giese et al., 1994). It is one of the main indoor air pollutants, present in tobacco smoke, furniture, industrial adhesives, and varnishes. It has been classified as a mutagen and suspected carcinogen (Wippermann et al., 1999) and as causing headaches and irritation of the mucosa. Formaldehyde is considered a toxic compound because of its ability to react with proteins, nucleic acids, and lipids. The World Health Organization has established an air quality guideline of 100 μg m⁻³ for formaldehyde (European Commission, 1994).

Several studies have demonstrated that exogenous formaldehyde can be incorporated into the metabolism of photosynthetic cells and be used as a carbon source. For example, feeding the common spider plant (Chlorophytum comosum) with ¹⁴C-formaldehyde resulted in ¹⁴C-labeled products derived from the C1 metabolism, such as Ser and phosphatidylcholine (Giese et al., 1994). Removal of formaldehyde can occur by different in vivo pathways, through the formation of adducts with glutathione, Arg, Asn, and phosphatidylcholine (Giese et al., 1994). Removal of formaldehyde can also take place through the detoxification pathway catalyzed by the glutathione-dependent formaldehyde dehydrogenase (FALDH). This enzyme can be active in animal and plant tissues and catalyzes the following reaction:

\[ \text{S-hydroxymethylglutathione} + \text{NAD} \rightarrow \text{FALDH} \]

\[ \text{S-formylglutathione} + \text{NADH} + \text{H}^+ \]
where S-hydroxymethylglutathione is a nonenzymatically formed adduct of glutathione and formaldehyde.

S-formylglutathione is then hydrolyzed to formate and glutathione by S-formylglutathione hydrolase:

\[
\text{Hydrolase} \quad \text{Formate + Glutathione} \quad (2)
\]

Formate can give rise to C1 folates, but a quantitatively more important fate is oxidation to CO₂ mediated by formate dehydrogenase (Cossins, 1964). NAD-dependent formate dehydrogenase has been cloned from Arabidopsis, which is predicted to have a mitochondrial and a chloroplastic localization (Olson et al., 2000). As is the case in the photosynthetic bacteria *Rhodobacter sphaeroides* (Barber and Donohue, 1998), it might be that in plants the Calvin cycle assimilates in part the products of FALDH activity.

In addition to formaldehyde, FALDH can also oxidize long-chain alcohols and ω-hydroxy fatty acids, such as octanol and 12-hydroxydecanoic acid. Furthermore, it has been demonstrated recently that it is very active in the reduction of N-nitrosoglutathione, the condensation product of glutathione and NO (Jensen et al., 1998; Liu et al., 2001; Sakamoto et al., 2002).

FALDH from Arabidopsis has been characterized, and its cDNA has been cloned (Martínez et al., 1996). This enzyme exhibits features similar to those of their animal counterparts and shows a structure highly conserved in plants and animals. FALDH is expressed in low amounts in Arabidopsis (5 × 10⁻³ units mg⁻¹, Martínez et al., 1996), which is approximately the same amount that has been observed in dry pea (*Pisum sativum*) seeds (4 × 10⁻³ units mg⁻¹, Shaqf et al., 1996) and about twice the amount present in the spider plant (Giese et al., 1994). The gene is expressed at similar levels in all plant organs, as assessed by northern-blot analysis (Martínez et al., 1996), in analogy with the ubiquitous distribution of the corresponding enzyme in animal tissues (Smith, 1988). FALDH is encoded in the Arabidopsis genome by a single gene, which maps in chromosome 5 (Dolfersus et al., 1997).

In the present work, we have investigated the potential role of FALDH in detoxification of exogenous formaldehyde. We have generated a yeast strain (*Saccharomyces cerevisiae*) mutant strain (sfa1Δ) by in vivo deletion of the *SFA1* gene that codes for the endogenous FALDH. We have investigated the effect of the overexpression of Arabidopsis FALDH in this mutant, regarding its capacity to detoxify exogenous formaldehyde. To investigate the role of the enzyme in plants, we have generated transgenic Arabidopsis lines transformed with either sense or antisense constructs of FALDH gene, and we have measured the rates for formaldehyde uptake and detoxification by both transgenic plants overexpressing FALDH and plants with reduced FALDH levels. Our results confirm the central role of FALDH in formaldehyde metabolism in plants.

**RESULTS**

**Construction of a Yeast sfa1Δ Strain**

To explore the physiological role of the Arabidopsis FALDH, we made complementation experiments by transforming a yeast strain lacking FALDH activity. This strain was constructed by in vivo deletion of the *SFA1* gene, coding for the yeast glutathione-dependent FALDH (Wehner et al., 1993). A linear fragment containing the *HIS3* gene flanked by the flanking sequences of *SFA1* gene was used to transform a haploid strain (His⁻), derived from the diploid W303D, to prototrophy (His⁺). Isoelectrofocusing analysis stained by activity confirmed that none of the His⁺ transformants contained glutathione-dependent FALDH activity (Fig. 1A, lane 3). The *sfa1::HIS3* replacement was also confirmed by Southern analysis, using as probe a *Pst*1/*Nco*I fragment containing the *SFA1* gene (Fig. 1, B and C).

**Figure 1.** Analysis of the yeast sfa1Δ strain (*sfa1::HIS3*). A, Isoelectric focusing and FALDH activity staining on homogenates of wild-type W303D (lane 1) and *sfa1::HIS3* strain transformed with (lane 2) and without (lane 3) the ADH2-pYes2 plasmid. B, Southern-blot analysis of genomic DNA digested with *Pst*1 and *Eco*RI from *sfa1::HIS3* (lane 1) or W303D (lane 2) strain. The *HIS3* gene contains an internal *Pst*1 restriction site that is not present in the *SFA1* gene. C, Schema of the homologous recombination procedure used to construct the sfa1Δ yeast strain. The *Pst*1/*Nco*I fragment containing the *SFA1* gene (at the bottom) was used as probe for Southern analysis.
Figure 2. Electrophoretic analysis of purified Arabidopsis FALDH and specificity of the anti-FALDH antibodies. A, Purified FALDH (1.4 µg) was analyzed by SDS-PAGE and revealed by silver staining (lane 1). B, Specificity of the antibodies. Twenty micrograms of Arabidopsis protein extracts was electrophoresed, blotted to a membrane, stained with Coomassie Blue (lane 1), and developed with the anti-FALDH antibodies (lane 2) or the pre-immune antiserum (lane 3). C, Competition experiments for the antibodies binding. Fifty nanograms of purified FALDH (lanes 1, 3, and 5) or 20 µg of Arabidopsis protein extracts (lanes 2, 4, and 6) was electrophoresed, and the blotted membranes were incubated either with the antibodies (lanes 1 and 2), the pre-immune antiserum (lanes 3 and 4), or the antibodies pre-incubated with 30 µg of the protein used for immunization (lanes 5 and 6).

Overexpression of Arabidopsis FALDH in Yeast. Purification of the Enzyme and Generation of Specific Antibodies

Arabidopsis FALDH cDNA was cloned into the yeast pYes2 expression vector, under the control of the GAL1-GAL10 promoter (ADH2-pYes2 plasmid), and introduced into the sfa1::HIS3 strain. The transformed strain was grown in the presence of Gal as carbon source and using His and uracil as markers. The selected colonies exhibited FALDH activity in the crude extracts, confirmed by the presence of a band of the expected size in electrofocusing gels stained by activity (Fig. 1A, lane 2), indicating both that it was correctly expressed and that it was functional in this heterologous system. To purify the enzyme from this source, we used three consecutive chromatographic steps. From 500 g of yeast cell pellet, 2.24 mg of purified protein was obtained that appeared as a single band of M_r 45,000 on SDS-PAGE (Fig. 2A). This band was recognized by an anti-rat FALDH antiserum (result not shown). The specific activity of the purified enzyme against S-hydroxymethylglutathione was found to be 15 units mg⁻¹. Fold purification and recovery of the process are shown in Table I.

The purified enzyme was inoculated into rabbits to raise polyclonal antibodies. The antibodies recognized a single band of the expected size (45 kD) in Arabidopsis crude protein extracts that was not detected using the pre-immune sera (Fig. 2B, lanes 2 and 3, respectively). Those immunoreactions were specific because the binding of the antibodies could be competed with the protein used for immunization (Fig. 2B, lanes 4–9).

Enzymatic Properties of Arabidopsis FALDH

The Arabidopsis enzyme expressed in yeast showed a K_m value of 7 µM. The deduced turnover number (K_cat) was 1,351 min⁻¹, calculated for a M_r = 90,000 (dimer), and the catalytic efficiency (K_cat/K_m) was 193,000 mm⁻¹ min⁻¹. The enzyme exhibited high activity at pH 10 toward several plant alcohols, such as farnesol and geraniol. However, at more physiological pH (pH 7.5), only farnesol showed a significant activity, with a K_cat/K_m of 780 mm⁻¹ min⁻¹. Other medium-chain alcohols and ω-hydroxy fatty acids were also substrates of the enzyme, though their activity was much lower (Table II).

Overexpression of Arabidopsis FALDH in Yeast Greatly Improves Its Ability to Grow in the Presence of Formaldehyde

The yeast sfa1::HIS3 strain could grow at the same rate as the wild type in rich medium but could not grow in the presence of 0.6 mM formaldehyde. In contrast, the wild-type yeast strain could grow in the presence of 0.6 mM formaldehyde, though after a long lag phase (data not shown). These results confirm the essential role of FALDH for yeast formaldehyde metabolism. The sfa1::HIS3 strain transformed with the ADH2-pYes2 plasmid was then grown both in the absence and in the presence of different concentrations of formaldehyde added exogenously. Figure 3A shows that both the wild type and the transformed strains grew equally well in the absence of formaldehyde, reaching saturation at around 35 h after subculturing. In contrast, at 1 mM formalde-
hyde, yeast wild type was unable to grow during the time of the experiment (60 h), whereas the transformed strain showed the same growth rate as in the absence of formaldehyde. At 2 mM formaldehyde, the transformed strain still grew very efficiently.

We also determined the kinetics of formaldehyde elimination from the culture medium (Fig. 3B). Our results show that exogenous formaldehyde disappeared very rapidly in the case of the transformed yeast strain and was completely metabolized in 50 to 60 h, which is consistent with the cell’s growth rate shown in Figure 3A. However, in the case of the wild-type strain, the concentration of formaldehyde in the culture medium remained constant for long periods of time, which proves its inability to metabolize it efficiently and, as a consequence, to grow in the presence of this toxic compound.

Detoxification of Exogenous Formaldehyde by Transgenic Arabidopsis Plants

We addressed the question of whether plants overexpressing FALDH might be able to cope better with moderately high concentrations of environmental formaldehyde. We generated transgenic Arabidopsis lines transformed with FALDH cDNA under the control of the 35S cauliflower mosaic virus promoter. A total of 40 independently transformed plants were obtained (T1 progeny) after infiltration of Columbia plants. The T1 plants were self-fertilized, and 16 individuals of the T2 progeny showing a Mendelian segregation of kanamycin resistance were brought to homozygosis (T3 generation). Western-blot analysis of seven individuals from the T3 generation is shown in Figure 4A. One of them is a cosuppression line (line 13), whereas the others show from a moderate to a high increase of FALDH protein levels. In the same figure, it can be seen that the increase in FALDH levels correlated well with increased values of FALDH enzymatic activity.

To test the ability of the transgenic plants to metabolize exogenous formaldehyde, we selected three of the homozygous lines (T3 generation) showing the highest expression of the transgene (lines 1, 4, and 6). Equal amounts of seeds (3 mg) were germinated and grown in liquid medium (5 mL), and formaldehyde at the desired concentration was added to 6-d-old plantlets. Subsequently, aliquots were taken to measure the variation of formaldehyde concentration.
during a time course. A control of liquid medium with formaldehyde but without plants was also performed to discard losses of formaldehyde by evaporation. At least three independent experiments were carried out with each line.

At 2 mM formaldehyde, we observed significant differences in the rate for formaldehyde uptake (Fig. 5A). The three transgenic lines tested were able to completely detoxify the formaldehyde in 48 h, whereas Arabidopsis wild-type metabolized it at a slower rate (80% of the total in 48 h). In view of the fact that the plants could cope well with this concentration of exogenous formaldehyde (no visible defects were observed as a consequence of the addition of this compound), we decided to perform the same experiment using a concentration of 5 mM formaldehyde. As can be seen in Figure 5B, more significant differences were observed in this case. Line 1, showing the highest rate for formaldehyde uptake, could achieve a 50% decrease in formaldehyde concentration in the medium after 48 h (in contraposition to the 25% by the Arabidopsis wild type). This higher rate was evidenced very early (at 6 h, there was already a 13% differential rate), and subsequently increased (at 24 and 48 h, there was a 25% differential rate).

To corroborate the above data that suggest a correlation between the amount of FALDH expressed by the plant and its ability to detoxify exogenous formaldehyde, we performed the same experiments with transgenic lines showing reduced levels of FALDH. One line showing a cosuppression phenotype (line 13, Fig. 4A) and three lines bearing antisense constructs (lines 5a, 10a, and 17a, Fig. 4B) were incubated with 2 mM formaldehyde added exogenously. Aliquots from the culture medium were removed during a time course to measure the formaldehyde concentration. The results in Figure 5A demonstrate that the lines with reduced levels of FALDH show a 20% decrease in their ability to detoxify exogenous formaldehyde.

DISCUSSION

Plant FALDHs have been isolated from pea seeds (Uotila and Koivusalo, 1979; Shafqat et al., 1996), Arabidopsis (Martínez et al., 1996), and maize (Zea mays; Wippermann et al., 1999). Their molecular and kinetic properties are remarkably similar to those found in the homolog enzymes from mammals, invertebrates, and microorganisms, providing evidence of the high degree of structural and functional conservation through evolution. The enzyme shows very low expression levels in Arabidopsis plants (Martínez et al., 1996); thus, a system to produce a recombinant enzyme was desirable. We have used a yeast expression system to purify the enzyme to homogeneity and to study its functional significance in the detoxification of exogenous formaldehyde.

Arabidopsis FALDH, purified as a recombinant enzyme from yeast, has a specific activity of 15 units mg\(^{-1}\) and a \(K_m\) value of 7 \(\mu\)M. The polypeptide chain...
of the enzyme has a molecular mass of 45 kD, calculated by SDS-PAGE, which is consistent with both the molecular mass deduced from the cDNA sequence and with that of the enzyme purified from Arabidopsis plants (Martínez et al., 1996). The FALDH purified from a variety of different sources consists of two identical subunits and, thus, is a homodimer (Uotila and Koivusalo, 1989). Based on a dimeric structure for Arabidopsis FALDH, we calculated a \( K_{\text{cat}} \) value of 1,351 min\(^{-1} \) and a \( K_{\text{cat}}/K_m \) of 193,000 mM\(^{-1} \) min\(^{-1} \). This last value, which is the catalytic efficiency, is comparable with those of pea and other eukaryotes (Shafqat et al., 1996; Fernández et al., 1997).

SFA1 deletion in yeast is not lethal but impairs its growing in the presence of formaldehyde (Fernández et al., 1999; this work). We have demonstrated in this work that Arabidopsis FALDH is able to complement a knockout yeast mutant that has the SFA1 gene replaced by the HIS3 marker gene. Our results show that overexpression of Arabidopsis FALDH in this mutant results in high resistance to formaldehyde, demonstrating the functionality of the Arabidopsis enzyme and its essential role in formaldehyde metabolism. This effect was quite remarkable because at 1 mM formaldehyde, yeast wild type was unable to grow, whereas the strain overexpressing Arabidopsis FALDH grew at the same rate as in the absence of formaldehyde. At 2 mM formaldehyde, the growing rate of the transformed strain still was only slightly affected.

Additional support for the important role of FALDH in detoxifying formaldehyde was obtained by the generation of transgenic Arabidopsis lines. Three independent lines, exhibiting from 11- to 18-fold the wild-type FALDH activity, were used to test the capacity of plants overexpressing FALDH to take up and detoxify exogenous formaldehyde. We observed an increase in the detoxification rate of 25%, both at 2 and 5 mM formaldehyde (64 and 132.5 mL L\(^{-1} \), respectively). At 2 mM formaldehyde, transgenic plants could completely detoxify the exogenous formaldehyde in 48 h or less, without any visible damage. These results were corroborated by generating transgenic lines with decreased levels of FALDH enzyme (cosuppression and antisense lines). At 2 mM formaldehyde, these plants showed a 20% decrease in their ability to detoxify formaldehyde, as compared with the wild-type plants.

At 5 mM formaldehyde, which is an extremely toxic concentration, the transgenic lines showed a good rate for formaldehyde uptake during the first 24 h. From this time on, we observed important phytotoxic effects, the leaves starting to display chlorotic patches that finally spread to the whole tissue. At the same time, the rate decreased significantly, and the plants could not achieve a complete detoxification of the exogenous formaldehyde. This phytotoxic effect of formaldehyde at such high concentration is not surprising and might be due to the formaldehyde itself or to the products of its oxidation. The chlorotic phenotype of the plants and the observation of leaf sections under electron microscopy (data not shown) suggest that chloroplasts are seriously damaged after long expositions to 5 mM formaldehyde. This might be due to a process of photooxidation caused by an accumulation of FALDH oxidation products that are toxic and have effects on the glutathione/redox homeostasis.

It has been reported that foliar application of methanol can stimulate plant growth (Nonomura and Benson, 1992) and that methanol is quickly oxidized in most plant tissues successively to formaldehyde, formic acid, and CO\(_2\) (Cossins, 1964). Formaldehyde can be incorporated to plant C1 metabolism mainly by two metabolic pathways that involve folate-mediated and -independent reactions (Hanson and Roje, 2001). In the pathway involving folate-mediated reactions, formaldehyde can be converted to 5,10-methylene-tetrahydrofolate by a nonenzymatic reaction with tetrahydrofolate or to formate by the successive reactions catalyzed by FALDH and S-formylglutathione hydrolase. The formate is then incorporated into the C1-tetrahydrofolate pool via the 10-formyl-tetrahydrofolate. In the folate-independent pathway, formaldehyde is successively converted to formic acid and CO\(_2\), and CO\(_2\) is then assimilated by the Calvin-Benson cycle in the photosynthetic tissues (Cossins, 1964). Formate might also give rise to glyoxylate, in a reaction catalyzed by glyoxylate synthetase that is exclusively found in plants (Janave et al., 1993). Our results showing that the capacity to detoxify exogenous formaldehyde is proportionally related to the level of FALDH activity in the plants strongly suggest that the major metabolic flux of formaldehyde is via formate. However, our data do not allow the elucidation of the final fate of this formate. S-formylglutathione hydrolase and formate dehydrogenase have been cloned from Arabidopsis (Olson et al., 2000; Kordic et al., 2002), demonstrating that all the enzymes necessary for this metabolic pathway are present.

In summary, our results show that overexpression of the enzyme FALDH in plants confer a high resistance to formaldehyde, thus supporting the “green liver” concept of plant xenobiotic metabolism (Sandermann, 1992). The availability of transgenic plants with modified amounts of FALDH will allow us to investigate the mechanisms of formaldehyde toxicity at the cellular level in the future. This knowledge is necessary to improve the detoxification properties of the plants and to better understand C1 metabolism in higher plants.

**MATERIALS AND METHODS**

**Biological Material**

Yeast (*Saccharomyces cerevisiae*) W303D strain was grown at 30°C in Wickerham’s medium (United States Biochemical Corp., Cleveland). Arabi-
Bio-Gel HT (Bio-Rad Laboratories, Hercules, CA) column (16 × 110 cm, 1 × 10 mm) equilibrated with 200 mL of 25 mM Tris/HCl (pH 8), 0.5 mM sodium dodecyl sulfate, 1 mM phenylmethylsulfonyl fluoride, and 1 mM benzamidine, and loaded onto a Blue-Sepharose 6B column (1.8 × 20 cm) equilibrated with the same buffer. The active material was eluted with a 600-ml linear gradient of 0 to 2.0 mM sodium acetate. Protein concentrations were determined by the Bradford assay (Bio-Rad), using bovine serum albumin as a standard.

Construction of Transgenic Arabidopsis Plants

To prepare the sense construct, an EcoRI/XhoI restriction fragment containing the Arabidopsis FALDH coding sequence (Martinez et al., 1996) was purified and subcloned downstream of the cauliflower mosaic virus 35S promoter into the Smal site of pDH51 (Pietrzak et al., 1986). To prepare the antisense construct, a PstI/BglII fragment (425 bp) was purified and subcloned into pDH51 cut with BamHI and PstI. After verifying the correct orientation of the insert, the resulting 35S:ADH2 expression cassettes were excised as EcoRI restriction fragments and subcloned into the EcoRI site of the Agrobacterium tumefaciens binary plasmid pBin19 (Bevan, 1984). The recombinant plasmids were then introduced into A. tumefaciens C58C1 strain by the freeze-thaw method. Arabidopsis plants were transformed by the vacuum infiltration method (Bechtold et al., 1993). Transgenic plants were selected on Murashige and Skoog solid media containing 40 mg L⁻¹ kanamycin and later transferred to soil seedlings. Two lines of transgenic plants, with pBin19 empty vector were also produced to be used as controls. Segregation tests were performed with the T₃ seeds obtained by self-fertilization of independent primary transformants. Homozygous, monogenic T₃ seeds were obtained by self-fertilization of T₂ plants using kanamycin resistance as the marker.

Electrophoretic Analysis and Immunochemical Procedures

SDS-PAGE was performed as described (Laemmli, 1970), with subsequent Coomassie Blue or silver staining (Merrill et al., 1981). Isoelectric focusing was performed on polyacrylamide gels in a pH 3 to 9 gradient (PhastSystem, Amersham-Pharmacia Biotech), and bands were visualized by staining for glutathione-dependent FALDH activity with Nitro Blue tetrazolium/phenazine methosulphate, 4.8 mM formaldehyde, and 1 mM glutathione. For production of the antibodies, Arabidopsis FALDH purified as a recombinant enzyme from yeast was used to inoculate rabbits by conventional methods (Harlow and Lane, 1988). For western blotting, proteins were electrophoresed on 12.5% (w/v) SDS-PAGE gels, transferred to immobilon-P membranes (Millipore), and incubated with the antisera (dilution 1:3,000 [v/v]). The immunocomplexes were revealed using the Immun-Star detection kit system (Bio-Rad). Loading of equal amounts of proteins was controlled by Bradford analysis and by gel and membrane staining. Competition experiments to prove the specificity of the antibodies were performed by pre-incubation of the antibodies with 30 μg of the same protein used for rabbit inoculation for 1 h at room temperature.

Enzymatic Activity

Enzyme activity was determined spectrophotometrically at 25°C by monitoring the production of NADH at 340 nm (ε₅₄₀ = 6.22 mm⁻¹ cm⁻¹) with a Hewlett-Packard 8452A diode array spectrophotometer (Hewlett-Packard, Palo Alto, CA). In some experiments, 0.6 mM 3-acetylpyridine adenine dinucleotide (ε₅₄₀ = 9.10 mm⁻¹ cm⁻¹; Sigma, St. Louis) was used as a cofactor instead of NAD. One unit of activity corresponds to 1 μmol reduced cofactor formed per minute. FALDH activity was measured in 0.1 M sodium phosphate (pH 7.5) as described by Martinez et al. (1996). Kinetic constants for FALDH were determined with S-hydroxymethylglutathione. Concentrations of S-hydroxymethylglutathione were calculated using its dissociation constant (Koivusalo et al., 1989) and keeping free glutathione at a constant concentration of 1 mM, changing accordingly the formaldehyde concentration. Kinetic constants for alcohols were determined with 2.4 mM NAD, in 0.1 M sodium phosphate at pH 7.5, or in 0.1 M Gly buffer at pH 10. Seven substrate concentrations, in duplicate, were used for each kinetic measurement. Kinetic results were analyzed using the nonlinear regression data analysis program Enzfitter (Elsevier, Biosoft, Cambridge, UK), and the computer output was expressed in terms of two independent estimations. Formaldehyde concentration was determined by the conversion of NAD to NADH in the presence of FALDH from Pseudomonas putida with an excess of NAD.
Distribution of Materials

Upon request, all novel materials described in this publication will be made available in a timely manner for noncommercial research purposes, subject to the requisite permission from any third party owners of all or parts of the material. Obtaining any permission will be the responsibility of the requestor.

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LITERATURE CITED


