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STUDIES ON AN ENZYME, *S*-FORMYLGLUTATHIONE HYDROLASE, OF THE DISSIMILATORY PATHWAY OF METHANOL IN *CANDIDA BOIDINII*

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Summary

In *Candida boidinii*, *S*-formylglutathione formed by reaction of the glutathione-dependent formaldehyde dehydrogenase is hydrolyzed to formate and glutathione by a special enzyme, *S*-formylglutathione hydrolase which is induced in *C. boidinii* along with the other enzymes of the dissimilatory pathway during growth on CH_3OH. The *S*-formylglutathione hydrolase was purified to apparent homogeneity and a specific activity of 1390 U/mg. The molecular weight of the native enzyme was determined as 61 000 by gel filtration and 64 000 by sedimentation-diffusion equilibrium. It is composed of two non-identical polypeptide chains of 35 000 and 25 000 daltons.

The K_m-value of *S*-formylglutathione was found to be 0.21 mM. Glutathione is a competitive inhibitor with a K_i value of 18.5 mM. The enzyme is very specific for *S*-formylglutathione; *S*-acetylglutathione gave 1.3% of the reaction rate, *S*-propionyl- and *S*-glycolylglutathione 0.4 and 0.3%, respectively. Other glutathione derivatives of hydroxyacids tested were not split by the *S*-formylglutathione hydrolase.

Introduction

In methanol-utilizing yeasts, the methanol is successively oxidized to carbon dioxide by the induced enzymes methanol oxidase, formaldehyde dehydrogenase and formate dehydrogenase [1—4]. We have purified and characterized these enzymes from the facultative methylotrophic organism *Candida boidinii* in recent years [5,6]. We observed that the true substrate of the formaldehyde

dehydrogenase from *C. boidinii* is not formaldehyde but the hemimercaptal spontaneously formed by reaction of formaldehyde with glutathione. Similar results were also found for some other methanol-utilizing yeast strains. Consequently, the primary reaction product of the formaldehyde dehydrogenase of these yeasts was shown to be *S*-formylglutathione rather than formate [6]. The *S*-formylglutathione formed is not a stable intermediate and is hydrolyzed also non-enzymatically in aqueous solutions to give formate and free glutathione. Van Dijken et al. [7] reported that in *Hansenula polymorpha* the formate dehydrogenase hydrolyzed *S*-formylglutathione in the presence of NAD^+, suggesting that this enzyme has two functions, the hydrolysis of the *S*-formylglutathione and the oxidation of formate to give carbon dioxide and NADH. We had observed a hydrolase activity in crude extracts of *C. boidinii* [6] earlier, and investigated it in detail. In the following paper we describe the purification and properties of an *S*-formylglutathione hydrolase from *C. boidinii*. Such an enzyme has been previously described only by Uotila and Koivusalo [8] from human liver, where the conversion of formaldehyde to formate also requires glutathione and is catalyzed by a dehydrogenase and a hydrolase.

Materials and Methods

Organism and cultivation

For the induction experiments, *C. boidinii* (ATCC 32 195) was grown on glucose in rich medium in shake flasks at 30°C according to Sahm and Wagner [9]. Cells were harvested by centrifugation, washed twice with 0.9% NaCl and resuspended in minimal medium containing 1% CH_3OH as the sole carbon and energy source. Growth was estimated by turbidity measurements at 546 nm. After appropriate times, samples were taken for the determination of the dissimilatory enzymes. For enzyme preparations *C. boidinii* was grown in 80-1 or 250-1 fermenters in minimal medium with CH_3OH as the sole carbon and energy source as described earlier [9]. Cells were harvested by centrifugation and stored frozen until use.

Preparation of crude extracts

In small scale experiments, *C. boidinii* cells were suspended in 50 mM potassium phosphate buffer pH 7.5, and broken in an X-Press (AB Biox, Nacka, Sweden) at least −20°C under high pressure. The suspension was thawed and centrifuged at 25 000 × *g* for 30 min. For preparative purposes a Dyno Mill was employed to disintegrate the cells as described earlier [19].

Enzyme assays

Methanol oxidase, formaldehyde dehydrogenase, and formate dehydrogenase were assayed as described earlier [5,6]. *S*-formylglutathione hydrolase activity was measured by following the absorbance of the thioester bond at 240 nm using a Zeiss photometer M4QIII equipped with temperature-controlled cuvettes and recorder. The reaction mixture contained 90 μmol potassium phosphate buffer (pH 7.1), 1 μmol *S*-formylglutathione and limiting amounts of enzyme in a total volume of 1 ml. The assay was carried out at 25°C. All measurements were corrected for nonenzymatic hydrolysis of the substrate. An

extinction coefficient $\epsilon_{240} = 3300\ cm^{-1} \cdot M^{-1}$ was used for calculation. 1 unit *S*-formylglutathione hydrolase corresponds to the amount of protein hydrolyzing 1 μmol *S*-formylglutathione per min.

Substrate and substrate analogs

S-Formylglutathione was chemically synthesized according to Uotila [10]. The product was finally purified by gel chromatography on a Sephadex G-10 column and eluted with 10 mM sodium acetate buffer (pH 4.5). *S*-formylglutathione was freeze-dried, redissolved in water to give a concentration of 0.1 M and stored frozen in aliquots of 10 μmols. Samples were used within 14 days after synthesis. *S*-Lactoyl-, *S*-glycolyl-, *S*-glyceryl-, and *S*-mandelyl-glutathione were synthesized enzymatically according to Racker [11] using the enzyme glyoxalase I, obtained from Boehringer (Mannehim, F.R.G.).

The reaction was followed at 240 nm, the products were purified as described for *S*-formylglutathione. Yields were found to be 80, 55, 65, and 60%, respectively, using the following values for the molar extinction coefficient of the compounds at 240 nm: *S*-lactoyl-glutathione, 3310; *S*-glyceryl-glutathione, 3370; *S*-glycolyl-glutathione, 3260; and *S*-mandelyl-glutathione, 4200 $M^{-1} \cdot cm^{-1}$, respectively.

S-acetyl-glutathione was chemically prepared by the method of Kielley [12] and *S*-propionyl-glutathione was prepared as described by Uotila [10]. The molar extinction coefficients at 240 nm were taken as 2980 and 3070 $M^{-1} \cdot cm^{-1}$, respectively. Protein was determined by the method of Lowry et al. [13] using bovine serum albumin as standard.

Preparation of glutathione-Sepharose 4B

Reduced glutathione was coupled to Sepharose 4B activated with CNBr [14]. The resin was thoroughly washed with 0.1 M $NaHCO_3$ (pH 9.5), 1 M NaCl, 1 mM EDTA followed by 0.1 M sodium acetate, 1 M NaCl (pH 4.3), 1 mM EDTA, and finally with 10 mM sodium phosphate (pH 6.2).

Preparation of Procion green H-E4BD-Sepharose 4B

40 g Sepharose 4B were suspended in 200 ml water, 20 ml 20% NaCl solution were added and the suspension titrated with 10 N NaOH to pH 11—11.5. 500 mg Procion green H-E4BD (ICI, Frankfurt, F.R.G.) were added with careful stirring and the mixture heated for 2 h at 60—65°C. The resin was recovered by filtration and washed thoroughly with 1 M NaCl and distilled water. A known aliquot of the resin was hydrolyzed with 6 N HCL at 40°C for 1 h and the amount of dye in solution measured at 625 nm. Approximately 0.35 mg of the dye were bound per ml resin.

Isoelectric focusing

Isoelectric focusing was carried out in cylindrical 5% polyacrylamide gels containing ampholytes (LKB, Bromma, Sweden) (pH 3—10), and the following proteins were used for calibration: ovalbumin (p*I* 4.65), bovine serum albumin (p*I* 4.80), β-lactoglobulin (p*I* 5.1), myoglobin (p*I* 6.99), and chymotrypsinogen (p*I* 9.5). Proteins used for calibration were obtained from Boehringer (Mannheim, F.R.G.). 0.2 M H_2SO_4 and 0.4 M ethylene diamine served as electrode

buffers. Separation was carried out at 500 V for 3 h. Gels were removed from the glass cylinders, treated with 10% trichloroacetic acid overnight and stained with Coomassie brilliant blue. A second isoelectric focusing was performed in a flat bed of granulated gel according to Radola [15]. The pH in the gel bed was measured with a special electrode purchased from Desaga (Heidelberg, F.R.G.).

Determination of molecular weight

Gel filtration. The molecular weight of the native protein was estimated by gel filtration by the method of Andrews [16]. Sephadex G-100 was packed in a column (2.5 × 100 cm) equilibrated with 50 mM potassium phosphate buffer (pH 7.5), 0.2% β-mercaptoethanol. The column was operated in the cold room at a flow rate of 9.4 ml/h. The following proteins were used for calibration: cytochrome *c* (12 400), desoxyribonuclease I (30 000), ovalbumin (43 000), bovine serum albumin (68 000) and aldolase (147 000).

Sodium dodecyl sulfate electrophoresis. The molecular weight of the polypeptide chains were determined by the method of Shapiro et al. [17].

Ultracentrifuge. A Beckman Spinco Model E analytical centrifuge was used, equipped with RTIC temperature control unit, monochromator and photoelectric scanner. The enzyme was dialyzed against 50 mM potassium phosphate buffer (pH 7.5), 1% dithioerythritol. Protein concentrations were determined from the absorbance at 280 nm or another suitable wavelength.

Experiments were performed at 60 000 rev./min for the determination of the sedimentation coefficient and at 10 000 rev./min for the determination of the molecular weight. Centrifugation was carried out in a four-place rotor AN-F and six-place rotor AN-G, respectively, using KelF double sector cells (12 mm) and sapphire windows. The partial specific volume was determined according to Flossdorf and Süssenbach [18] by means of a sensitive density balance (model DMA 02, A. Paar, Graz, Austria).

Results

Induction of the S-formylglutathione hydrolase

When *C. boidinii* is grown on glucose, no *S*-formylglutathione hydrolase can be detected in crude extracts. After transfer of the cells into a methanol-containing medium, the activity of the hydrolase appears with approximately the same time course as the other enzymes of the dissimilatory pathway of methanol (Fig. 1), indicating that the observed activity is induced by methanol and correlated with its metabolism. Surprising is the rather high catalytic activity of the *S*-formylglutathione hydrolase, which greatly exceeds all other enzymes of the methanol metabolism.

Purification of S-formylglutathione hydrolase

All operations were carried out at 4°C. A summary is given in Table I.

DEAE-cellulose column chromatography

The centrifuged crude extract obtained from 1 kg wet cells of *C. boidinii* was diluted with cold deionized water to a conductivity of 1.5 mS (equal to 10 mM potassium phosphate buffer, pH 7.5) and applied to a DEAE-cellulose column

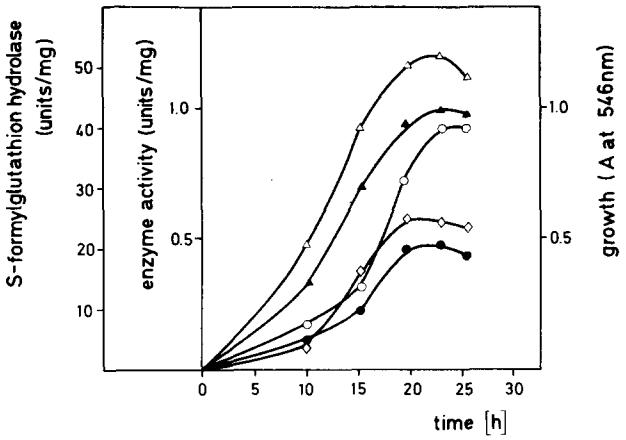


Fig. 1. Growth (○———○) and induction of *S*-formylglutathione hydrolase (△———△), methanol oxidase (◇———◇), formaldehyde dehydrogenase (▲———▲), and formate dehydrogenase (●———●), in *C. boidinii* during growth on methanol. The label 'enzyme activity (unigs/mg)' in the figure only refers to the three latter enzymes. Cells grown on glucose were washed and used as an inoculum in a basal medium with 1% (v/v) methanol as carbon and energy source.

(10 × 80 cm) previously equilibrated with 10 mM potassium phosphate buffer, 0.2% β-mercaptoethanol (pH 7.5). The column was washed with 1 column volume of starting buffer. Then elution was carried out by a linear gradient between 10 l starting buffer and 10 l 0.15 M potassium phosphate buffer, 0.2% β-mercaptoethanol (pH 7.5). The flow rate was 800 ml/h and 400 ml fractions were collected and analyzed for enzymatic activity. In the first ultraviolet peak after the breakthrough of the column, *S*-formylglutathione hydrolase eluted close together with formate dehydrogenase and other material (Fig. 2). The pooled fractions were concentrated by ultrafiltration to 435 ml using a hollow fiber cartridge with a cut-off range of 10 000 daltons.

Gel filtration

The concentrated enzyme solution from the previous step was applied in three equal portions to a column (10 × 90 cm) packed with Sephadex G-75

TABLE I

PURIFICATION OF *S*-FORMYLGLUTATHIONE HYDROLASE

Sample	Volume (ml)	Protein (mg/ml)	Hydrolase (U/ml)	Specif. (U/mg)	Yield (%)
Crude extract	1.550	18.5	$0.98 \cdot 10^3$	53	100
DEAE-cellulose chromatography	435	6.4	$2.32 \cdot 10^3$	363	66
Gel filtration G-75	365	4.2	$1.98 \cdot 10^3$	475	48
Glutathione-Sepjarose-affinity chromatography *	1	1.5	$1.25 \cdot 10^3$	830	(75% relative)
Procion green H-E4BD-Sepharose chromatography *	1	2.2	$3.03 \cdot 10^3$	1.392	(92% relative)

* Only aliquots after step 3 were processed.

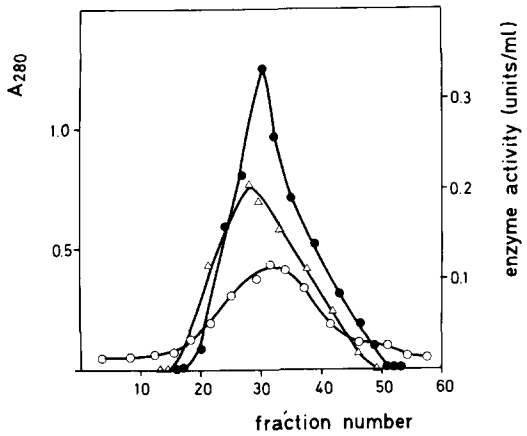


Fig. 2. DEAE-cellulose ion-exchange chromatography for separating *S*-formylglutathione hydrolase from crude extract of *C. boidinii*. The enzyme solution (1550 ml) obtained after centrifugation of the disrupted cells was applied to a column (10 × 80 cm) packed with DEAE-cellulose equilibrated against 10 mM potassium phosphate buffer (pH 7.5), 0.2% β-mercaptoethanol. The column was washed with starting buffer and elution carried out by a linear gradient between 10 l starting buffer and 10 l of 150 mM potassium phosphate buffer (pH 7.5), 0.2% β-mercaptoethanol. (○———○), absorbance at 280 nm; activity of *S*-formylglutathione hydrolase $\times 10^{-3}$ (△———△); activity of formate dehydrogenase (●———●).

equilibrated with 50 mM potassium phosphate buffer, 0.2% β-mercaptoethanol (pH 7.5). The column was eluted with the same buffer at a flow rate of 85 ml/h. The effluent was analyzed for enzymatic activity. *S*-formylglutathione hydrolase was not separated very well from formate dehydrogenase, but high molecular weight material was removed in the void volume of the column. The separation between formate dehydrogenase and hydrolase could be improved only slightly by gel chromatography over Sephadex G-200. The enzyme solution was concentrated by ultrafiltration, mixed with an equal volume of glycerol and stored frozen at −25°C for further experiments.

Affinity chromatography on glutathione-Sepharose 4B

Reduced glutathione is one of the reaction products of the *S*-formylglutathione hydrolase. Since it was noted that glutathione was a competitive inhibitor of the hydrolase, an affinity chromatography over glutathione coupled to Sepharose 4B was investigated, after more conventional methods had failed to separate the enzyme completely from formate dehydrogenase.

The resin was packed in a column (1.6 × 25 cm) and washed with 10 mM potassium phosphate buffer (pH 6.2). 1 ml of a solution containing 1665 U *S*-formylglutathione hydrolase, 3.12 U formate dehydrogenase, and a total of 3.5 mg protein, dialyzed against the equilibration buffer, was applied to the column. The column was eluted with the same buffer at a flow rate of 8 ml/h. Fig. 3 shows that formate dehydrogenase ran through the column while *S*-formylglutathione hydrolase was tightly bound and could not be removed even by 500 mM potassium phosphate buffer. Elution was finally accomplished by 20 mM potassium phosphate buffer (pH 7.3) and 10 mM glutathione. *S*-formylglutathione hydrolase eluted in a rather broad peak. The enzyme-

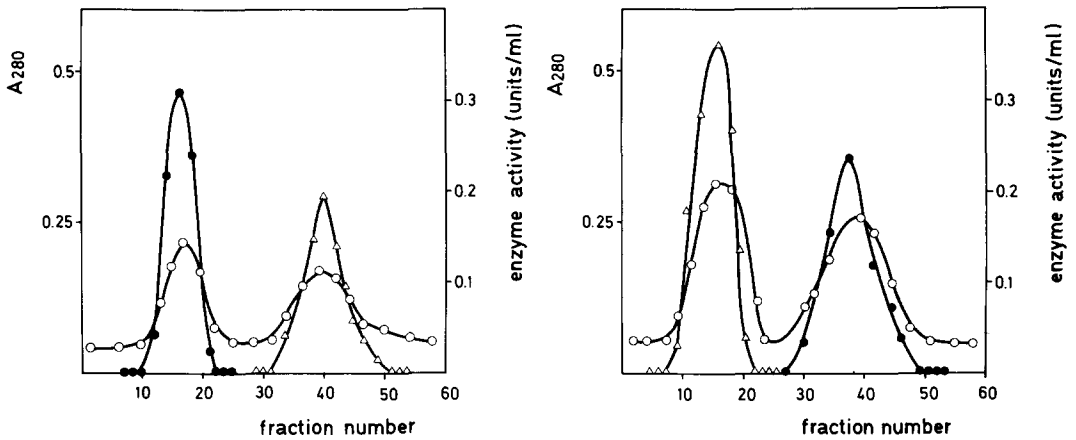


Fig. 3. Separation of *S*-formylglutathione hydrolase and formate dehydrogenase by affinity chromatography on glutathione-Sepharose 4B. The column (1.6 × 25 cm) was equilibrated with 10 mM potassium phosphate buffer (pH 6.2). 1 ml of sample was applied at a flow rate of 8 ml/h. Formate dehydrogenase was not retained. *S*-formylglutathione hydrolase could be eluted with 20 mM potassium phosphate buffer (pH 7.3) containing 10 mM glutathione. (○———○), absorbance at 280 nm; activity of *S*-formylglutathione hydrolase $\times 10^{-3}$ (△———△); activity of formate dehydrogenase (●———●). Buffer change at fraction number 23.

Fig. 4. Separation of *S*-formylglutathione hydrolase and formate dehydrogenase by chromatography on ICI-Procion green H-E4BD-Sepharose 4B. The column (1.6 × 35 cm) was equilibrated with 10 mM potassium phosphate buffer (pH 6.2), 0.2% β-mercaptoethanol. 2 ml of sample were applied at a flow rate of 8 ml/h. Formate dehydrogenase was bound to the column under these conditions while *S*-formylglutathione hydrolase run through. Formate dehydrogenase could be eluted with 500 mM potassium phosphate buffer (pH 8.7), 0.2% β-mercaptoethanol. (○———○) absorbance at 280 nm; activity of *S*-formylglutathione hydrolase $\times 10^{-3}$ (△———△); activity of formate dehydrogenase (●———●). Buffer change at fraction number 22.

containing fractions were concentrated on a Amicon CEC concentrator using a PM 10 membrane. Any residual glutathione was removed by gel filtration employing a column (1.6 × 60 cm) packed with Sephadex G-25 and eluted with 100 mM potassium phosphate buffer.

Chromatography on Procion green H-E4BD-Sepharose

From several triazine dyes tested a resin of Procion green H-E4BD covalently linked to Sepharose 4B proved best for the separation of *S*-formylglutathione hydrolase from formate dehydrogenase. The resin was packed in a column (1.6 × 35 cm) and equilibrated with 10 mM potassium phosphate buffer (pH 6.2), 0.2% β-mercaptoethanol. 2360 U *S*-formylglutathione hydrolase, 6.5 U formate dehydrogenase, and a total of 7 mg protein were applied to the column in a volume of 2 ml. The column was washed with equilibration buffer at a flow rate of 8 ml/h. *S*-formylglutathione hydrolase was not absorbed on the resin under these conditions and ran through the column while formate dehydrogenase was bound and could be eluted only by a gradient of increasing ionic strength and pH above 8 (Fig. 4). Both enzymes could be recovered in high yield (92% of the *S*-formylglutathione hydrolase and 88% of the formate dehydrogenase) from this unconventional resin.

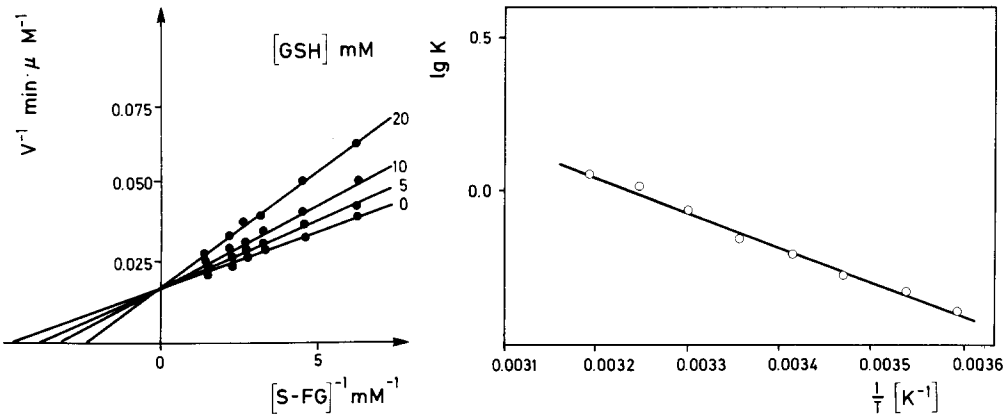


Fig. 5. Inhibition of *S*-formylglutathione hydrolase by glutathione. The enzyme activity was measured under standard conditions, varying the concentration of glutathione (GSH).

Fig. 6. Determination of the activation energy. The velocity of the enzymatic hydrolysis of *S*-formylglutathione was measured at various temperatures in the interval from 5—40° C. The logarithm of the reaction rate is plotted as a function of the reciprocal temperature. From the slope, the activation energy of the enzymatic hydrolysis was calculated to be 21.3 J/mol.

Properties of S-formylglutathione hydrolase

The catalytic activity of *S*-formylglutathione hydrolase was measured as a function of the pH in the interval pH 3.5—9.5; the maximal reaction rate was observed at pH 7.05 as a rather narrow peak. The enzyme lost activity quite rapidly above 40°C but could be stored at 4°C for 2—3 weeks with negligible losses of activity. During longtime storage in 50% glycerol at —20°C, the enzyme lost no activity in a period of 6 months. The temperature dependency of the enzymatic and nonenzymatic hydrolysis of the substrate *S*-formylglutathione was measured and the activation energy calculated according to the equation

$$\log k = \log k_{max} - \frac{A}{2.303 \cdot R \cdot T}$$

where k is the reaction rate, R the universal gas constant and T the temperature. As shown in Fig. 5, a linear relationship between log k and the reciprocal temperature was observed for the enzymatic hydrolysis of *S*-formylglutathione between 5 and 40°C. From the slope of Fig. 6, the activation energy was calculated as 21.3 Joule/mol. Similarly, the activation energy of the nonenzymatic hydrolysis of *S*-formylglutathione was determined in the interval between 25 and 55°C and found to be 59.0 Joule/mol. The enzyme is quite specific for the substrate *S*-formylglutathione. Only 1.3% of the reaction rate was found for the enzymatic hydrolysis of the analogue *S*-acetylglutathione, 0.4% with *S*-propionyl-, and 0.3% with *S*-glycolylglutathione, respectively, while *S*-lactyl-, *S*-glyceryl-, *S*-mandelylglutathione, and formylthioglycolate were not hydrolyzed.

The K_m value of *S*-formylglutathione was determined according to Lineweaver and Burk and found to be 0.21 mM. Reduced glutathione is a competi-

tive inhibitor of the enzyme with respect to *S*-formylglutathione as shown in Fig. 6. The K_i value of glutathione was determined from a Dixon plot and found to be 18.5 mM.

The isoelectric point of *S*-formylglutathione hydrolase was found as 5.28 in cylindrical gels and 5.25 in a granulated gel bed. Under identical conditions formate dehydrogenase exhibited a p*I* of 5.43 and 5.37, respectively; the close similarity of the p*I* values explains the difficulties in separation on ion exchange columns.

Molecular weight and subunit structure of S-formylglutathione hydrolase

S-formylglutathione hydrolase sedimented in the ultracentrifuge as a homogeneous protein with a sedimentation coefficient $S_{w,20} = 4.63$ s. No concentration dependence of the sedimentation coefficient was observed. The molecular weight of the enzyme was determined by sedimentation diffusion equilibrium. A plot of ln c vs. r^2 yielded straight lines from which a molecular weight of 64 000 ± 5000 was calculated. The partial specific volume was obtained from a plot of solution density versus concentration as $\bar{v} = 0.729$ ($cm^3 \cdot g^{-1}$). From gel chromatography using a calibrated Sephdex G-100 column, the molecular weight of *S*-formylglutathione hydrolase was estimated as 61 000, which is in good agreement with the data obtained by other methods.

Gel electrophoresis in the presence of sodium dodecyl sulfate yielded two protein bands corresponding to a molecular weight of 35 000 and 25 000, respectively, in approximately equal amounts as judged from the staining intensity (Fig. 7). Therefore it follows that *S*-formylglutathione hydrolase is composed of two nonidentical subunits. The native enzyme has an $\alpha\beta$-structure,

Fig. 7. SDS-polyacrylamide gel electrophoresis of 10 μg purified *S*-formylglutathione hydrolase. The gel was stained with Coomassie brilliant blue after 3 h migration from top to bottom.

as gel filtration and ultracentrifugation yield a molecular weight which is equal to the sum of the two polypeptide chains. We observed that the larger subunit of *S*-formylglutathione hydrolase cannot be distinguished from the subunit of the formate dehydrogenase by gel electrophoresis which may or may not be just coincident in size.

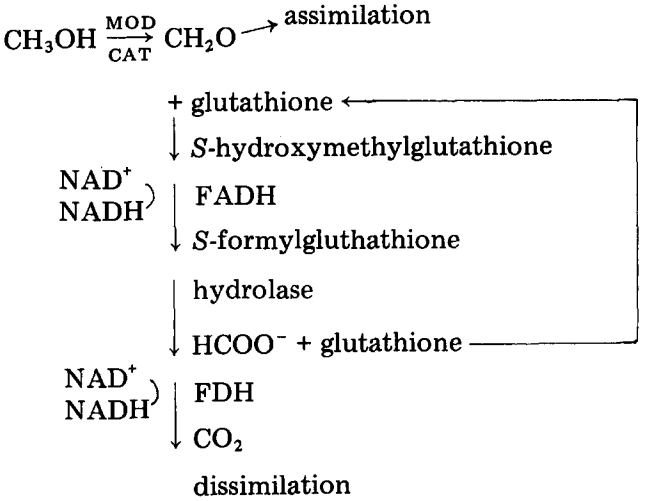
Test for S-formylglutathione hydrolase activity in formate dehydrogenase

Formate dehydrogenase samples purified according to Schütte et al. [6] still contained measurable activities of *S*-formylglutathione hydrolase. Due to its very high specific activity (1390 U/mg), small amounts of the hydrolase can easily be detected. This hydrolase activity however, did not depend on the addition of NAD^+ as described by van Dijken et al. [7] for the formate dehydrogenase from *Hansenula polymorpha*. Formate dehydrogenase, purified as described above by affinity chromatography or chromatography over the new resin Procoin green H-E4BD coupled to Sepharose, does no longer exhibit hydrolase activity towards *S*-formylglutathione as observed by following the absorbance of the thioesterbond at 240 nm, and does not utilize *S*-formylglutathione as a substrate in the oxidation to CO_2 as no reduction of NAD^+ can be detected at 340 nm either. NADH appeared only after the addition of purified *S*-formylglutathione hydrolase to the system.

Discussion

Attempts to separate the *S*-formylglutathione hydrolase and the formate dehydrogenase completely proved difficult by conventional procedures. But the distribution of the activities in the efluent of a DEAE-cellulose column (Fig. 1) and a gel filtration column already indicated that in *C. boidinii* we are dealing with two different enzymes. *S*-formylglutathione hydrolase and formate dehydrogenase could be separated completely by employing either affinity chromatography on glutathione-Sepharose columns or perhaps more conveniently by using a new resin, Procion green H-E4BD coupled to Sepharose (Fig. 4). With the highly purified enzymes, hydrolysis of *S*-formylglutathione and oxidation of formate could be studied separately. In a reconstituted system, the coupled reaction proceeds fairly slow as the K_m for formate of the formate dehydrogenase (13—16 mM) is in the same order of magnitude as the K_i for glutathione (18.5 mM) of the *S*-formylglutathione hydrolase which is accumulating if formate is derived solely from the enzymatic hydrolysis of *S*-formylglutathione. Under physiological conditions in the cells growing on methanol, free glutathione would be utilized to form *S*-hydroxymethyl glutathione by reaction with formaldehyde to serve as substrate for formaldehyde dehydrogenase. The level of free reduced glutathione may be part of the regulatory mechanism to channel the C1 intermediate into the dissimilatory or assimilatory pathway of the cells.

The dissimilatory pathway should be written as follows for *C. boidinii*:



In contrast to the situation in *Hansenula polymorpha*, the hydrolysis of *S*-formylglutathione is catalyzed by a separate enzyme in *C. boidinii* growing on methanol. This enzyme is induced together with the other enzymes of the dissimilatory pathway and does not depend on the concomitant oxidation of the formate to CO_2. No other acceptor than water has yet been found during the enzymatic cleavage of *S*-formylglutathione. It remains unclear whether the hydrolase has also transferase activities. A transferase function was discussed by Uotila and Koivusalo [8] for the *S*-formylglutathione hydrolase in human liver to utilize some of the energy of the thioester bond, but experimental evidence is lacking.

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