Purification and Properties of Glycerol:NAD⁺ 2-Oxidoreductase (Glycerol Dehydrogenase) from *Schizosaccharomyces pombe*

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Glycerol:NAD⁺ 2-oxidoreductase (glycerol dehydrogenase, EC 1.1.1.6) from *Schizosaccharomyces pombe* has been purified to homogeneity. The protein has a molecular weight of about 400 000; it can be dissociated into identical subunits of molecular weight 47 000, and is probably an octamer. The pH optimum for glycerol oxidation is 10.0 or higher and for the reverse reaction is 6.0. Oxidation occurs specifically at C₂ of glycerol to produce dihydroxyacetone and not glyceraldehyde. Several diols with hydroxyls on adjacent carbon atoms can be oxidized and corresponding carbonyl compounds reduced, 1,2-propanediol being oxidized 1.6 times more rapidly than glycerol. The forward reaction has a specific requirement for NAD⁺ as coenzyme whereas NADPH shows about one-third of the activity of NADH for the reverse reaction. The monovalent cations K⁺ and NH₄⁺ activate the enzyme, while Na⁺ and Li⁺ counteract this effect. Some thiol and chelating agents are inhibitory while thiol antagonists, Mn²⁺, and to a lesser extent Zn²⁺, stimulate activity. Apparent *K_m* and *V_{max}* values have been determined. The enzyme is similar to but not identical with the glycerol dehydrogenases isolated from *Escherichia coli* and *Klebsiella aerogenes* (*K. pneumoniae*).

INTRODUCTION

The fission yeast *Schizosaccharomyces pombe* has been shown to produce four different enzymes capable of catalysing pyridine nucleotide-linked breakdown or formation of glycerol (Kong *et al.*, 1985). The glycerol dehydrogenase from *S. pombe* reported originally (May & Sloan, 1981) catalyses the first step in glycerol utilization when glycerol is used as a growth substrate, and is subject to catabolite repression by glucose. In this paper we report the purification and characterization of this enzyme (previously designated GDH2), confirm that it is a glycerol:NAD⁺ 2-oxidoreductase (EC 1.1.1.6) and compare it with similar enzymes isolated from other sources.

METHODS

General methods. The organism used and its maintenance and method of growth, the preparation of cell extracts, enzyme assay methods, methods for protein estimation, enzyme purification procedures and methods for disc gel electrophoresis including the location of protein-containing and enzymically active bands have already been described (Kong *et al.*, 1985).

Growth of cells. Batches of cells for extraction and purification of GDH2 were grown in 72 l batches on modified Edinburgh minimal medium no. 2 containing 1% (v/v) glycerol. During the work it became clear that better yields could have been obtained by growing cells on glucose to the stationary growth phase.

Enzyme assays. The purification of GDH2 was monitored by measuring the rate at which it catalysed the oxidation of NADH with DHA as substrate at pH 9.0. Where measurements of substrate oxidation were carried out, activity was measured by the rate of NAD⁺ reduction at pH 10.0.

Molecular weight and subunit size determinations. The molecular weight of the enzyme was estimated by comparing its mobility on Sephacryl S-300 with that of known standards (Andrews, 1965). Standard proteins (with

Abbreviations: DHA, dihydroxyacetone; GDH2, glycerol:NAD⁺ 2-oxidoreductase.

molecular weights indicated) were obtained from Boehringer ('Combithek' calibration proteins) and included cytochrome *c* (12500), chymotrypsinogen A (25000), ovalbumin (45000), bovine serum albumin (68000), rabbit muscle aldolase (158000), beef liver catalase (240000) and ferritin (450000). Subunit size was estimated by heating the enzyme with SDS before comparing its mobility in SDS-PAGE with that of known standards following the general method of Laemmli (1970).

Determination of pH optima. For this the buffers in the standard assay systems were replaced by alternative buffers adjusted to appropriate pH values. Buffer mixtures used (at 45 mM) were potassium hydrogen phthalate/KOH (pH 4.0–6.0), maleic acid/KOH (pH 6.0–7.0), imidazole/HCl/KCl (pH 6.5–7.5), triethanolamine/KOH (pH 7.0–8.5), glycine/KOH (pH 8.5–10.5) and $\text{KHCO}_3/\text{K}_2\text{CO}_3$ (pH 10.0–12.0). Tris/HCl (pH 7.5–9.0) and MES/KOH (pH 5.5–7.5) were also tested but were found to exert some inhibitory effect on enzyme activity.

Kinetic data. Apparent K_m and V_{max} values for substrates and for coenzymes were determined from Lineweaver–Burk plots made from the results of experiments in which a fixed concentration of substrate or coenzyme and an appropriate range of concentrations of the other reactant were used.

TLC. Glycerol and trioses were separated by TLC on silica gel (Merck, 60HR, reinst) on glass plates; the solvent was ethyl acetate/acetic acid/water (12:1:1, by vol.). After drying, spots were located by either (i) spraying with aniline phosphate and heating at 100 °C for 5 to 10 min when trioses gave a reddish-brown spot, or (ii) spraying with 5 M- H_2SO_4 and heating at 160 °C. Aniline phosphate was prepared fresh by mixing equal volumes of 0.1 M-aniline and 0.1 M-orthophosphoric acid, both made up in water saturated with butan-1-ol (Dickens & Williamson, 1958). R_f values observed were: methylglyoxal 0.95, DHA 0.62, glycerol 0.35, pyruvic acid 0.29, DL-glyceraldehyde 0.09.

Chemicals. In addition to chemicals previously listed (Kong *et al.*, 1985), tetramethylammonium hydroxide was from BDH, pyruvaldehyde (methylglyoxal), D-glyceraldehyde, L-glyceraldehyde, *i*-erythritol and imidazole were from Sigma, MES was from Calbiochem–Behring, hydroxyacetone (acetol), 1,2-propanediol, 1,3-propanediol, 1,2-butanediol, 2,3-butanediol and 1,2,3-butanetriol were from Tokyo Kasei Kogyo Co. (Tokyo, Japan) and L-lactate dehydrogenase was from Boehringer.

1,3-Propanediol. The supplier's analysis reported a purity of 95%. It was further purified by treatment with periodate to remove any contaminating 1,2-diols.

Glyceraldehyde. While DL-glyceraldehyde can be obtained as a crystalline preparation (as the dimer) there are no reports of crystalline preparations of either D- or L-glyceraldehyde; the samples purchased were both syrups claimed to be 70% pure. When this D-glyceraldehyde was examined by TLC with aniline phosphate as spray reagent, it gave, in addition to the main spot at R_f 0.09, a second fainter spot with an R_f corresponding to that of DHA.

Fresh samples of D-glyceraldehyde were prepared by two methods. One was by oxidation of D-fructose with lead tetra-acetate according to Perlin (1962). The 3-*O*-formyl-2-*O*-glycolyl-D-glyceraldehyde produced was hydrolysed with 0.05 M- H_2SO_4 , and the acids removed with a strong anion exchange resin (De-Acidite FF) in the bicarbonate form, leaving D-glyceraldehyde in aqueous solution. The second method was by oxidation of D-glucose with periodate according to Schöpf & Wild (1954) to give 2-*O*-formyl-D-glyceraldehyde which was then hydrolysed and the product obtained as in the first method. Both products, when examined by TLC and spraying with aniline phosphate, gave a single spot, R_f 0.09, with no faster running impurity detectable.

RESULTS

Purification of enzyme

Cell extracts were fractionated by the methods described previously (Kong *et al.*, 1985). Following the removal of nucleic acids (step 1), activity was precipitated with 1.8 M- $(\text{NH}_4)_2\text{SO}_4$ (step 2). Preliminary experiments showed that fractionation with narrower ranges of $(\text{NH}_4)_2\text{SO}_4$ concentrations gave no significant improvement in purification. The precipitate was dissolved in a small volume of 20 mM-Tris/HCl, pH 8.0, and desalted on a column of Sephadex G-25 before chromatography on DEAE-cellulose (step 3). Stepwise elution was carried out with Tris buffer containing increasing concentrations of KCl, activity being eluted with 0.1 M-KCl. Fractions containing activity were pooled, and activity again precipitated with 1.8 M- $(\text{NH}_4)_2\text{SO}_4$. By following the procedure to this stage a purification of 70- to 100-fold could be obtained but disc gel electrophoresis showed that besides the main enzymically active protein, other proteins were still present. Final purification was by gel permeation chromatography (step 4) of pooled samples from several batches which had been stored at –70 °C after step 3, and which were concentrated by ultrafiltration before being applied to the

Table 1. *Summary of purification procedure*

Activity is expressed as $\mu\text{mol min}^{-1}$, specific activity as $\mu\text{mol min}^{-1} (\text{mg protein})^{-1}$, measuring NADH-dependent DHA reduction. The crude extract contained 10.9 g protein.

Fraction	Volume (ml)	Total activity	Specific activity	Purification factor	Yield (%)
Crude extract	435	218	0.020	1	100
Ethodin treatment	435	194	0.026	1.3	89
1st $(\text{NH}_4)_2\text{SO}_4$ precipitation	19	204	0.72	36	94
DEAE-cellulose chromatography	25	120	1.02	51	55
2nd $(\text{NH}_4)_2\text{SO}_4$ precipitation	6.0	52	1.41	70	24
Sephadex G-200 chromatography*	5.0	87	9.3	465	—

* Pooled material from several batches used for final stage.

column. Sephadex G-200 was used for the first batches, Sephacryl S-300 after the size of the enzyme was known. The final product now gave a single protein band on disc gel electrophoresis which coincided with the band of enzyme activity. A summary of the steps followed in a representative purification procedure is shown in Table 1.

Stability of enzyme

The enzyme was best stored as a suspension in 2 M- $(\text{NH}_4)_2\text{SO}_4$ at 4 °C, under which conditions no loss of activity was observed over several months; long term storage was at –70 °C. Considerable protection was given to enzyme solutions by 1.0 M-glycerol. In the absence of glycerol, standing at pH 7.0 and 24 °C for 60 min led to 90% loss of activity while 50 °C for 5 min caused complete inactivation. In the presence of 1.0 M-glycerol, the corresponding losses were 25% and none, while the loss at 60 °C was 50% and at 70 °C 84%.

Molecular weight and subunits

The molecular weight as determined by chromatography on Sephacryl S-300 was estimated to be 400 000. Determination of subunit size by electrophoresis under dissociating conditions with SDS gave a single protein band with molecular weight 47 000, indicating that the undissociated protein must be an octamer, made up of eight identical subunits.

pH optima of forward and reverse enzyme reactions

Glycerol oxidation was most rapid under alkaline conditions, being at a maximum at pH 10 to 12 and decreasing to zero at pH 6. DHA reduction was most rapid near pH 6, falling to zero at pH 4.5, and more slowly to 25% of its peak value at pH 10 (Fig. 1).

Effects of monovalent cations

A summary of the results obtained with different combinations of monovalent cations is shown in Table 2. In addition to the monovalent cations (as chlorides) added to the assay systems, the buffers (phthalate at pH 6.0 and glycine at pH 10.0) also contained monovalent counter-ions which contributed to the total ionic concentration. The highest enzyme activity in both forward and reverse reactions was found with K^+ , 30 mM being optimal. For glycerol oxidation the order of activity was $\text{K}^+ > \text{NH}_4^+ > (\text{CH}_3)_4\text{N}^+$ (tetramethylammonium ion) $> \text{Na}^+ > \text{Li}^+$ with $(\text{CH}_3)_4\text{N}^+$ showing a little more than half the activity of K^+ . Activities observed with mixtures of ions suggest that competition may be occurring in some mixtures, and that ionic ratios rather than absolute concentrations may be the governing factor. For DHA reduction the order of activity was $\text{K}^+ > \text{NH}_4^+ > \text{Na}^+ > \text{Li}^+ = (\text{CH}_3)_4\text{N}^+$. In this direction Na^+ , Li^+ and $(\text{CH}_3)_4\text{N}^+$ appeared more strongly inhibitory than in the forward reaction, activity with Li^+ or $(\text{CH}_3)_4\text{N}^+$ being reduced 14-fold compared with K^+ , and again there was evidence of competitive interactions. In similar studies, both McGregor *et al.* (1974) and Tang *et al.* (1979) used the tetraethylammonium ion on the assumption that it could be regarded as neither activating nor inhibiting and we included the tetramethylammonium ion, hopefully, as a similar reference ion. If this assumption is accepted, the results for the forward reaction show

Fig. 1. Variation of GDH2 activity with pH. O, Forward reaction (glycerol oxidation); ●, reverse reaction (DHA reduction). Activities were measured as in Table 2 except that buffer A and buffer B were replaced by other buffer systems appropriate for each specific pH value (see Methods). The specific activity of the enzyme was $1.05 \mu\text{mol min}^{-1} (\text{mg protein})^{-1}$ at pH 11.5 for the forward reaction and $7.8 \mu\text{mol min}^{-1} (\text{mg protein})^{-1}$ at pH 6.0 for the reverse reaction.

Table 2. *Effect of monovalent cations on GDH2 activity*

The system for measuring glycerol oxidation contained 0.6 mM-NAD^+ , 10 mM-glycerol , $2 \mu\text{g enzyme ml}^{-1}$ in 45 mM-buffer A ; for measuring DHA reduction 0.3 mM-NADH , 5 mM-DHA , $2 \mu\text{g enzyme ml}^{-1}$ in 45 mM-buffer B . Buffer A was glycine adjusted to pH 10.0 with the appropriate monovalent cation hydroxide which gave a cation concentration of 28 mM . Buffer B was phthalic acid adjusted to pH 6.0 with the appropriate monovalent cation hydroxide which gave a cation concentration of 84 mM . The activities equivalent to 100% were 2.0 and $11 \mu\text{mol substrate consumed min}^{-1} (\text{mg protein})^{-1}$ for glycerol oxidation and DHA reduction respectively.

Glycerol oxidation			DHA reduction		
Cation in buffer A (28 mM)	Cation added as chloride (30 mM)	Relative activity	Cation in buffer B (84 mM)	Cation added as chloride (30 mM)	Relative activity
K^+	—	100	K^+	—	100
$(\text{CH}_3)_4\text{N}^+$	—	58	$(\text{CH}_3)_4\text{N}^+$	—	7
	Li^+	19		Li^+	7
	Na^+	42		Na^+	26
	K^+	100		K^+	100
	NH_4^+	90		NH_4^+	93
Li^+	—	10	Li^+	—	5
	Na^+	14		Na^+	6
	K^+	93		K^+	50
	NH_4^+	66		NH_4^+	50
Na^+	—	21	Na^+	—	4
	K^+	100		K^+	78
	NH_4^+	84		NH_4^+	76
	$\text{Na}^+ + \text{K}^+$	74		Li^+	0

K^+ and NH_4^+ to be activators and Na^+ and Li^+ to be inhibitors. The assumption is clearly not valid for the reverse reaction where activity with $(\text{CH}_3)_4\text{N}^+$ is no higher than with Li^+ , and Li^+ antagonizes the activation by K^+ or NH_4^+ , though $(\text{CH}_3)_4\text{N}^+$ does not. The general conclusions which can be drawn from these complex interactions are that the enzyme is activated by K^+ and almost equally well by NH_4^+ , and that Na^+ and Li^+ act as competitive inhibitors.

Effect of thiol and chelating compounds and of divalent cations

The thiol compounds cysteine, dithiothreitol and 2-mercaptoethanol were strongly inhibitory while the thiol antagonists iodoacetamide, *N*-ethylmaleimide and *p*-chloromercuribenzoic acid

Table 3. *Effect of thiol and chelating compounds on GDH2 activity*

Enzyme and compound were mixed and incubated at 20 °C for 10 min. Activity (glycerol oxidation) was assayed as in Table 4. The value equivalent to 100% is also given in Table 4.

Compound	Concn (mM)	Relative activity
None	-	100
L-Cysteine	1	14
Dithiothreitol	1	18
	10	4
2-Mercaptoethanol	1	55
	10	2
Iodoacetamide	1	140
	10	220
N-Ethylmaleimide	0.1	145
	1	97
p-Chloromercuribenzoic acid	0.1	133
	1	106
8-Hydroxyquinoline	10	3
EDTA	1	45
	10	0

Table 4. *Substrate specificity of GDH2*

Oxidation reactions were measured in 40 mM-glycine/KOH, pH 10.0, with 0.6 mM-NAD⁺ (or NADP⁺), 5 mM-substrate and 2 µg enzyme ml⁻¹; the specific activity with glycerol was 2.9 µmol min⁻¹ (mg protein)⁻¹. Reduction reactions were measured in 40 mM-potassium hydrogen phthalate/KOH, pH 6.0, with 0.3 mM-NADH (or NADPH), 5 mM substrate and 2 µg enzyme ml⁻¹; the specific activity with DHA was 16.1 µmol min⁻¹ (mg protein)⁻¹.

Oxidation reaction			Reduction reaction		
Coenzyme	Reduced substrate	Relative activity	Coenzyme	Corresponding oxidized substrate	Relative activity
NAD ⁺	Glycerol	100	NADH	DHA	100
	Ethenediol	30		DL-Glyceraldehyde	0
	1,2-Propanediol	160		Glycolaldehyde	20
	1,2-Butanediol	90		Hydroxyacetone	7
	2,3-Butanediol	60		(1-Hydroxybutan-2-one)	NT
	1,2,3-Butanetriol	50		3-Hydroxybutan-2-one	5
	DL-Glyceraldehyde	30		(1,3-Dihydroxybutan-2-one)	NT
	(Lactaldehyde)	NT		(3-Hydroxypyruvaldehyde)	NT
	Other alcohols*	0		Pyrvaldehyde	18
NADP ⁺	Glycerol	0	NADPH	Other carbonyl compounds†	0
				DHA	30

NT, Not tested.

* Includes *L*-erythritol, 1,3-propanediol, methanol, ethanol, propan-1-ol, propan-2-ol, glycerol 3-phosphate.

† Includes D-glyceraldehyde, formaldehyde, acetaldehyde, butyraldehyde, dihydroxyacetone phosphate.

increased activity. The chelating compounds 8-hydroxyquinoline and EDTA were also inhibitory. When the enzyme was incubated at 20 °C for 10 min with various divalent cations (0.1 mM), Ca²⁺, Cu²⁺, Fe²⁺ and Mg²⁺ produced no significant change, but activity was increased by Zn²⁺ to 170% and by Mn²⁺ to 330% of the control value.

Substrate specificity

The enzyme can catalyse the oxidation of a number of alcohols with structures similar to that of glycerol (Table 4). Greatest activity (1.6 times that found with glycerol) was found with 1,2-propanediol. Activity was also found with several other dihydroxy compounds, and the presence of two hydroxyls on adjacent carbon atoms appears to be a necessary but not a sufficient

Table 5. *Kinetic data for GDH2* K_m and V_{max} values were calculated from Lineweaver-Burk plots.

Substrate tested	Other reactant (mM)	pH	K_m (mM)	V_{max} [$\mu\text{mol min}^{-1}$ (mg protein) $^{-1}$]
Glycerol	NAD ⁺ (0.6)	10.0	0.5	4.8
1,2-Propanediol	NAD ⁺ (0.6)	10.0	0.07	4.8
NAD ⁺	Glycerol (5)	10.0	0.13	5.0
NAD ⁺	1,2-Propanediol (5)	10.0	0.05	5.0
DHA	NADH (0.33)	6.0	0.06	25
NADH	DHA (5)	6.0	0.12	33

condition for activity. Our sample of 1,3-propanediol, when first tested, appeared to be an exception to this rule since it showed activity which, however, only persisted for a short time and suggested that an active impurity was present; after further purification it showed no activity. Note that in this system glyceraldehyde is not being reduced, but oxidized, the product being presumably 3-hydroxypyruvaldehyde. A specific requirement for the oxidation is NAD⁺; when it was replaced by NADP⁺, with glycerol as substrate, no oxidation occurred.

Activities with different substrates in the reverse reaction are also shown in Table 4. Not all the presumed products of the oxidation reaction were available for testing, but of those that were tested the enzyme was most active with DHA, the next most active compounds, glycolaldehyde and pyruvaldehyde (methylglyoxal) showing only one-fifth as much activity. No activity was found with DL-glyceraldehyde but a commercial sample of D-glyceraldehyde did show considerable activity. However, as described in Methods, there was good evidence that this sample contained some DHA and two fresh samples each synthesized independently by different methods, and free from this impurity, were completely inactive. In contrast to the inability of NADP⁺ to replace NAD⁺ in the forward reaction, NADPH was about one-third as active as NADH in the reverse reaction.

Kinetic properties

Values for apparent Michaelis constants and for V_{max} , measured at optimal pH values, are given in Table 5. The K_m for 1,2-propanediol (0.07 mM) was considerably lower than that for glycerol (0.5 mM). The K_m value of 0.12 for NADH was based on measurements over a concentration range of 0.033–0.33 mM; at higher concentrations v decreased and Michaelis-Menten kinetics were not applicable.

Identity of the oxidation product

Oxidation of glycerol linked to NAD⁺ is not favoured thermodynamically (equilibrium constant approximately 10^{-12}). To obtain sufficient product for identification, the reaction was coupled to the NADH-linked reduction of pyruvate by lactate dehydrogenase, a reaction which similarly strongly favours substrate reduction. The reaction mixture, containing 0.4 mmol glycerol, 0.6 mmol sodium pyruvate, 2 μmol NAD⁺, 36 μg purified GDH2 and 50 μg lactate dehydrogenase in 2.0 ml 20 mM-Tris/HCl, pH 8.0, was incubated at 30 °C, and samples (0.5 ml) were taken at 0, 1, 2 and 3 h and were cooled immediately and kept on ice. They were then analysed by TLC on duplicate plates, one of which was sprayed with aniline phosphate, the other with H₂SO₄. DHA (R_F 0.62), which was absent from the initial sample, appeared in significant amounts after 1 h and the intensity of the spot increased progressively after 2 h and 3 h. Glycerol (R_F 0.35) was present in largest amounts in the initial sample and the intensity of the glycerol spot decreased as incubation progressed. No spot corresponding to glyceraldehyde (R_F 0.09) was detected in any of the samples. We conclude that GDH2 catalyses oxidation of the glycerol molecule at C₂ exclusively and must therefore be classed as a 2-oxidoreductase.

Table 6. Comparison of GDH2 enzymes isolated from several sources

Source of enzyme	<i>K. pneumoniae</i> *	<i>E. coli</i> †	<i>S. pombe</i>	NT 3060‡
Molecular weight§	160 000 (79 000)	310 000 (81 000)	400 000	390 000
Subunit size	40 000	39 000	47 000	42 500
Number of subunits	4 (2)	8 (2)	8	8 or 9
pH optimum for				
(1) glycerol oxidation	8–9	9.5–10	10.5–12	–
(2) DHA reduction	4–8	5.5–6	6	–

* The major dissimilatory enzyme of the *dha* pathway. A minor enzyme protein (designated GDH II) appears to be more closely related to the *E. coli* enzyme (Tang *et al.*, 1982a).

† Tang *et al.* (1979).

‡ Yamada *et al.* (1980).

§ Molecular weights have also been quoted for commercially available enzymes: 230 000 for the *B. megaterium* enzyme (Calbiochem–Behring catalogue, 1984) and 340 000 for the *E. aerogenes* enzyme (Boehringer, Biochemica News no. 4).

DISCUSSION

Previous studies of GDH2 (EC 1.1.1.6) have been done almost entirely with enzymes of bacterial origin. While there are a number of reports of some type of glycerol dehydrogenase being produced by several bacterial species (Lin, 1976), GDH2 has been purified and characterized only from some mutant strains of *Escherichia coli* (Tang *et al.*, 1979, 1982b), and from *Aerobacter aerogenes* 1033, later referred to as *Klebsiella aerogenes* and currently *K. pneumoniae* (Burton & Kaplan, 1953; McGregor *et al.*, 1974; Ruch *et al.*, 1980; Tang *et al.*, 1982a), together with some related strains. In addition a crystalline preparation of the enzyme has been prepared from an unidentified Gram-positive bacterium, NT 3060, by Yamada *et al.* (1980). Preparations of the enzyme are also available commercially from Boehringer who prepare it from *Enterobacter aerogenes*, from Sigma who list preparations from both *E. aerogenes* and a *Cellulomonas* sp. and from Calbiochem–Behring who prepare it from *Bacillus megaterium*, but there appears to be no published information about these enzymes. The only report of NAD⁺-linked glycerol dehydrogenase in yeasts, apart from our initial report of the *S. pombe* enzyme (May & Sloan, 1981), is that of Babel & Hofmann (1982) who found this type of activity in 14 out of 15 strains from seven different genera, while only four of these strains possessed glycerol kinase. This suggests that the ability to use glycerol by initial oxidation by GDH2 may be more widely distributed in yeasts than previously thought.

The enzyme we have obtained from *S. pombe* resembles fairly closely preparations from other organisms; some of their characteristics are compared in Table 6. The enzyme appears to be composed of eight identical polypeptides whose molecular weight (47 000) is somewhat larger than that of the subunits of the *E. coli* (39 000) and *K. pneumoniae* (40 000) enzymes. The *E. coli* enzyme exists mainly as an octamer with small amounts of dimer while the NT 3060 enzyme is also probably an octamer (or possibly a nonamer); the main dissimilatory enzyme of *K. pneumoniae*, however, appears to be a tetramer accompanied by small amounts of dimer, but there is also a second enzyme in this organism (GDH II) which shows close homology with the *E. coli* enzyme (Tang *et al.*, 1982a). The pH optima for both glycerol oxidation and DHA reduction for the *S. pombe* enzyme are also almost the same as those for the *E. coli* enzyme and slightly different from those for the *K. pneumoniae* enzyme. Properties of the *S. pombe* enzyme which are also shared by the bacterial enzymes include similar ranges of substrate specificities; higher activity with and greater affinity for 1,2-propanediol than glycerol; activation by the monovalent cations K⁺ and NH₄⁺ but inhibition by Li⁺ and Na⁺ [Strickland & Miller (1968) reported a similar inhibition of the *K. pneumoniae* enzyme]; inhibition by 8-hydroxyquinoline and EDTA but activation by Mn²⁺ or Zn²⁺ [activation by Mn²⁺ of a *K. aerogenes* enzyme was reported by Huetting *et al.* (1978)]. The *S. pombe* enzyme showed no activity in the forward direction with NADP⁺ as coenzyme but could use NADPH in the reverse reaction at about one-

Table 7. *Relation between structure and GDH2 activity*

The activities are relative rates of oxidation of R¹.CHOH.CHOH.R². The value equivalent to 100% was 2.9 μ mol substrate consumed min⁻¹ (mg protein)⁻¹.

R ¹	R ²	Activity
H	CH ₂ OH	100
H	CH ₃	160
H	C ₂ H ₅	90
H	H	30
CH ₃	CH ₃	60
CH ₃	CH ₂ OH	50
CH ₂ OH	CH ₂ OH	0

third of the activity shown with NADH, behaviour which again is the same as that reported for the *E. coli* enzyme (Tang *et al.*, 1979). On the other hand the *S. pombe* enzyme differs from the bacterial enzymes in showing inhibition by some thiol compounds and activation by thiol antagonists such as iodoacetamide, and by lack of inhibition by various divalent cations.

The enzyme can catalyse the NAD⁺-linked oxidation of several substrates structurally related to glycerol and the relation between structure and activity is shown in Table 7. The essential requirement would seem to be two hydroxyl groups on adjacent carbon atoms with additional groups being preferably non-polar and the optimum size a three-carbon chain.

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