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Methylglyoxal and the polyol pathway

Three-carbon compounds are substrates for sheep liver sorbitol dehydrogenase

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Methylglyoxal, 1,2-propanediol and glycerol are shown to be substrates for sheep liver sorbitol dehydrogenase. With 1,2-propanediol the enzyme-catalyzed reaction occurs specifically with the *R*(−)-enantiomer. The maximum velocities and the specificity constants obtained for the three-carbon substrates are considerably lower than those reported previously for sorbitol, and suggest that rate-determination is imposed by catalytic steps other than the enzyme–coenzyme product dissociation. The present findings are discussed in terms of substrate specificity and stereospecificity, and may indicate novel aspects of sorbitol dehydrogenase function in relation to glucose metabolism and diabetic pathogenesis.

Methylglyoxal; Polyol pathway; Sorbitol dehydrogenase; Sheep liver

1. INTRODUCTION

In the polyol pathway, sorbitol dehydrogenase (SDH) and aldose reductase mediate the conversion of glucose to fructose via sorbitol [1,2]. Pathological changes in various tissues have been attributed to polyol accumulation, including cornea [3–5], kidney [3,6] and heart [7].

The glyoxalase system has been found in all tissues analysed for its presence [8]. It consists of two enzymes, glyoxalase I and glyoxalase II, which catalyze the reduction of methylglyoxal to D-lactic acid via *S*-D-lactoylglutathione. Although the function of the glyoxalase system remains to be defined exactly, roles in detoxification and control of cell proliferation have been suggested [9]. The metabolites of the system may be involved in disease mechanisms, including the development of diabetic complications [9–11].

Methylglyoxal (CH_3COCHO), which is the primary physiological substrate for glyoxalase I, is formed from dihydroxyacetonephosphate and glyceraldehyde-3-phosphate by most glucose-metabolizing cells [12]. It can also be formed from acetone after hydroxylation to hydroxyacetone (acetol) [13]. The toxic and growth-arrest effects of methylglyoxal in both mammalian and microbial cells in vitro and in vivo have been known for many years [14–16]. Toxicity induced by methylglyoxal is selective in rapidly proliferating cells, which makes glyoxalase inhibitors interesting as anti-tumour, anti-microbial and anti-malarial agents [17–19].

Detoxification of methylglyoxal can occur by the glyoxalase system, or by several alternative pathways involving a number of different enzymes [11,20]. Aldose reductase is known to catalyze the reversible reduction of methylglyoxal to hydroxyacetone with the concomitant consumption of NADPH [11]. The present communication shows that methylglyoxal can also serve as a substrate for SDH, and further that this enzyme is capable of interconverting hydroxyacetone and 1,2-propanediol as well as dihydroxyacetone and glycerol. This suggests a role for SDH in acetone, methylglyoxal and triose metabolism.

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Enzymes. sorbitol dehydrogenase, L-iditol:NAD 2-oxidoreductase (EC 1.1.1.14), aldose reductase, alditol:NAD(P) 1-oxidoreductase (EC 1.1.1.21), glyoxalase I (EC 4.4.1.5), glyoxalase II (EC 3.1.2.6).

2. MATERIALS AND METHODS

The source and purity of sheep liver sorbitol dehydrogenase, as well as the determination of the concentration of active enzyme in solution, were as described previously [21]. Merck provided glycerol and the two enantiomers of 1,2-propanediol. All other substrates and the coenzymes were from Sigma. Due to the instability of the coenzyme in acid media, stock solutions of NADH were prepared in 10 mM phosphate buffer pH 7.4 or 10 mM glycine-NaOH buffer pH 9.8. Stock solutions of NAD were prepared in water. All reagents were of analytical grade and triple-distilled water was used for all solutions.

Initial rates were determined spectrophotometrically by measuring the rate of change in absorbance at 340 nm for NAD reduction or NADH oxidation. Measurements were made using a Gilford 260 spectrophotometer connected to a single pen recorder and a constant-temperature water bath at 23.5°C. Reactions were initiated by adding an aliquot of enzyme stock solution to a 3.0-ml reaction mixture in a 10-mm light-path quartz cuvette. Initial rate measurements were performed in duplicate with a precision ≤5%. The relative standard deviations obtained in the determination of the K_m and V_{max} values were ≤10%.

3. RESULTS

Fig. 1a–d shows for pH 7.4 the double-reciprocal plots of the data for racemic 1,2-propanediol oxidation and hydroxyacetone reduction. Fig. 2a,b shows the double-reciprocal plots for *R*-1,2-propanediol oxidation at pH 7.4 and pH 9.8. No enzymic activity was detected with the *S*-enantiomer at either pH. In accordance with this, while V_{max} is the same for racemic and *R*-1,2-propanediol, the ratio of the K_m values is 1:2, as expected.

Table I lists the kinetic parameters for the oxidation and the reduction of the three-carbon substrates by sheep liver sorbitol dehydrogenase. The product of methylglyoxal reduction by SDH is considered to be *R*-lactaldehyde. Reduction of hydroxyacetone or dihydroxyacetone was not performed at high pH. This was because of an increase in absorbance at 340 nm due to the formation of addition-compounds. These resulted from nucleophilic attack on the C_4 position of the nicotinamide ring of NAD, produced concomitantly with substrate reduction, by *enol*-derivatives of these species at high pH [22]. At pH 7.4, no such reaction was observed.

4. DISCUSSION

4.1. *Substrate specificity*

The following requirements have previously been outlined for the polyol substrates of SDH: (i) the pres-

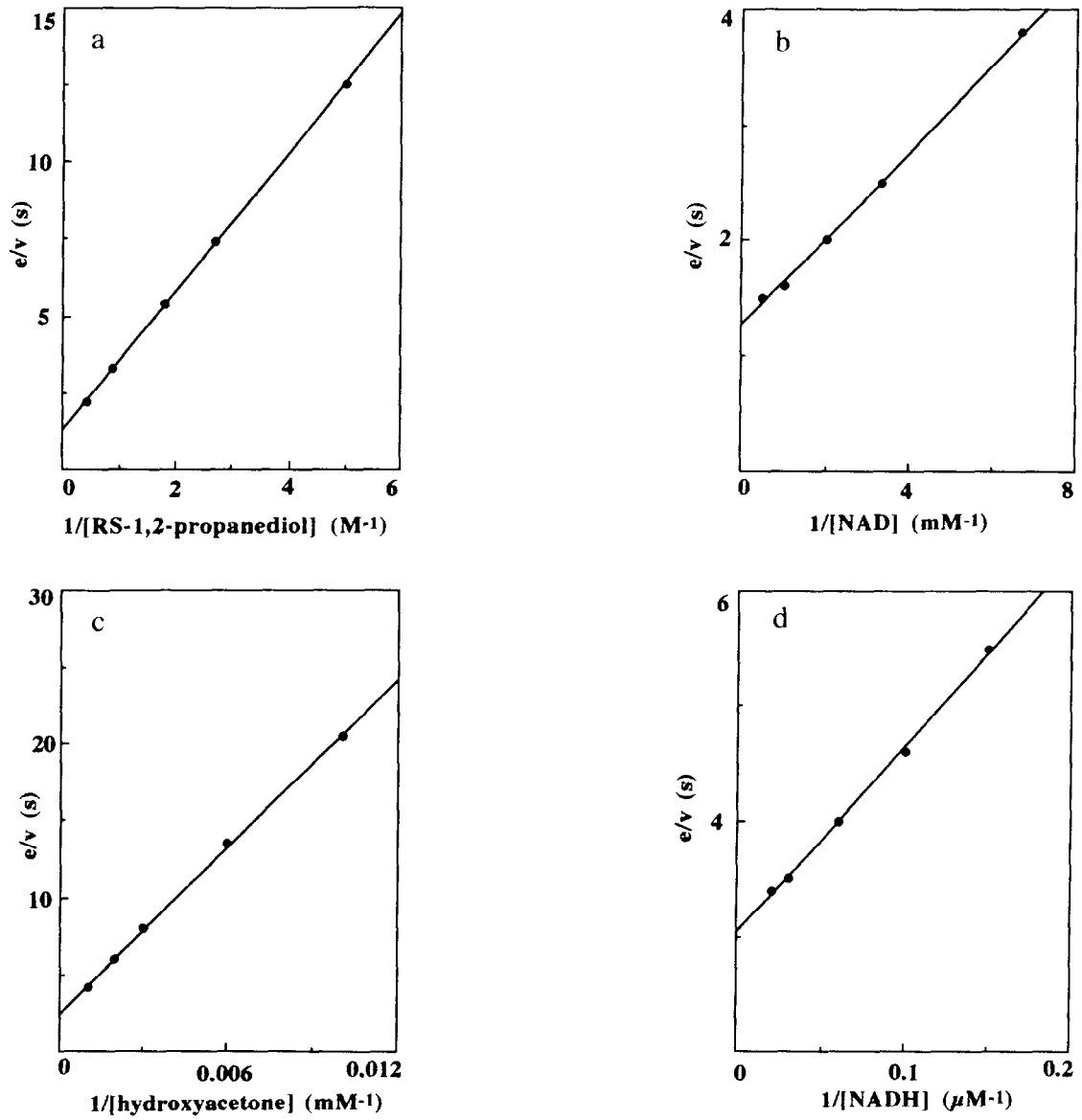


Fig. 1. Double-reciprocal plots for racemic 1,2-propanediol oxidation and hydroxyacetone reduction, pH 7.4. (a) Varied [*RS*-1,2-propanediol], [NAD] = 1.0 mM. (b) Varied [NAD], [*RS*-1,2-propanediol] = 4.0 M. (c) Varied [hydroxyacetone], [NADH] = 100 μM. (d) Varied [NADH], [hydroxyacetone] = 1.6 M.

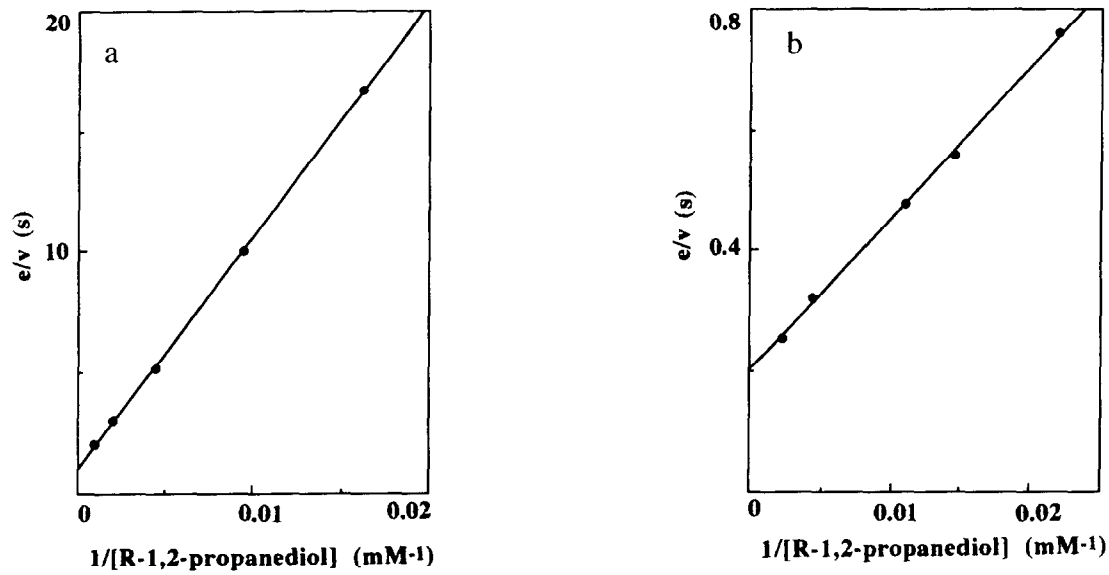


Fig. 2. Double-reciprocal plots for *R*-1,2-propanediol oxidation. (a) Varied [*R*-1,2-propanediol], [NAD] = 1.0 mM, pH 7.4. (b) Varied [*R*-1,2-propanediol], [NAD] = 1.0 mM, pH 9 8.

ence of a hydroxyl group at C-1; (ii) *S* configuration on C-2; and (iii) *R* configuration on C-4 [23–26]. Hexitols and pentitols are generally the best substrates for SDH [2,21,23–27] but several four-carbon chain compounds, including (2*R*,3*R*)-2,3-butanediol, have been reported to serve as substrates for the enzyme from human liver [27]. The present findings show that the presence of the $C_4H(OH)$ moiety per se is not essential, and the substrate specificity of sorbitol dehydrogenase can thus be extended to include di- and trifunctional alcohols with a three-carbon chain.

The reversible oxidation of *R*-1,2-propanediol to hydroxyacetone and of glycerol to dihydroxyacetone is in harmony with SDH being specific for reaction at the C_2 of a 1,2-dihydroxy compound [25,26]. The presence of a primary hydroxyl group adjacent to the oxidation site is considered essential for positioning the reactive 2-hydroxyl at the catalytic zinc atom of SDH, probably by hydrogen bond formation between this functional group and the Glu-154 residue [26]. That 1-deoxysorbitol or monofunctional secondary alcohols like propane-2-ol and butane-2-ol are not substrates for SDH

Table I

Kinetic parameters for C_3 substrates of sorbitol dehydrogenase

Substrate	pH 7.4			pH 9.8		
	V_m (s^{-1})	K_m (mM)	V_m/K_m ($s^{-1} \cdot M^{-1}$)	V_m (s^{-1})	K_m (mM)	V_m/K_m ($s^{-1} \cdot M^{-1}$)
RS-1,2-Propanediol	0.80	1,900	0.42	4.6	300	15.3
R-1,2-Propanediol	0.80	825	0.97	5.0	125	40
NAD	0.80	0.31	$2.6 \cdot 10^3$	4.7	0.25	$1.9 \cdot 10^4$
Hydroxyacetone	0.35	720	0.49			
NADH	0.35	0.0050	$7 \cdot 10^4$			
Glycerol	0.5	1,450	0.34	2.5	300	8.3
NAD	0.5	0.58	860	2.5	0.63	$4 \cdot 10^3$
Dihydroxyacetone	6.7	690	9.7			
NADH	6.7	0.0030	$2\,2 \cdot 10^6$			
Methylglyoxal	1.7	430	4.0			
NADH	1.7	0.060	$2\,7 \cdot 10^4$			

All measurements were made in either 50 mM phosphate buffer pH 7.4 or 50 mM glycine-NaOH buffer pH 9.8. For the determination of V_m and K_m, saturating concentrations (>2 K_m) of the constant reaction partner were used.

emphasizes the significance of this bonding interaction [25–27]. The aldehyde group of methylglyoxal is presumably hydrated when serving as a substrate. However, the 1-hydroxyl appears not to be essential for the oxidation of four-carbon chain substrates by human liver SDH [27]. It appears that this enzyme, which also oxidizes aromatic alcohols [28], differs somewhat from sheep liver SDH in terms of substrate specificity.

As reflected by their comparatively low specificity constants (V_m/K_m) in Table I, the C_3 substrates must be engaged in weaker active site–substrate bonding interactions than various pentitols and hexitols [21,26]. This may arise from the shorter consecutive sequence of hydroxyl groups in the three-carbon substrates, which precludes active site-substrate hydrogen bond formation. Furthermore, it is noticeable in Table I that those substrates having a methyl group in the 3-position are generally better substrates than those which have a hydroxyl group in this position. This is consistent with a nonessential role for the 3-hydroxyl, and lends strength to the concept that a hydroxyl group in this position interacts unfavourably with the nicotinamide ring of the coenzyme [26,29].

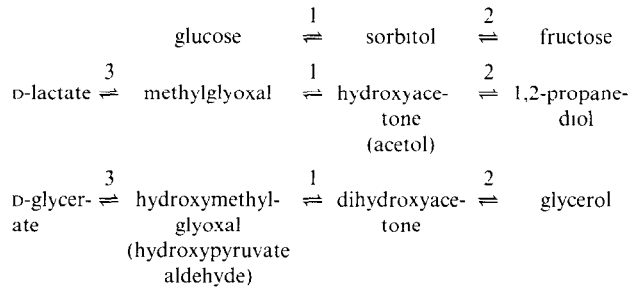
For sheep liver sorbitol dehydrogenase, several alternative polyol substrates have previously been shown to give the same maximum velocity as sorbitol, consistent with a compulsory order mechanism with rate-limiting enzyme–coenzyme product dissociation [21]. With the C_3 substrates this is not the case, probably due to the formation of binary enzyme–substrate and/or ternary enzyme–coenzyme-substrate complexes whose interconversion is rate-limiting and substrate-specific. Similar results have been obtained with human liver SDH [27].

4.2. *Stereospecificity*

The present work provides an exacting test of the stereospecificity of polyol oxidation by SDH. The 2*R*-configuration of *R*-1,2-propanediol corresponds with the 2*S*-configuration in sorbitol. This parallels the stereospecific oxidation of (2*S*,3*R*)-2,3-butanediol by human liver SDH [27]. D-Mannitol, which is the 2-epimer of sorbitol, has nevertheless been found to be a substrate for sheep liver SDH [21]. It has been suggested that an alkaline pH may induce racemisation and thus the configuration required for the oxidation of this polyol [30].

4.3. *C_3 metabolism*

Scheme 1 summarizes the results of the present study and includes some of the known metabolic pathways of acetone, acetol and methylglyoxal which, like 1,2-propanediol, can be gluconeogenic [8,11,13,31,32]. Acetol is formed from acetone by acetone monooxygenase. Methylglyoxal is formed from acetol by aldose reductase. It is also produced from triose phosphates either non-enzymatically or by methylglyoxal synthase.



Scheme 1 Methylglyoxal and the polyol pathway. 1, aldose reductase; 2, sorbitol dehydrogenase; 3, glyoxalase I and II

The polyol pathway has been the subject of considerable interest in recent years due to its putative role in the etiology of diabetic complications. Now it appears that both aldose reductase and sorbitol dehydrogenase can be assigned more general functions in metabolism, including that of acetone and other gluconeogenic precursors (Scheme I). Acetone, a product of fatty acid catabolism, can after hydroxylation to acetol be converted to glucose via lactate. This can occur by reduction of acetol to 1,2-propanediol, which is metabolized to lactate in the liver, or by oxidation of acetol to methylglyoxal. The latter is then converted to lactate either by the operation of the glyoxalase system or via lactaldehyde, which can be oxidized by aldehyde dehydrogenase [13,20].

The possibility of SDH being implicated in the metabolism of three-carbon compounds also suggests a novel role for this enzyme in diabetes, where the metabolic flux through the polyol, glycolytic and methylglyoxal pathways increases. As is the case with sorbitol, elevated levels of methylglyoxal, acetol and 1,2-propanediol are observed in uncontrolled diabetes, and these metabolites are assumed to be involved in the development of diabetic complications [11]. Like glucose, methylglyoxal and acetol can produce covalent modifications of proteins [11]. Furthermore, reductive catabolism of methylglyoxal can give rise to oxidative stress resulting from the depletion of NADPH [33,34]. Numerous studies support the concept that diabetics suffer from oxidative stress [35–37]. As methylglyoxal is a far better substrate for aldose reductase than is glucose, it has been suggested that a normal function of this enzyme in vivo is to protect against methylglyoxal-induced toxicity [11]. The present findings suggest the possibility that the polyol pathway enzymes together may constitute a system serving in the detoxification of methylglyoxal by converting it to the gluconeogenic precursors acetol and 1,2-propanediol. Furthermore, SDH can convert methylglyoxal to *R*-lactaldehyde, which is also gluconeogenic. It is interesting, that the reduction of methylglyoxal by an aldose reductase preparation from human placenta and skeletal muscle has been reported to produce a minor component of *R*-

lactaldehyde in addition to acetol [11]. Considering that SDH, unlike aldose reductase, is specific for the *R*-enantiomer it is possible that *R*-lactaldehyde was produced by sorbitol dehydrogenase.

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