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New substrate for galactose oxidase

Galactose oxidase (D-galactose oxygen oxidoreductase, EC 1.1.3.9) from *Polyporus circinatus* catalyzes the oxidation of galactose by molecular oxygen. In addition to the galactose, a number of compounds related to galactose were oxidized. The polymers containing galactose were oxidized much more rapidly than the galactose itself and showed much higher affinities for the enzyme[1].

Galactose oxidase was used as the reagent for the determination of galactose[2-4]. The present communication is concerned with dihydroxyacetone as the substrate for galactose oxidase.

Galactose oxidase purified from a culture medium of *P. circinatus* had a specific activity of 9180 units/mg protein. Dihydroxyacetone supplied by Sigma Chemical Co. was chromatographically pure. For the assay of galactose oxidase, Glucostat Reagent (Worthington Biochem. Corp.) was used without glucose oxidase as described by AVIGAD *et al.*[1]. Galactose oxidase was also assayed by the oxygen uptake at the oxygen electrode[5].

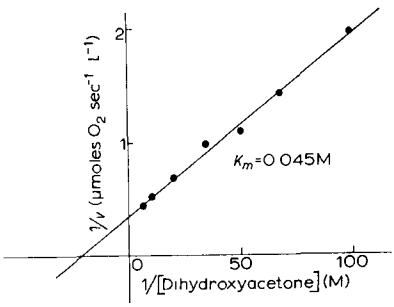
TABLE I

SPECIFICITY

The reaction mixture (1.0 ml) contained 0.5 ml of peroxidase–chromogen-buffered system, 1.85 μg of galactose oxidase, and 10 μmoles of the substrates. The samples were incubated for 10 min at 30°.

Substrate	*Relative activity*
D-Galactose	100
D-Sedoheptulose	0
D-Ribulose	0.25
D-Xylulose	0.33
Dihydroxyacetone	110
D-Glyceraldehyde	0
Dihydroxyacetone phosphate	0
DL-Glyceraldehyde 3-phosphate	0

Several keto compounds were assayed as substrates for galactose oxidase. Table I shows that dihydroxyacetone was oxidized much more rapidly than galactose itself. The Michaelis–Menten constant (K_m) for dihydroxyacetone was determined by employing peroxidase–*o*-dianisidine or by observing the oxygen uptake. From a Lineweaver–Burk plot of the data, the K_m value for dihydroxyacetone was found to be 0.031 M in the first method and 0.045 M in the last one (Fig. 1). This meant that the K_m for dihydroxyacetone was 10 times smaller than that for galactose and of the same order as that obtained for melibiose. Fig. 2 shows the oxygen uptake when dihydroxyacetone or galactose was present in the incubation medium in the concentration of substrate saturation. The initial velocity of dihydroxyacetone was 5 times greater than that of galactose. The oxidation of dihydroxyacetone by galactose



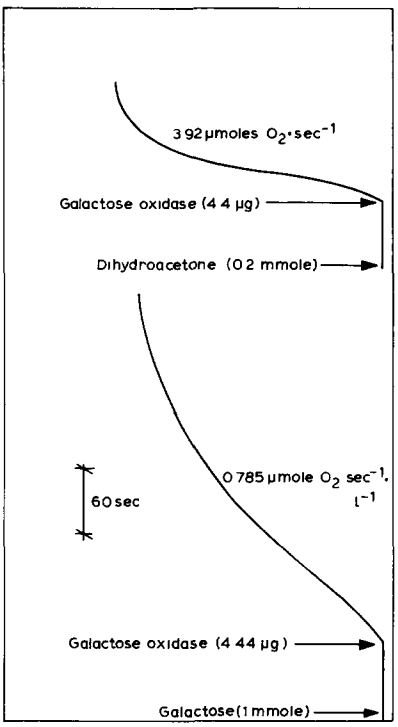


Fig 1 Effect of dihydroxyacetone concentration on enzyme activity as shown by Lineweaver–Burk plots The oxygen uptake was measured at the oxygen electrode[6] The incubation mixture contained 1 85 μg of galactose oxidase, dihydroxyacetone as indicated in the figure, and 0 1 M phosphate buffer (pH 7 2) to make up 2 ml

Fig 2. Comparative rates of dihydroxyacetone and galactose oxidation The oxygen uptake was measured at the oxygen electrode[6] The incubation mixture contained galactose, dihydroxyacetone, and galactose oxidase concentrations as indicated in the figure, and 0 1 M phosphate buffer (pH 7 2) to make up 2 ml

oxidase was 100% inhibited by 2 mM hydroxylamine and 2 5 mM cyanide as was the galactose oxidation

The results suggest that dihydroxyacetone is a better substrate for galactose oxidase than galactose

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