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Note

The interaction of deoxyfluoro substrate-analogues with D-galactose dehydrogenase from *Pseudomonas fluorescens**

ROBERT G. EDWARDS, PETER THOMAS, AND JOHN H. WESTWOOD
Institute of Cancer Research, Royal Cancer Hospital, The Haddow Laboratories, Clifton Avenue, Sutton, Surrey SM2 5PX (Great Britain)
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The electronegativity and bond length of fluorine bound covalently to a carbon atom are similar to those of the oxygen of a hydroxyl group bound to carbon[1,2]. The carbon-bound fluorine is also capable of accepting, although not donating, a hydrogen bond[3]. Studies of enzyme–substrate interactions where fluorine has replaced one or more hydroxyl groups on the substrate have proved useful in investigating both the mechanism of action and the substrate-binding properties of a number of enzymes[4–8].

D-Galactose dehydrogenase (D-galactose:NAD oxidoreductase) [EC 1.1.1.48], which catalyses the conversion of D-galactose into D-galactono-1,4-lactone, has been isolated from both *Pseudomonas fluorescens* and *Ps. saccharophilia*. The enzymes have been purified and some of their kinetic properties investigated[9,10].

We now report on studies of the reaction of the D-galactose dehydrogenase from *Ps. fluorescens* with a number of D-galactose derivatives in which the hydroxyl groups at C-2, C-4, and C-6 have been substituted by fluorine, and also on the kinetic isotope effects when D-galactose was replaced by D-galactose-*1-d* as the substrate.

Kinetic data obtained from the interaction of D-galactose dehydrogenase with the deoxyfluorogalactoses are shown in Table I. The double reciprocal plots were linear and indicate a sequential mechanism. The low K_m values for D-fucose (6-deoxy-D-galactose) and 2-deoxy-2-fluoro-D-galactose show that HO-6 is not important for binding of the substrate, and that if HO-2 is involved, it is as a H-bond acceptor. 2-Deoxy-D-*lyxo*-hexose (2-deoxy-D-galactose), however, is a substrate for the enzyme, although kinetic data have not been reported[10]. The very high K_m for 4-deoxy-4-fluoro-D-galactose shows the critical role played by HO-4 in the binding to the enzyme. D-Glucose, which is not a substrate for the enzyme, differs in configuration from D-galactose only at C-4. Thus, a H-bond donated from a correctly oriented hydroxyl group is probably the major factor in the binding of substrate to the enzyme. As the K_m for D-fucose is low, the value for the 6-fluoro sugar should also be low. However, from an inspection of models, it seems that the fluorine on C-6 may be able to form a

*Dedicated to the memory of Sir Edmund Hirst, C.B.E., F.R.S.

hydrogen bond with HO-4; as a hydrogen bond from HO-4 to the enzyme is important for substrate binding, this would have the effect of elevating K_m without causing a significant decrease in V_{max} (Table I). Both 2-deoxy-2-fluoro-D-galactose and 4-deoxy-4-fluoro-D-galactose have substantially diminished V_{max} values, which may reflect differences in substrate orientation causing a diminished rate of catalysis. The electron-withdrawing effect of fluorine at C-2 may also affect the rate of hydride transfer. Such an effect was observed when 2-deoxy-2-fluoro-D-glucose 6-phosphate was used as a substrate for D-glucose 6-phosphate dehydrogenase[6]. An isotope effect of 2.0 was found for the reaction of D-glucose-*1-d* 6-phosphate with D-glucose 6-phosphate dehydrogenase[11]. However, hydride transfer does not appear to be the rate-determining step in the reaction mediated by D-galactose dehydrogenase. The isotope effect observed when D-galactose-*1-d* was used as the substrate was small (1.33). This value is similar to that (1.34) reported for ethanol-d_5 and alcohol dehydrogenase[12], for which the dissociation of the E.NAD$^+$ complex is rate determining at high concentrations of acetaldehyde; at lower concentrations, some prior step becomes at least partly rate-determining[12]. It is possible that a similar mechanism operates for D-galactose dehydrogenase. It would be of interest to investigate the isotope effect with 2-deoxy-2-fluoro-D-galactose-*1-d* as a substrate in comparison with the parent fluoro sugar; in this case, due to the electron-withdrawing effect of the fluorine, it is possible that hydride transfer would become totally rate-determining.

TABLE I

KINETIC CONSTANTS FOR D-GALACTOSE DEHYDROGENASE FROM *Pseudomonas fluorescens*

Substrate	K_m (*sugar*) (*m*M)	K_m (*NAD*) (*m*M)	V_{max} (*Relative*)
D-Gal	0.93	0.4	1.0
D-Gal-d_1	1.0	0.36	0.75
D-Fuc	2.04	0.4	0.65
6F-D-Gal	57	0.4	0.85
2F-D-Gal	5.81	0.25	0.045
4F-D-Gal	125	0.25	0.035

EXPERIMENTAL

D-Galactose dehydrogenase from *Ps. fluorescens* was purchased from the Boehringer Corp. (London) Ltd. D-Fucose, D-galactono-1,4-lactone, and sodium borodeuteride (NaB^2H_4, 98 atom % ^2H) were commercial materials. The deoxy-fluoro-D-galactopyranoses and 4-deoxy-4-fluoro-D-glucose were synthesized in these laboratories[13].

Preparation of D-*galactose*-1-d. — A solution of sodium borodeuteride (0.23 g) in water (5 ml) was added to an ice-cold, aqueous solution (20 ml) of D-galactono-1,4-lactone (1.78 g) during 1.5 h. After a further 0.5 h at 0°, excess of sodium boro-

deuteride was decomposed with dilute acetic acid, and the resulting solution was stirred for 0.5 h with Amberlite IR-120(H^+) resin (3 g). After removal of the resin, the solution was concentrated to dryness, the residue was dissolved in methanol, and the solution was concentrated. This procedure was repeated 7 times. The crystalline residue (1.86 g) was recrystallised from water–methanol–ethanol (1:1:1 v/v), to give D-galactitol-*1-d* (0.85 g), m.p. 186–189°; lit. m.p. 188.5°. The identity of the compound, and the presence of ^2H at C-1, was confirmed by mass spectrometry. The mother liquor from the galactitol crystallisation was evaporated to dryness, and the residue was crystallised from water–methanol–ethanol–acetone (5:9:9:6, v/v) at −10° to give D-galactose-*1-d* (0.26 g), m.p. 157–162°. G.l.c. analysis showed only galactose to be present, and a mass spectrum of the penta-acetate gave a peak at *m/e* 391 for the molecular ion and indicated a >95% incorporation of deuterium.

Assay of D-*galactose dehydrogenase activity.* — Assays were performed in 0.1M Tris-HCl buffer (pH 8.6), by using the standard procedure of observing the rate of increase of absorption at 340 nm. Initial rates were measured with a Pye–Unicam SP500 monochromator and a Gilford model 220 absorbance indicator. The full-scale deflection of the recorder was set to correspond to a change in E_{340} of 0.1.

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REFERENCES

1 L. PAULING, *The Nature of the Chemical Bond*, Cornell Univesity Press, 3rd edition, 1963, p. 90.
2 L. E. SUTTON (Ed.), *Tables of Interatomic Distances and Configuration in Molecules and Ions*, The Chemical Society, London, 1965, pp. S14s–S23s.
3 F. BUCKLEY, P. A. GIGUENE, AND D. YAMAMOTO, *Can. J. Chem.*, 46 (1968) 2917–2923.
4 E. M. BESSELL, A. B. FOSTER, AND J. H. WESTWOOD, *Biochem. J.*, 128 (1972) 199–204.
5 E. M. BESSELL AND P. THOMAS, *Biochem. J.*, 131 (1973) 77–82.
6 E. M. BESSELL AND P. THOMAS, *Biochem. J.*, 131 (1973) 83–89.
7 P. THOMAS, E. M. BESSELL, AND J. H. WESTWOOD, *Biochem. J.*, 139 (1974) 661–664.
8 P. A. BRILEY, R. EISENTHAL, AND R. HARRISON, *Biochem. J.*, 145 (1975) 501–507.
9 F. WENGENMAYER, K.-H. VEBERSCHÄR, AND G. KURZ, *Eur. J. Biochem.*, 40 (1973) 49–61.
10 O. BLACHNITZKY, F. WENGENMAYER, AND G. KURZ, *Eur. J. Biochem.*, 47 (1974) 235–250.
11 I. A. ROSE, *J. Biol. Chem.*, 236 (1961) 603–609.
12 K. BUSH, V. J. SHINER, AND H. R. MAHLER, *Biochemistry*, 12 (1973) 4802–4805.
13 A. B. FOSTER AND J. H. WESTWOOD, *Pure Appl. Chem.*, 35 (1973) 147–168.