# RELATIVE ENANTIOMER BINDING AND REACTION RATES WITH PROPANEDIOL DEHYDRASE

Frederick R. Jensen and Richard A. Neese

Department of Chemistry
University of California, Berkeley
Berkeley, California 94720

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Summary: In the dioldehydrase reaction, L(+) propanediol reacts at a faster rate than the D(-)-isomer in competitive reactions, but when the diols are run independently the rate of the D(-)-isomer is 2.3 times greater than that of the L(+)-isomer. It is assumed that this reversal is due to a difference in the Michaelis-Menten constants. A ratio of $K_{mD(-)}/K_{mL(+)} = 3.2 \pm 0.3$ at 25° is calculated from a series of competitive reactions. It is concluded that the binding site is such that while both isomers are accomodated readily, the D(-)-isomer is hindered to some degree in its approach to the binding site. However, once the enzyme-substrate complex is formed, the D(-)-isomer is in a more favorable configuration for further reaction.

One of the rare cases wherein enzyme catalysis is not uniquely specific for one enantiomer is dioldehydrase (D,L-1,2-propanediol hydro-lyase, EC 4.2.1.28) from Aerobacter aerogenes (ATCC 8724) (1). This bacteria when appropriately conditioned produces the enzyme which converts propanediol to propionaldehyde. The rates of these reactions are comparable with the D(-)-isomer reacting at about twice the rate of the L(+)-isomer [D(-)/L(+) = 1.7 and 1.8, estimates from graphs given at 37°, respectively, by Yamane et al (2) and Lee and Abeles (3).]*

*In these reports data were taken from time zero for an indicated interval. Since these data were obtained over a time interval which included the induction period, these relative rates are not directly comparable with the results reported herein which were obtained in the linear portion of the reaction which occurs after the induction period.

Reported herein is a new aspect of this reaction which may greatly assist in formulating the precise configuration and binding at the reaction site. When an equimolar mixture (D,L pair) of the two propanediols is reacted with dioldehydrase, the L(+)-isomer present is converted to propionaldehyde at a faster rate than the D(-)-isomer. Isolation of the remaining propanediol after partial reaction shows that the diol has been enriched in the (-)-isomer.

A reasonable explanation for this apparent reversal of reactivity is that the Michaelis-Menten complex dissociation constants are different for the two isomers and is greater for the D(-)-isomer than for the L(+)-isomer as reflected in equations 1 and 2 [$S_L$ = L(+) substrate; $S_D$ = D(-) substrate; E = enzyme; $S_L$-E and $S_D$-E = enzyme-substrate complex; P = product].

$$S_L + E \underset{}{\overset{k_{1L}}{\rightleftharpoons}} S_L\text{-}E \xrightarrow{k_{2L}} P + E$$

$$S_D + E \underset{}{\overset{k_{1D}}{\rightleftharpoons}} S_D\text{-}E \xrightarrow{k_{2D}} P + E \qquad (1)$$

$$K_{mD} = \frac{k_{-1D} + k_{2D}}{k_{1D}} \qquad K_{mL} = \frac{k_{-1L} + k_{2L}}{k_{1L}} \qquad (2)$$

The ratio of the Michaelis-Menten constants for the two isomers was determined using equation 3 (4):

$$\frac{d\,S_L}{d\,S_D} = \frac{k_{2L}\,K_{mD}\,[S_L]}{k_{2D}\,K_{mL}\,[S_D]} \qquad (3)$$

and varying the relative amounts of the two isomers in successive reactions until no change in optical rotation for the initial part of the reaction was observed, i.e., $\frac{d\,S_L}{d\,S_D} = 1$. This ratio and that obtained for $k_{2L}$ and

$k_{2D}$ independently ($k_{2L}/k_{2D}$ = 2.3 ± 0.1) allowed determination of the Michaelis-Menten constant ratio $K_{mD}/K_{mL}$ = 3.2 ± 0.3 at 25°.

Thus, this ratio of Michaelis-Menten constants indicates that the L(+)-isomer is favored over the D(-)-isomer by a factor of 3.2 in competition for enzyme binding sites. However, once the diol is in the binding site, the D(-)-isomer is converted to propionaldehyde at a faster rate than the L(+)-isomer as shown by rate studies on the respective diols run independently.

These differences may arise from the geometry of the binding site and its relationship to the two isomers. The fact that the L(+)-isomer more easily enters the reaction site than the D(-)-isomer, but does not react as fast, may indicate that the position that the D(-)-isomer takes up in the enzyme site brings the reacting centers very close together, but this same proximity decreases the binding ability of this isomer relative to the L(+)-isomer. In any event, accomodation of the methyl groups of the two isomers into the same cavity indicates that the cavity is large or that reverse orientations occur for the two isomers.

Another interesting aspect of the interaction between the individual diols and the enzyme is the irreversible denaturation which occurs with both isomers, but after much longer reaction time in the case of the L(+)-isomer. Thus, after initiating the reaction by injecting coenzyme $B_{12}$ into the reaction solution, an induction period occurs during which, presumably, the haloenzyme is forming. After this lag period, zero order kinetics (approximate)

are observed until the denaturation reaction begins to occur and the diol dehydrase reaction rate falls off. The point at which this fall off occurs is different for the two isomeric diols. At 25° the fall off begins for the D(-)-isomer after about 40 minutes. For the L(+)-isomer, however, nearly 70 minutes passes before denaturation begins to occur. Once the fall off has begun, the D(-)-isomer reaches complete inactivity more rapidly than does the L(+)-isomer. The reasons for the increased stability with the L(+)-isomer are not clear, but may also have to do with the way in which the two isomers are bound to the enzyme.

## MATERIALS AND METHODS

A Bendix Automatic Polarimeter (type 143 A) was connected to a Hewlett Packard strip chart recorder and used for all optical rotation measurements. A 0.2 dcm cell equipped with a water jacket was kept at constant temperature.

Apoenzyme was prepared as described by Abeles and Lee (1). L(+)-propanediol was prepared by reducing ethyl lactate (Aldrich, $[\alpha]_D = -10.7°$, neat) with $LiAlH_4$ in tetrahydrofuran. The specific rotation was $[\alpha]_D = +19.9°$ [7.67 g/100 ml $H_2O$]. D(-)-propanediol was prepared by yeast reduction of hydroxy acetone (5). The specific rotation was $[\alpha]_D = -19.1°$ [7.9 g/100 ml $H_2O$].

Enzyme solution was dialyzed against diol-free buffer before reaction solutions were prepared. The final reaction solutions were 0.15 M in $K_2HPO_4$, 0.5 M in propanediol (D,L or various ratios of the two isomers)

and contained about 8 units of enzyme. Each reaction was initiated by injecting 0.1 ml of a solution of coenzyme $B_{12}$ (0.1 mg/ml $H_2O$) into the reaction solution making a total volume of two ml. The solution was shaken vigorously and pipetted into the polarimeter cell. Rates of reaction were calculated from the slopes during the linear decrease in optical rotation which occurs after the initiation period. The relative rates of reaction for the two isomers were independently determined for various diol concentrations at 25°. The D(-)-isomer rate decreased with increasing diol concentration, while the L(+)-isomer rate was independent of diol concentration. This decrease in rate with increased D-(-)-isomer concentration may have been due to the presence of a small amount of a difficult to remove impurity. Extrapolation to zero diol concentrations gave D(-)/L(+) = 2.3 ± 0.1.

In the competitive reactions solutions with ratios of the two isomers present all became more negative in sign of rotation below a ratio of 1.4/1 [D(-)/L(+)]. At a ratio of 1.4/1 a straight line was observed, showing no change in rotation (i.e. $\frac{d\ L(+)}{d\ D(-)} = 1$) with time for the initial reaction (before consumption of the diols could change the ratio of the remaining diols). At ratios above 1.4/1, solutions all became more positive with time. This ratio of 1.4/1 was used with equation 3 to give the ratio of the Michaelis-Menten constants of $K_{mD}/K_{mL} = 3.2 \pm 0.3$ at 25°.

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