Competitive Inhibition of an L-Fucose Isomerase Activity by Dithiothreitol

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Summary: Dithiothreitol was found to be a competitive inhibitor for a purified L-fucose isomerase from Aerobacter aerogenes, inhibiting enzyme activity for the isomerization of both L-fucose and D-arabinose. The kinetics of inhibition showed that the oxidized form was the more potent inhibitor. The K_i for both sugars was the same. Dithioerythitol and certain polyalcoholic sugars also appeared to function as competitive inhibitors of the enzyme.

Dithiothreitol has been described as a "new protective reagent for sulfhydryl groups"(1). Since its discovery in 1963 by W.W. Cleland (1) it has been used widely as a reducing agent in enzyme mediated reaction systems. Many investigators have found that dithiothreitol (DTT) inhibits the catalytic or binding activity of certain proteins. In most cases the nature of this inhibition appears to be due to the reduction of disulfide bonds.

While studying the properties of a purified L-fucose isomerase, which Aerobacter aerogenes must constitutively synthesize to grow on the unnatural sugar D-arabinose (3), we observed that reducing agents such as glutathione and mercaptoethanol appeared to protect the enzyme against loss of activity. The addition of dithiothreitol, however, resulted in an immediate decrease in activity.

The inhibition caused by dithiothreitol appeared to be competitive with substrate in that increased concentrations of either L-fucose or D-arabinose would restore activity (Fig. 1). Furthermore, free sulfhydryl groups were not required for the inhibition of enzyme activity, in fact the oxidized form of dithiothreitol showed greater activity than a fresh solution of the reduced form (Fig. 1). Figure 2 illustrates the reciprocal plot of enzyme velocity vs. L-fucose concentration with varying

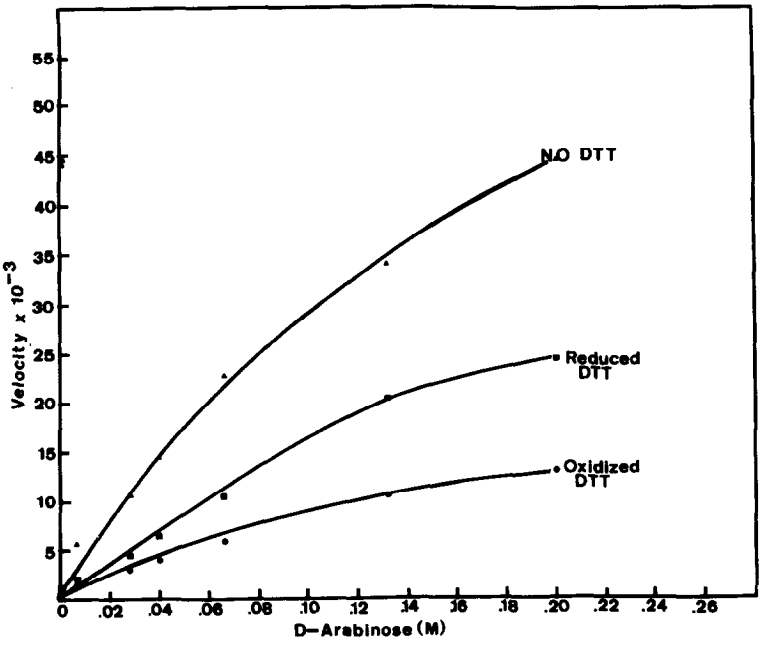

Fig. 1 Isomerase activity on D-arabinose in presence and absence of dithiothreitol. The assay system consisted of $MnCl_2$ (2.2×10^{-4}M), Trishydroxymethylamino Methane (pH = 7.0, 8.4×10^{-3}M), Glutathione (pH = 7.0, 6.7×10^{-3}M), Ribitol Dehydrogenase (excess), D-arabinose (6.6×10^{-2}M), NADH (3.20×10^{-4}M), DTT (10^{-3}M), and enzyme. Velocity was measured as the change in OD at 340 mμ on the Gilford Multiple Sample Absorbance Spectrophotometer. The extent of DTT reduction was determined by the nitroprusside method (3) and absorption spectra (2). This same assay is used throughout.

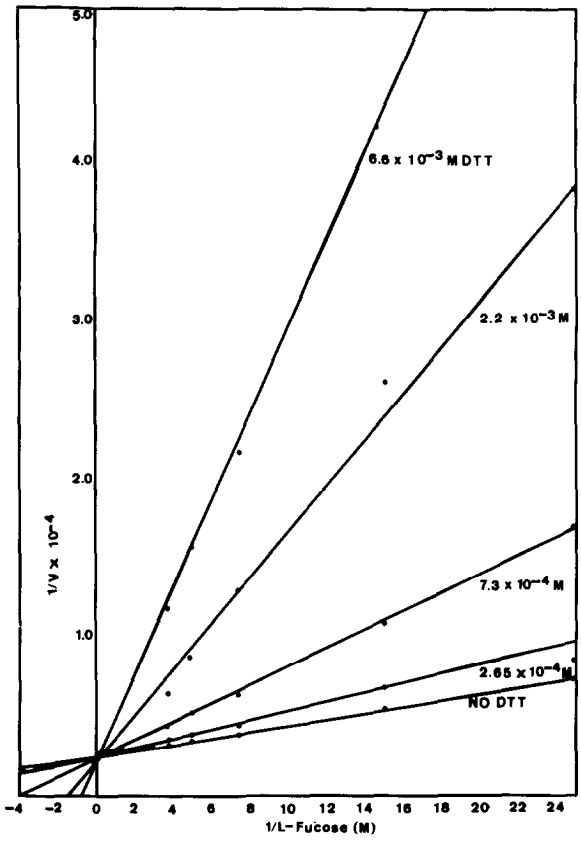

Fig. 2 Inhibition of L-Fucose Isomerization by DTT.

levels of dithiothreitol. Dithiothreitol appeared to function as a competitive inhibitor with either L-fucose or D-arabinose as substrates.

The reduced DTT was treated with air to produce the oxidized form. The extent of oxidation was determined by the nitroprusside method (1,2) and by its absorption spectra (1) as recorded on the Beckman DK-1A spectrophotometer.

The DTT was preincubated at room temperature with the reaction system for two minutes prior to the addition of substrate. The inhibition was not due to non-specific inactivation, however, since: (1) reaction velocity was linear, (2) reaction

velocity was recorded immediately, and (3) enzyme stored for over six months in the presence of DTT at 0°C had slightly higher activity than enzyme stored under similar conditions in its absence.

Figure 3 shows the reciprocal plot with velocity plotted against inhibitor concentration. The apparent K_i of inhibition with the two substrates was approximately the same, 4.6 and 2.6 x 10^{-6}M for D-arabinose and L-fucose respectively.

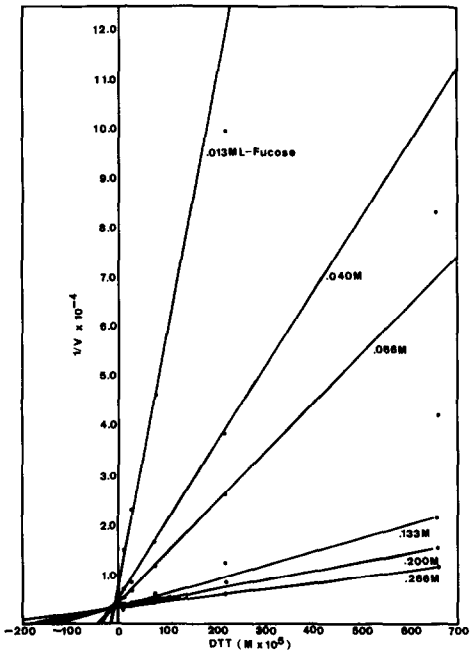

Fig. 3 Reciprocal plot of velocity vs. the inhibitor concentration with varying amounts of L-Fucose.

Table I shows the inhibition of L-fucose isomerase activity by sulfhydryl containing compounds and by polyols. Of all the compounds tested oxidized DTT and ditherythritol gave the highest percent of inhibition. Of the polyalcoholic sugars tested the best

COMPOUND	CONCENTRATION	% INHIBITION OF L-FUCOSE ACTIVITY
DTT oxidized	1×10^{-3}M	66.4
Dithioerythritol	1×10^{-3}M	76.5
Mercaptoethanol	1×10^{-3}M	0
L-cysteine	1×10^{-3}M	0
i-Erythritol	1×10^{-3}M	0
m-Erythritol	1×10^{-3}M	0
Ribitol	1×10^{-3}M	11.5
D-arabitol	1×10^{-3}M	4.5
L-arabitol	1×10^{-3}M	0
Xylitol	1×10^{-3}M	0
Mannitol	1×10^{-3}M	0
Sorbitol	1×10^{-3}M	0
Dulcitol	1×10^{-3}M	0

Table I. L-fucose isomerase inhibition by SH containing compounds and polyols. Assay the same as Fig. 1.

inhibitors were five carbon compounds with the 3 and 4 hydroxyls in the cis position. The inhibition observed with dithioerythritol and the pentitols was also reversible with higher concentrations of D-arabinose or L-fucose.

The competitive inhibition of pentose isomerases by pentitols has been reported previously (4). The inhibition of L-fucose isomerase by dithiothreitol and dithioerythritol could result from the structural similarity of these compounds with the enzyme substrates.

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