INHIBITION OF 5,10-METHYLENETETRAHYDROFOLATE DEHYDROGENASE BY THE d,L-DIASTEREOISOMER OF 5,10-METHYLENETETRAHYDROFOLATE*

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The existence of the enzyme 5,10-methylenetetrahydrofolate dehydrogenase in pigeon liver and beef liver extracts was demonstrated first by Jaenicke and Greenberg et al. (Jaenicke, 1955; Greenberg, et al., 1955; Jaenicke, 1956). Since then the enzyme has been partially purified from beef liver (Hatefi et al., 1957) and bakers' yeast (Ramasastri and Blakley, 1962).

In the previous studies the substrate for the reaction was either dl, L-5,10-methylenefolate-H_4, prepared by the addition of folate-H_4 to HCHO, or the 1,L-diastereoisomer of 5,10-methylenefolate-H_4 generated enzymatically from L-serine by the enzyme serine hydroxymethylase. It has been reported that with 5,10-methylenefolate-H_4 formed nonenzymatically the dehydrogenase reaction is slower than with the 1,L-diastereoisomer enzymatically generated (Hatefi et al., 1957). It has also been reported that the yeast enzyme is stereospecific for the 1,L-diastereoisomer (Ramasastri and Blakley, 1962). It seems desirous, therefore, to evaluate the effect of the d,L-diastereoisomer on the reaction rate now that both 1,L- and d,L- forms of 5,10-methylenetetrahydrofolate are available (Kaufman et al., 1963).

The present report, obtained with a purified preparation of 5,10-methylene-tetrahydrofolate dehydrogenase from Escherichia coli, confirms the stereo-

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specificity of substrate for the enzyme, in addition, it was observed that the d,L-diastereoisomer of 5,10-methylenefolate-H_4 inhibits the reaction in a non-competitive manner.

The enzyme was purified from extracts of acetone dried E. coli cells by ammonium sulfate fraction after removal of the nucleic acids by protamine sulfate. Further purification was obtained by alcohol fraction followed by batch absorption on Alcγ gel.

Purified preparations of l,L- and d,L- 5,10-methylenefolate-H_4 were obtained by the method of Kaufman et al. (Kaufman et al., 1963).

The requirements for the conversion of 1,L-5,10-methylenefolate-H_4 to 5,10-methenylfolate-H_4 are shown in Table I. The stereospecificity of the substrate for the enzyme is clearly established in the experiment in which the d,L-isomer was substituted for the 1,L-isomer. In this case no reaction occurred. Unlike the yeast enzyme which was stimulated by β-mercaptoethanol (Ramasastri and Blakley, 1962), the E. coli enzyme showed no requirement for this compound. In experiments not shown, dimercaptopropanol was similarly ineffective in stimulating the reaction.

TABLE I

Requirements for Conversion of 1,L-5,10-Methylenefolate to 1,L-5,10-Methenylfolate-H_4

Reaction System	Δ O.D. 340/min
Complete	66
- Mercaptoethanol	66
- 1,L-5,10-CH_2-FH_4	0
- 1,L-5,10-CH_2-FH_4 + d,l-CH_2-FH_4	0
- TPN	0
- TPN + DPN	6
- Enzyme	0

The reaction mixture consisted of 1.5 ml . 2M glycine buffer pH 8.5, 10 μg of purified enzyme, .6 ml of substrate (.45 x 10^{-3}M), .2 ml TPN 2 μmoles/ml, .1 ml β-mercaptoethanol 10^{-3}M and water to 3 ml.

The identity of the reaction product was established as 5,10-methenylfolate-H_4 by spectral comparison, after acidification of the reaction mixture and its control. The characteristic u.v. spectrum of 5,10-methenylfolate-H_4 with absorption maximum at 365 mμ was observed. In Fig. 1 are shown the saturation curve for the l,L-diastereoisomer and the effects of two different concentrations of the d,L-isomer on this curve. Fifty percent inhibition is observed with 10^{-3}M of the d,L-isomer. Increasing concentration of the active l,L-isomer was ineffective in abolishing the inhibition. This establishes the nature of the inhibition as non-competitive, and is borne out by the Lineweaver-Burke plots shown by the insert (Fig. 1). The Michaelis constant for the E. coli enzyme was calculated to be 2.5×10^{-5}M.

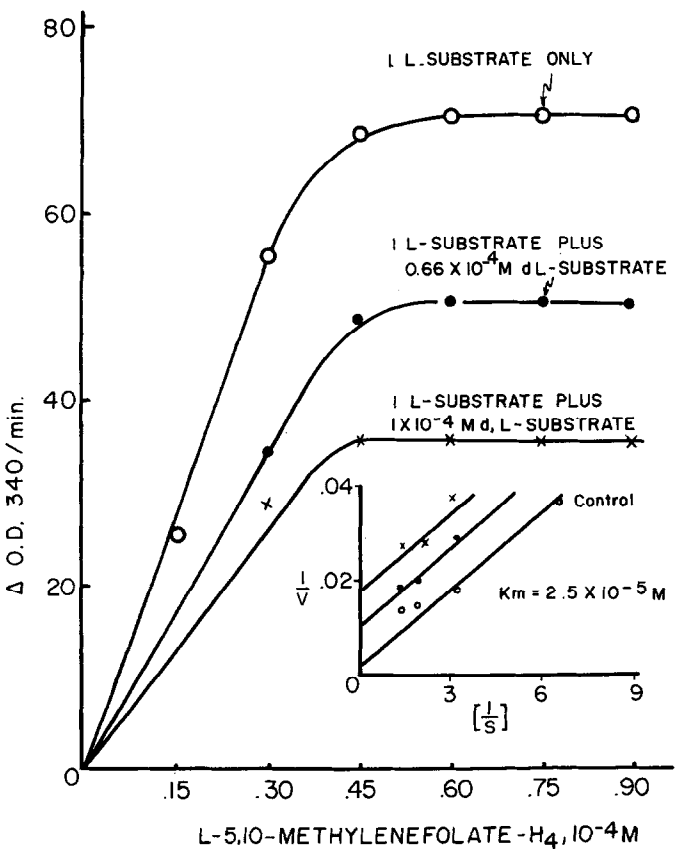

Fig. 1.- Effect of d-L-5,10-methylenefolate-H_4 on the rate of substrate oxidation. The reaction mixture was the same as that of Table I except that it contained 12 μg of enzyme and the designated amount of substrate and d-L-isomer.

Hatefi et al. (Hatefi et al., 1957) reported that the beef liver enzyme showed a much slower rate of TPNH production with the nonenzymatic or dl,L-5,10-methylenefolate-H_4 than with the enzymatically generated l,L-isomer. Ramasastri and Blakley attributed this reduced rate of TPNH formation to inhibition by excess formaldehyde. (Formaldehyde inhibition is also observed with the E. coli enzyme.) However, the fifty percent inhibition of TPNH formation observed by Hatefi et al. with the dl-L-isomer was probably not due to HCHO inhibition alone. Assuming that beef liver enzyme and the yeast enzyme are inhibited to the same extent by HCHO, then one should expect at most a twenty-five percent inhibition (calculated from the data of Hatefi et al., 1957 and Ramasastri and Blakley, 1962). The greater inhibition observed by these investigators is probably caused by inhibition by the d,L-isomer.

Failure to observe reduced rate of TPNH production by the dl,L-isomer with the yeast enzyme may be due to species differences. Indeed, some differences have been observed, for example, the stimulation of the yeast enzyme by dimercaptopropanol and β-mercaptoethanol and by certain cations have not been observed with the E. coli enzyme. The latter enzyme also shows a greater affinity for the substrate than the same enzyme from other sources. The Km for this enzyme is 2.5×10^{-5}M.

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