INHIBITION OF N^5-METHYLTETRAHYDROFOLATE - HOMOCYSTEINE TRANSMETHYLASE BY A VITAMIN B_{12}-ANTIMETABOLITE

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SUMMARY - Compound 102804 isolated from Bacillus cereus has been found to be a potent inhibitor of the N^5-methyltetrahydrofolate-homocysteine transmethylase isolated from Escherichia coli B. This inhibition was noted when 102804 was added to the enzyme reaction mixture after the reaction started or concurrently with the preparation of the mixture. Chemically inactivated 102804 has no activity as an inhibitor of this enzyme system.

A search for microorganisms producing substances inhibiting vitamin B_{12}-stimulated growth of Escherichia coli (Davis 113-3) resulted in selection of a strain of Bacillus cereus which has this capability. The vitamin B_{12}-antimetabolite (designated as compound 102804) was isolated in crystalline form and found to be a heat labile substance with empirical formula $C_{12}H_{16}N_2O_5$ (1). It is a competitive inhibitor for vitamin B_{12} in the E. coli (Davis 113-3) system and ineffective when the E. coli is grown in L-methionine containing media. This suggested that the mechanism of action involves inhibition of the formation of L-methionine by the E. coli cells.

Independent studies in several laboratories established that N^5-methyltetrahydrofolate - homocysteine transmethylase is a vitamin B_{12}-containing protein and several procedures have been published for its purification from E. coli auxotrophs (2 - 4). We have used the Taylor - Weissbach system (2) in our studies on the mechanism of action of compound 102804.

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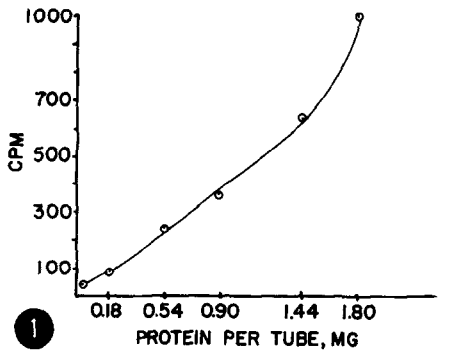


Figure 1. Formation of Methionine as a Function of Enzyme Protein

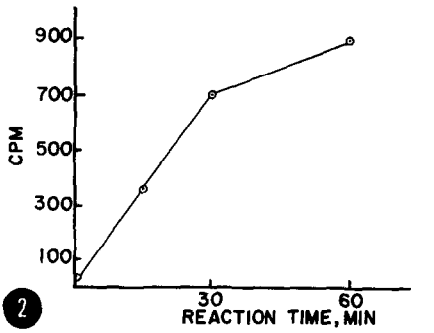


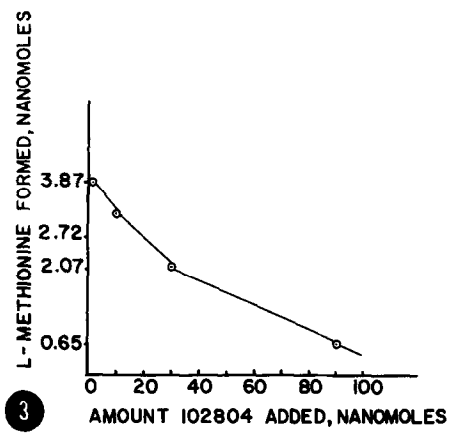
Figure 2. Formation of Methionine as Related to Incubation Interval

MATERIALS AND METHODS

Chemicals: D,L-N^5-methyl-^{14}C-tetrahydrofolate was purchased from Calbiochem. S-Adenosyl-L-methionine was obtained from Sigma Chemical Company. L-Homocysteine was prepared from the thiolactone (purchased from Calbiochem) according to Greenberg's procedure (5). All other chemicals and reagents were purchased from usual commercial sources.

Preparation of E. coli cell-free extract: Escherichia coli B was grown in a medium containing 1.1% K_2HPO_4, 0.85% KH_2PO_4, 0.6% Difco yeast extract, and 1.0% glucose and incubated at 37° C in shaken flasks. When the culture reached the mid-log phase of growth the cells were collected by centrifugation and the paste washed with a solution containing 0.5% NaCl and 0.5% KCl and the suspension recentrifuged. 5 grams of cells (wet weight) were suspended in 10 ml of 0.01 M Tris·HCl buffer (pH 7.4) and the cells disrupted in a Bronson sonic oscillator for 10 minutes. The treated suspension was centrifuged at 10,000 rpm for 20 min and the supernatant used for experimental studies. It contained 18 mg/ml of protein (measured as bovine serum albumin equivalents) as determined by the method of Lowery et al. (6).

Determination of N^5-methyltetrahydrofolate - homocysteine transmethylase activity : The procedure of Taylor and Weissbach (2) adapted as follows: The standard assay mixture (total volume of 0.20 ml) contained K_2HPO_4 (pH 7.4), 20 umoles, S-adenosyl-L-methionine, 10 nmoles, homocysteine, 50 nmoles, D,L-N^5-methyl-^{14}C-tetrahydrofolate, 30 nmoles (5,000 to 5,500 cpm), and enzyme solution. Compound 102804 dissolved in water was added as indicated. After aerobic incubation for 30 min at 37° C (or longer as indicated) the reaction was terminated by addition of 0.5 ml of cold water and the radioactive methionine formed isolated by chromatography on Dowex-1 (Cl^-) resin as described by Weissbach et al. (7). The radioactivity of the methionine-containing eluate was determined in a Packward Tri-Carb spectrometer using Bray's naphthalene - dioxane scintillation fluid (7). The identi-



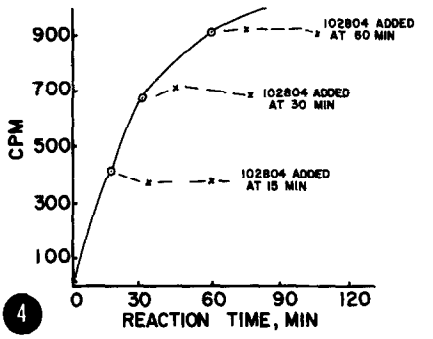


Figure 3. Dose-response Curve of Inhibition of Methionine Formation Caused by Compound 102804

Figure 4. Kinetics of Inhibition of Methionine Formation Caused by Compound 102804

fication of the methionine formed was carried out by thin layer chromatography on Merck silica gel plates using a n-butanol - acetic acid - water system (4:1:2) and detecting the methionine with ninhydrin spray and then determining the radioactivity of that area of the chromatogram.

RESULTS AND DISCUSSION

Standardization experiments with this crude enzyme preparation are summarized in Figures 1 and 2. In the former the amount of L-methionine formed in the 30 min reaction period is shown to be directly proportional to the amount of enzyme protein added. In the latter the rate of formation of L-methionine was found nearly constant over a 60 min incubation period. (In this study reaction mixture contained 0.90 mg of enzyme preparation per tube).

Addition of compound 102804 to the reaction mixture at 0 time resulted in inhibition of formation of L-methionine, and the inhibition was proportional to the amount of inhibitor added. Some of the data collected are summarized in Figure 3. When heat in-

activated compound 102804 was prepared (by heating a water solution at 100° C for 60 min) and added at 90 nmoles per tube, no significant inhibition of the transmethylase was noted. Data collected in an experiment where addition of compound 102804 (at 100 nmoles per tube) was delayed until the formation of L-methionine had been initiated are summarized in Figure 4. It is apparent that the addition of compound 102804 to the reaction mixture stopped the formation of L-methionine immediately.

These studies show that compound 102804 is a potent inhibitor of N^5-methyltetrahydrofolate - homocysteine transmethylase with an inhibition index relative to vitamin B_{12} of approximately 3. Compound 102408 is apparently able to displace the vitamin from the enzyme protein and does not react with vitamin B_{12} or the N^5-methyltetrahydrofolate.

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REFERENCES

1. Kageyama,M., Burg, K.A., and Perlman, D. (1977) J. Antibiotics 30 (in press).
2. Taylor,R.T., and Weissbach, H. (1967) J. Biol. Chem. 242, 1502
3. Kisliuk, R.L. (1961) J. Biol. Chem. 236, 817
4. Foster,M.A., Jones, K.M., and Woods, D.D. (1961) Biochem. J. 80, 519
5. Greenberg, D.M. (1962) Methods in Enzymology V, 943.
6. Lowery,O.H., Rosebrough,N.J., Farr, A.L., and Randall, R.J. (1951) J. Biol. Chem. 193, 265.
7. Weissbach, H., Peterkofsky,A., Redfield,B.G., and Dickerman, H. (1963) J. Biol. Chem. 238, 3318.
8. Bray, G.A. (1960) Anal. Biochem. 1, 129.