FUNCTION OF METHYL COBALAMIN IN METHIONINE SYNTHESIS*

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Reaction (1), which represents the terminal step in the

$$\text{5-Methyl tetrahydrofolate} + \text{homocysteine} \longrightarrow \text{tetrahydrofolate} + \text{methionine} \qquad (1)$$

biosynthesis of methionine (Larrabee et al., 1961; Sakami and Ukstins, 1961), is catalyzed by "methionine synthetase" (5-methyl tetrahydrofolate:L-homocysteine methyl transferase). This enzyme has been purified extensively from Escherichia coli and contains a bound form of vitamin B_{12} (Takeyama and Buchanan, 1961). Recent evidence has suggested that methyl cobalamin (methyl-B_{12}) may be the actual coenzyme for methionine synthetase (Weissbach et al., 1963; Foster et al., 1964).

The study of reaction (1) has been complicated by the observation that the synthetase must be supplemented with other enzymes and cofactors (DPNH, FAD, ATP and Mg^{++}) in order to demonstrate methionine synthesis (Hatch et al., 1961). Some clarification of the multiple cofactor requirement is provided by the finding that ATP and Mg^{++} can be

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replaced by adenosyl methionine (Mangum and Scrimgeour, 1962; Rosenthal and Buchanan, 1963; Weissbach et al., 1963; Foster et al., 1964; Mangum et al., 1964). The present communication describes experiments which further define the role of methyl-B_{12} and other cofactors in a methionine-synthesizing system from pig liver.

The pig liver enzyme system, which catalyzes reaction (1), has been purified previously by high speed centrifugation, treatment with protamine, and fractionation with ammonium sulfate (Sakami and Ukstins, 1961; Mangum et al., 1964). In typical preparations, the ammonium sulfate fraction (30-50% precipitate), supplemented with adenosyl methionine, catalyzed the formation of 0.01-0.03 μmoles of methionine per mg. of protein per hour. There was no synthesis of methionine when adenosyl methionine was omitted or replaced by methyl-B_{12}.

A requirement for methyl-B_{12} can be demonstrated, however, after resolution of the system into two fractions, A and B, by chromatography of the ammonium sulfate preparation on DEAE-cellulose (3 x 30 cm. column; gradient of 0.1 M $\longrightarrow$ 1.0 M phosphate buffer, pH 7.5; 3 ml. fractions). Fraction A (tubes 13-20) has a low activity for methionine synthesis, even in the presence of adenosyl methionine (Expt. 1 in Table 1). However, when methyl-B_{12} is added to fraction A, the activity is increased substantially (Expt. 2). Fraction B, which shows only slight activity with methyl-B_{12} (Expt. 3), exerts a stimulatory effect upon the AM-supplemented fraction A (cf. Expt. 4 with Expt. 1). It should be noted that the amount of methionine synthesized in Expt. 4 is greater than that in Expt. 2, even though the amount of fraction A has been reduced. The results in Table 1 were confirmed by a separate experiment in which C^{14}-labeled methyl tetrahydrofolate was used, and the radioactivity in methionine measured after its separation by column chromatography.

Our interpretation of the data in Table 1 is that fraction A contains three forms of the methionine synthetase: an active holoenzyme

Table I

Components for Methionine Synthesis

Expt.	Fraction	Supplement	Methionine Synthesized
			μmoles
1	5.4 mg. of A	AM	0.03
2	5.4 mg. of A	Me-B_{12}	0.11
3	0.9 mg. of B	Me-B_{12} (or AM)	0
4	1.9 mg. of A plus 0.9 mg. of B	AM	0.18

The complete system contained: 1 μmole of 5-methyl tetrahydrofolate, 50 μmoles of phosphate buffer, pH 7.5, 10 μmoles of DL-homocysteine and indicated amounts of fractions A and B in a total volume of 2.0 ml. The following supplements were added as indicated: 0.1 μmole of adenosyl methionine (AM), and 0.013 μmole of methyl-B_{12} (Me-B_{12}). Methyl-B_{12} was preincubated with enzyme, buffer, 0.72 μmoles of ATP, and 1.33 μmoles of glutathione for 15 minutes under N_2 in the dark at 37° C. before addition of the other components.

Reaction mixtures were placed in a Dubnoff shaker and kept in the dark under N_2 at 37° C. for 2 hours. Methionine was assumed to be equivalent to the amount of tetrahydrofolate formed. The latter was measured after its chemical conversion to 5,10-methenyl tetrahydrofolate, according to an unpublished method of Dr. J. M. Buchanan.

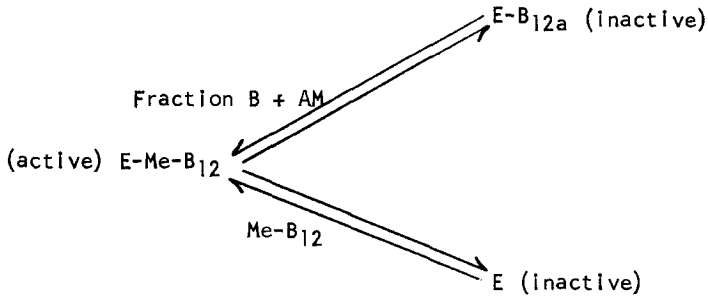
containing bound methyl-B_{12}, an apoenzyme that can be reactivated by added methyl-B_{12}, and a second inactive form of the enzyme in which there is a bound breakdown product of methyl-B_{12}, possibly B_{12a}. Reactivation of this last form, via ancillary enzymes present in fractions A and B, may involve the following sequence*:

$$B_{12a} \longrightarrow B_{12r} \longrightarrow B_{12s} \qquad (2)$$

$$B_{12s} + \text{adenosyl methionine} \longrightarrow \text{methyl-}B_{12} + \text{adenosyl homocysteine} \qquad (3)$$

* The oxidation state of the cobalt atom is +3, +2, and +1 in B_{12a}, B_{12r}, and B_{12s}, respectively.

Evidence has been presented elsewhere for the enzymatic (Foster et al., 1964) and non-enzymatic (Friedrich and Konigk, 1962; Muller and Muller, 1963) catalysis of reaction (3), and a B_{12} reductase, which utilizes reduced lipoic acid (or DPNH plus FAD) in reaction (2), has been described in Clostridium tetanomorphum (Vitols et al., 1964). A requirement for oxido-reduction cofactors, however, has not been demonstrated in the present experiments. The relationship of the three forms of the synthetase may be illustrated in the following diagram in which E, Me-B_{12}, and AM represent the apoenzyme, methyl-B_{12}, and adenosyl methionine, respectively.



The above experiments, as well as those from other laboratories (Weissbach et al., 1963; Foster et al., 1964), provide evidence that methyl-B_{12} is the coenzyme for methionine synthetase. Both methyl-B_{12} and its precursor, adenosyl methionine, participate catalytically in the methyl transfer reaction (equation 1). For example, in Expt. 2 of Table I, 0.013 μmoles of methyl-B_{12} was responsible for the synthesis of 0.11 μmoles of methionine. This is consistent with our previous data, obtained with the enzyme system prior to DEAE chromatography, in which adenosyl methionine stimulated the synthesis of 10-fold larger amounts of methionine (Mangum and Scrimgeour, 1962).

If equations 1-3 represent the correct formulation of the complex methionine-synthesizing system, methyl-B_{12} is not an intermediate in the process of methyl transfer. Conversely, Weissbach et al. (1963) and Foster et al. (1964) have reported that methyl-B_{12} is able to transfer

TABLE II

Effect of 5-Methyl Tetrahydrofolate on the Transfer of the Methyl Group from Methyl-B_{12} to Homocysteine

Expt.	Methyl Tetrahydrofolate	Methionine
	μmoles	cpm
1	0	5630
2	0.2	942
3	0.6	455
4	1.0	364

The complete system contained: 0.046 μmoles of methyl-B_{12}-methyl-C^{14} (27,700 cpm), 50 μmoles of phosphate buffer, pH 7.5, 10 μmoles of DL-homocysteine, 5-methyl tetrahydrofolate as indicated, and 5.25 mg of fraction A in a total volume of 0.65 ml. Mixtures were incubated in the dark for 2 hours at 37° C. under N_2. Methionine was isolated by descending paper chromatography using ethanol: H_2O: ammonia (90:5:5) as the solvent system, and radioactivity was counted using Bray's method (1960) in a liquid scintillation spectrometer.

its methyl group to homocysteine. Our experiments (Table II) confirm this finding, although methyl-B_{12} is a poor methyl donor compared to methyl tetrahydrofolate. Thus, in Expt. 1 the actual amount of methionine synthesized from methyl-B_{12} is small (ca. 0.01 μmole). Addition of methyl tetrahydrofolate to this system (Expts. 2-4) severely depresses the transfer of C^{14}-methyl groups from methyl-B_{12}. For example, in Expt. 4 the amount of methionine synthesized was 0.1 μmole, of which only 0.0006 μmole originated from methyl-B_{12}. Since methyl-B_{12} does not appear to be the primary methyl donor in reaction (1), its function is most probably related to labilization of the methyl group from methyl tetrahydrofolate.

REFERENCES

Bray, G., Anal. Biochem., 1, 279 (1960).
Foster, M. A., Dilworth, M. J., and Woods, D. D., Nature, 201, 39 (1964).
Friedrich, W. and Königk, E., Biochem. Z., 336, 444 (1962).
Hatch, F. T., Larabee, A. R., Cathou, R. E., and Buchanan, J. M., J. Biol. Chem., 236, 1095 (1961).
Larrabee, A. R., Rosenthal, S., Cathou, R. F., and Buchanan, J. M., J. Am. Chem. Soc., 83, 4094 (1961).
Mangum, J. H., and Scrimgeour, K. G., Federation Proc., 21, 242 (1962).
Mangum, J. H., Scrimgeour, K. G., and Huennekens, F. M., J. Biol. Chem., In preparation.
Müller, O., and Müller, G., Biochem. Z., 337, 179 (1963).
Rosenthal, S., and Buchanan, J. M., Acta Chem. Scand., 17, 5288 (1963).
Sakami, W., and Ukstins, I., J. Biol. Chem., 236, PC50 (1961).
Takeyama, S., and Buchanan, J. M., J. Biochem. (Tokyo), 49, 578 (1961).
Vitols, E., Walker, G., and Huennekens, F. M., Biochem. Biophys. Res. Comm., 15, 372 (1964).
Weissbach, H., Peterkofsky, A., Redfield, B. G., and Dickerman, H., J. Biol. Chem., 238, 3318 (1963).