DIMETHYLTHETIN AND DIMETHYL-β-PROPIOTHETIN IN METHIONINE SYNTHESIS*

By JACOB W. DUBNOFF and HENRY BORSOOK

(From the Kerckhoff Laboratories of Biology, California Institute of Technology, Pasadena)

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In a previous communication it was shown that choline and betaine are effective in promoting methionine synthesis from homocysteine in tissue homogenates (1). Data presented in this paper indicate that dimethylthetin, $(CH_3)_2^+ SCH_2COO^-$, which has been shown by Welch (2) to be lipotropic and has been reported by du Vigneaud (3) to promote growth on a methionine-free, homocysteine-containing diet, is 20 times as active as betaine in methionine formation. Dimethyl-β-propiothetin, $(CH_3)_2^+S(CH_2)_2COO^-$, recently isolated from *Polysiphonia fastigiata* by Challenger and Simpson (4) is also highly active. The enzyme for this transmethylation is found in the liver and kidney of all animals tested. Its high activity and general distribution suggest its biological importance in methionine synthesis.

Methods

Viobin extracts were prepared by stirring 5 gm. of Viobin[1] in 100 ml. of water for half an hour and filtering. Fresh extracts were prepared from organs of animals which had been killed by stunning and thoroughly bled. The organs were chilled, homogenized with 2 parts of buffer in the homogenizer of Potter and Elvehjem (5), and strained through cheese-cloth. The buffer (6) is composed of 0.0128 M sodium phosphate, pH 7.4, 0.123 M sodium chloride, 0.005 M potassium chloride, and 0.003 M magnesium sulfate.

Methylmercaptoacetic acid was prepared by Dr. M. Fling according to the method of Larsson (7).

Dimethylthetin was kindly given us by Dr. A. D. Welch.

Dimethyl-β-propiothetin was prepared by the method of Biilmann and Jensen (8).

DL-Homocystine was prepared from DL-methionine by the method of

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[1] Viobin preparations are commercially prepared desiccated and defatted raw tissues manufactured by the Viobin Corporation, Monticello, Illinois.

Butz and du Vigneaud (9) and reduced to DL-homocysteine as described by Riegel and du Vigneaud (10).

Dimethylglycine was prepared by the method of Schubert (11).

4 ml. of buffer solution containing the enzyme and substrates were incubated in 20 ml. beakers in an apparatus especially designed for non-manometric studies (12). In this apparatus as many as thirty 20 ml. beakers employed as reaction vessels are held in a stainless steel container fitted with a cover through which any gas mixture may be passed. The container is incubated at 38° and shaken at 90 oscillations per minute in a small water bath.

After incubation the solutions were deproteinized by the addition of 0.5 ml. of 30 per cent trichloroacetic acid and 1 ml. of water to each beaker. With guinea pig liver homogenates after the addition of trichloroacetic acid, the solutions were brought to a boil in the incubation apparatus in order to get filtrates which would remain clear in the analytical procedure. Filtrates of other organs offered no difficulty.

Methionine was determined by a modification of the method of McCarthy and Sullivan (13). The procedure was as follows: To 2 ml. of the trichloroacetic acid filtrate were added 0.2 ml. of 5 N NaOH followed by 0.1 ml. of 1 per cent freshly made sodium nitroprusside. The solutions were incubated at 38° for 5 to 10 minutes and then 1 ml. of an acid mixture consisting of 9 volumes of concentrated hydrochloric acid and 1 volume of 85 per cent phosphoric acid was added. After 10 minutes the solutions were read in a Klett-Summerson colorimeter with a green filter.

If the solutions are cooled in ice before the addition of strong acid, homocysteine reduces the color by an amount which increases with increasing concentration of methionine. When this cooling step is omitted as described, the decrease in color due to homocysteine is a small and constant value over a wide range of methionine concentrations, and no difficulty is encountered with gas bubbles during the measurement of the color. 1 mole of methylmercaptoacetic acid formed by the demethylation of dimethylthetin gives a color equivalent to 0.6 mole of methionine in this determination. Accordingly, all apparent increases of methionine due to the addition of dimethylthetin must be divided by 1.6 to compensate for the equivalent amount of methylmercaptoacetic acid formed in the reaction. Dimethyl-β-propiothetin is chromogenic, but it can be destroyed by allowing the solutions to stand overnight after the addition of 5 N NaOH. The nitroprusside is added the following day and the determination continued as described. The chromogenic power of methylmercaptopropionic acid, the demethylated product of dimethyl-β-propiothetin, is approximately 0.9 that of methionine on a mole basis.

Results

In rat liver choline, betaine, dimethyl-β-propiothetin, and dimethylthetin show significant activity in methionine formation (Table I). Dimethylethanolamine, dimethylglycine, and methylmercaptoacetic acid, the compounds formed by the removal of one methyl group from choline, betaine, and the dimethylthetin, respectively, are inactive.

If homocysteine is present in excess, it can be directly shown that only one methyl group is transferred per mole of dimethylthetin (Fig. 1). The reaction with betaine is too slow to reach equilibrium; only about 0.6 mole equivalent of methyl is transferred per mole of betaine in 24 hours.

TABLE I

Methionine Formation in Rat Liver Homogenate

1 ml. of 1:4 homogenate. Homocysteine 25 mg. per cent; all other substrates 12.5 mg. per cent. Total volume 4 ml.; gas phase, nitrogen; time, 3 hours; temperature, 38°. Methionine values are the average of three determinations.

Reaction mixture	Methionine found
	mg. per cent
Homocysteine	1.0 ± 0.05
" + choline	2.6 ± 0.05
" + betaine	4.2 ± 0.01
" + dimethyl-β-propiothetin	4.5* ± 0.1
" + dimethylthetin	8.4* ± 0.05
" + methylmercaptoacetic acid	1.0 ± 0.0
" + dimethylglycine	1.0 ± 0.1
" + dimethylethanolamine	1.1 ± 0.05
" + monomethylethanolamine	1.3 ± 0.2

* Corrected for chromogenic value of demethylated product as indicated in section on methods.

Table II shows the distribution of the betaine- and dimethylthetin-transmethylating enzymes and approximate $Q_{\text{methionine}}$ values for various tissues. Only liver and kidney show activity with betaine and dimethylthetin.

The effect of pH on the activity of the dimethylthetin enzyme is shown in Fig. 2. The optimum pH is about 7.8.

Neither the betaine nor the dimethylthetin enzyme is inactivated by simple dialysis.

The formation of methionine from dimethylthetin, dimethyl-β-propiothetin, betaine, or choline was not inhibited by cyanide, azide, arsenate, or arsenite.

The two enzymes can be distinguished by the greater stability of the dimethylthetin enzyme at pH 4.0 as shown in Fig. 3. The variation of the

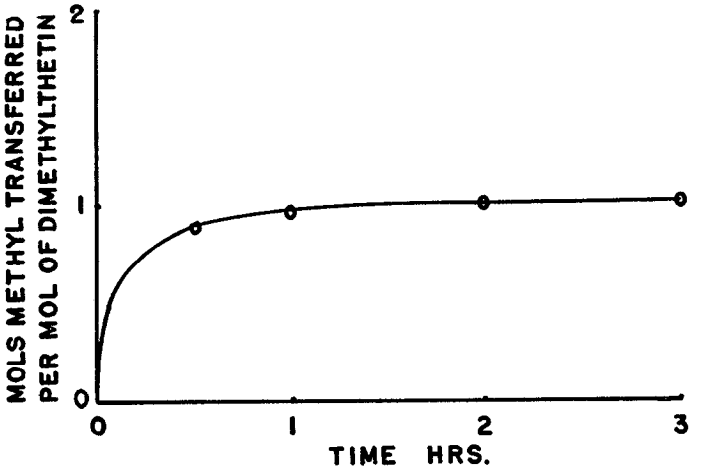

FIG. 1. Number of methyl groups transferred per mole of dimethylthetin. 1 ml. of 5 per cent solution of Viobin liver in buffer (6); dimethylthetin 5 mg. per cent; L-homocysteine 12.5 mg. per cent. Temperature, 38°; gas phase, nitrogen.

TABLE II

Distribution and Activity of Methionine-Forming Enzyme Systems

The figures are $Q_{methionine}$; average of two determinations; probable error ±5 per cent.

Each vessel contained 1 ml. of homogenized guinea pig or rat tissue diluted 1:4, or 1 ml. of a 5 per cent Viobin solution. DL-Homocysteine 25 mg. per cent, betaine or dimethylthetin 12.5 mg. per cent. Total volume, 4 ml.; gas phase, nitrogen; time, 3 hours; temperature, 38°.

	Guinea pig			Rat			Hog (Viobin)		
	Dimethylthetin	Betaine	Dimethylthetin / Betaine	Dimethylthetin	Betaine	Dimethylthetin / Betaine	Dimethylthetin	Betaine	Dimethylthetin / Betaine
Liver	1.5	0.09	17	1.5	0.11	14	1.3	0.14	9
Kidney	0.3	0.06	5	0.2	0.03	7	0.9	0.03	30
Spleen							0	0	
Muscle	0	0							
Pancreas							0	0	

ratio of $Q_{dimethylthetin}$ to $Q_{betaine}$ (Table II) also points to two different enzymes for the two methylators.

The dimethylthetin enzyme may be purified free of betaine enzyme and concentrated about 100-fold by precipitating a 5 per cent aqueous solution

of Viobin[1] liver with alcohol. The fraction precipitated at between 11.5 and 18 per cent alcohol contains almost all the activity of the whole extract. While the crude homogenate is effective with homocystine and

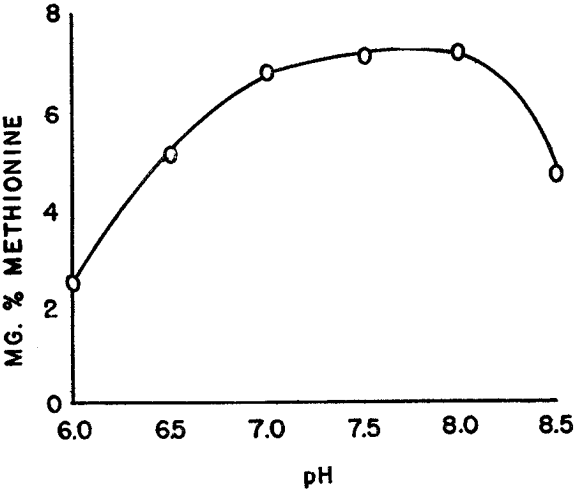

FIG. 2. Effect of pH on methionine formation from dimethylthetin. 1 ml. of 5 per cent Viobin; dimethylthetin 12.5 mg. per cent, DL-homocysteine 12.5 mg. per cent. Temperature 38°; gas phase, nitrogen; time, 1 hour.

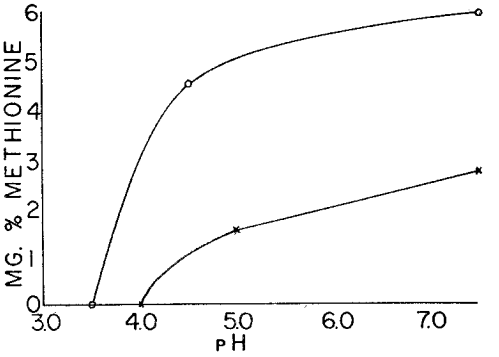

FIG. 3. Stability of enzyme to pH. Upper curve, dimethylthetin transmethylase; lower curve, betaine transmethylase. 5 per cent Viobin allowed to stand 24 hours at 5° at given pH, and relative activity at pH 7.5 determined at 38°. Substrates 12.5 mg. per cent. Gas phase, nitrogen; total volume, 4 ml.; time, 3 hours.

homocysteine, this fraction reacts only with homocysteine. Although the dimethylthetin transmethylase can be prepared free from the betaine transmethylase by further alcohol fractionation, all active betaine transmethylase preparations have had dimethylthetin transmethylase activity.

Whether the latter enzyme is required for betaine activity is still uncertain.

An attempt was made to demonstrate the presence of dimethylthetin in animal tissues. Neutral and acidified aqueous and alcoholic extracts of Viobin[1] pancreas, spleen, kidney, and liver, fresh beef pancreas, and guinea pig and rat liver and kidney were inactive with a partially purified dimethylthetin enzyme. Liver and kidney homogenates of the rat and guinea pig were allowed to stand at pH 4.0 at 5° for 18 hours to destroy all but the dimethylthetin methylating system. Any increase in methionine on addition of homocysteine in such a system could be considered as evidence of preformed dimethylthetin. No increase in methionine occurred. These experiments seem to exclude any significant quantity of preformed dimethylthetin in these tissues. On the addition of labile methyl donors to homogenates there is a slight synthesis of methionine in most experiments, suggesting the presence of small amounts of preformed homocysteine.

None of these reactions is reversible under our conditions; *i.e.*, methionine will not remethylate dimethylglycine, dimethylethanolamine, or mercaptoacetic acid aerobically or anaerobically in the presence of high energy-yielding metabolites. These reactions were studied by measuring the change in methionine concentration in the presence of these putative methyl acceptors.

Although dimethylthetin is very effective with homocysteine, it will not methylate glycocyamine in rat or guinea pig liver homogenates.

DISCUSSION

Evidence has been presented that there are at least four compounds which can furnish methyl groups for methionine synthesis in tissue homogenates. These compounds, dimethylthetin, dimethyl-β-propiothetin, betaine, choline, are all "onium" compounds characterized by the coordination of an additional methyl group to sulfur or nitrogen, and they all react in the absence of oxygen or energy donors. It has been directly demonstrated in these and previous studies (1) that the methyl groups in dimethylglycine, dimethylethanolamine, and methylmercaptoacetic acid are not transferred under conditions in which a methyl of the "onium" compound is labile. This confirms the findings in feeding experiments on the availability of methyl in dimethylglycine and dimethylaminoethanol (14).

The methyl of methionine, which is held by a covalent bond to sulfur, but is nevertheless labile, requires energy for its transfer to glycocyamine (15) and to nicotinamide (16).

Du Vigneaud and his collaborators have proved rigorously that the methyl groups of choline, betaine, and methionine constitute a dietary

"pool" of physiologically interchangeable methyl groups. The evidence reported here indicates that in the tissues there does not exist a "pool" of labile methyl groups in the sense that the transfer is directly between any two members of the dietary pool of labile methyl compounds. It seems likely that rather than a "pool" there are specific methyl donors for each methyl acceptor (*i.e.*, the methylation of glycocyamine by methionine (15)), and that a given methyl compound may be related to another only indirectly through a series of methyl transfer reactions.[2]

We have so far failed to find any methyl transfer reaction which is reversible in the usual chemical sense. Methionine, for example, does not directly remethylate dimethylethanolamine, dimethylglycine, or methylmercaptoacetic acid.

The present findings indicate that the physiological transfer from methionine to choline must be a cyclic process in which some and possibly all steps are irreversible. In some stages of the cycle oxidation and presumably, therefore, energy is required; other stages may proceed anaerobically. In such a dynamic state a given labile methyl-containing compound cannot be utilized or depleted to the same extent by all methyl acceptors. This is in accord with the fact that not all methyl donors are equally effective in overcoming growth inhibition by such compounds as glycocyamine (17, 18) and nicotinamide (19, 20).

The effectiveness of dimethylthetin suggests its importance in the biological synthesis of methionine. Its apparent absence from animal tissues may be due to its rapid demethylation in the presence of homocysteine, or it may be present and function in only catalytic amounts. The occurrence of dimethylthetin in the dietary sources has not been studied. Its homologue, dimethyl-β-propiothetin, has been isolated from algae (4) and may be present in pineapple (21). This compound may, therefore, prove to be more important biologically than dimethylthetin despite its lower activity in methionine synthesis.

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SUMMARY

1. An enzyme has been isolated in a partially purified state which transfers a methyl group from either dimethylthetin or dimethyl-β-propiothetin to homocysteine.

[2] The data presented here do not establish a direct methyl transfer from all four compounds to homocysteine. Unpublished evidence suggests that choline is first oxidized to betaine. Whether the latter compound transfers directly or through a methylthetin derivative has not yet been determined.

2. Dimethylthetin is 10 to 20 times as effective as betaine as a methyl donor in tissue homogenates. Dimethyl-β-propiothetin is more effective than betaine.

3. The enzyme is found in the liver and kidney of rat, guinea pig, and hog, but is absent from muscle, pancreas, and spleen.

4. The reaction proceeds until one methyl group has been transferred from dimethylthetin to homocysteine. Mercaptoacetic acid is inactive.

5. Homocystine is completely inactive as a methyl acceptor in purified extracts.

6. The reaction is independent of O_2 and is not inhibited by oxidative poisons.

7. The dimethylthetin transmethylase is distinguished from the betaine transmethylase by its stability at pH 4.5.

8. The possible rôle of dimethylthetin and dimethyl-β-propiothetin in the biological formation of methionine is discussed.

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