ORIGIN OF PHOTOLABILE METHYL GROUPS IN METHIONINE BIOSYNTHESIS*

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The present experiments were designed to approach the question of whether methyl-B_{12} is an intermediate in the biosynthesis of methionine from methyl tetrahydrofolate ($MeFH_4$) and homocysteine. In this process, S-adenosyl methionine (SAM) is a required co-factor (Mangum and Scrimgeour, 1962) although its role is still obscure. In addition, methyl-B_{12} is the coenzyme for methionine synthetase (Weissbach, et al., 1963).

For these experiments an enzyme preparation was obtained from a pig liver homogenate and fractionated between 30 and 50% of saturation with ammonium sulfate (Kerwar et al., 1966). This preparation was chosen since this system is independent of the addition of methyl-B_{12} for enzymatic activity, yet requires SAM for the synthesis of methionine. At this stage of purification there is no demonstrable requirement for an exogenous reducing agent such as reduced flavin (Kerwar et al., 1964). The specific activity of the enzyme as measured by methionine formation (Weissbach et al., 1963) was from 1.6 - 3.0 mμ moles of methionine synthesized per hour per mg. of enzyme.

The basic approach was to use the known photolability of methyl-B_{12} as evidence of the synthesis of this compound. According to a recent report, (Hogenkamp, 1966), formaldehyde is a

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major product of the photolysis of methyl-B_{12} in an excess of oxygen. The present assay is based upon measurement of radioactivity in the dimedone derivative of formaldehyde using appropriately labeled precursors.

$MeFH_4$, SAM and methionine, all labeled in the methyl group, were subjected to photolysis (one hour at 10 cm. from a 150 watt tungsten lamp under an oxygen atmosphere). There was no production of labeled formaldehyde under these circumstances. In addition, methyl labeled methionine in the presence of the enzyme gave no formaldehyde following photolysis. When either the labeled $MeFH_4$ or labeled SAM were incubated in the dark with the "basic system" (described in Table I), no labeled formaldehyde was detected unless the incubation was followed by aerobic photolysis. If the enzyme was boiled prior to incubation, no labeled formaldehyde was produced under any circumstances.

Incubations were carried out in the dark for 60 minutes at 37° under nitrogen. Afterward, 2 mmoles of carrier formaldehyde were added and the solution flushed with oxygen. The stoppered flasks were photolyzed for 1 hour at a distance of 10 cm. from a 150 watt tungsten lamp. At the end of this time, 560 mg. of dimedone dissolved in ethanol was added with a drop of piperidine and the insoluble derivative allowed to form. In order to remove protein, the precipitate was centrifuged, taken up in boiling ethanol and then filtered through a fritted glass funnel. The derivative was allowed to crystallize from the filtrate after addition of a small amount of water. The crude crystals were then recrystallized to constant specific radioactivity. C^{14} was measured at 64% and H^3 at 16.5% efficiency in a liquid scintillation spectrometer.

The data show that under conditions where methionine is synthesized, both $MeFH_4$ and SAM serve as precursors of formaldehyde (methyl-B_{12}). The experiments suggest that in the pig liver enzyme system there must be a precursor of a photolabile methyl group which is not of itself photolabile yet which can be alkylated by either $MeFH_4$ or SAM, without prior reduction. An example would be a complex of B_{12a} with a thioredoxin type molecule (Kerwar et al., 1966).

It can also be seen that addition of SAM is not an absolute

System	Addition	Experiment 1	2	3	4
basic	---	148	118		
basic	SAM	163	122	69	91
basic	SAM (-) enzyme	0	0		
basic	H^3 SAM (-) $MeFH_4$			H^3 990	H^3 2090
basic	H^3 SAM			H^3 242 C^{14} 64	H^3 268 C^{14} 86
basic	H^3 SAM (-) enzyme			H^3 0 C^{14} 0	H^3 0 C^{14} 0

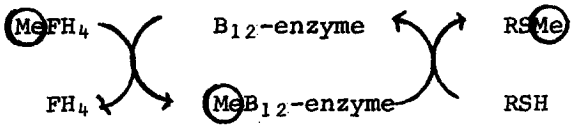
All data in dpm/mg of dimedone derivative
Theoretical yield is 584 mg.

Table I. Enzymatic Synthesis of Photolabile Methyl Group.

Basic system contains: enzyme protein, (different preparation for each experiment), 200 mg; methyl-C^{14}-$MeFH_4$, 1.5 x 10^6 dpm and 0.93 µ moles; methyl-B_{12}, 85 mµ moles; homocysteine, 100 µmoles; and sodium phosphate buffer, pH 7.5, 1000 µmoles. Where indicated, methyl-H^3-S-adenosyl methionine was added as 1.0 µmoles and 8.9 x 10^6 dpm, or unlabeled SAM, was added as 1.0 µmoles. All incubations were for one hour under nitrogen in the dark in a total volume of 10 ml.

requirement for the production of formaldehyde from $MeFH_4$. It is, however, a necessary cofactor for the synthesis of methionine from the same substrate, (Mangum and Scrimgeour, 1962). While it appears that SAM is a good precursor of the photolabile methyl group in the absence of $MeFH_4$, it is important to note that in the presence of $MeFH_4$, SAM is unable to compete as effectively as the natural precursor of the methyl group of methionine.

This suggests that the path of carbon in the overall reaction is:



Inhibition studies with propyl iodide and preliminary cofactor experiments (J. D. Brodie, unpublished) suggest that there may be two B_{12} sites of which one (alkylated only by SAM) affects the transfer of methyl from methyl B_{12} to homocysteine. Quantitative experiments to examine this possibility are in progress. A recent communication (Taylor and Weissbach, 1966) demonstrates that in the E. coli system, SAM does alkylate the enzyme with the formation of a "labile" methylated product.

Summary: The data clearly show that both methyl tetrahydrofolate and S-adenosyl methionine can serve as methyl group donors for the enzyme-dependent synthesis of a photolabile methyl group, almost certainly methyl-B_{12}. These experimental results support the concept that, in the biosynthesis of methionine from methyl tetrahydrofolate, the path of carbon is through the methyl group of methyl-B_{12}.

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