ALKYLATION OF AN ENZYME IN THE METHANE-FORMING SYSTEM OF METHANOBACILLUS OMELIANSKII

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Recent studies in our laboratory have established that N^5-methyltetrahydrofolate is an intermediate in the formation of CH_4 from carbon 3 of serine in cell-free extracts of Methanobacillus omelianskii (Wood and Wolfe, 1965; Wood, Allam, Brill and Wolfe, 1965). No evidence has been found to implicate a free cobamide compound as the natural methyl-donor in CH_4 formation by M. omelianskii, though methyl-B_{12}, (Wolin, Wolin and Wolfe, 1964) has been shown to be an excellent substrate for the CH_4-forming reaction. Brot and Weissbach (1965) have shown recently that a cobamide-enzyme-complex is involved in methyl-transfer in methionine biosynthesis by partially purified extracts of Escherichia coli. Using the same techniques as the above authors evidence has been obtained that a similar enzyme may be involved in CH_4 formation. This communication is concerned with the chemical alkylation of the active site of an enzyme in the methane-forming system, with propyl iodide; the inactive enzyme can be reactivated by light to give propane as the photolysis product.

Cell-free extracts of M. omelianskii were prepared by sonic disintegration, and both CH_4 and CH_3-CH_2-CH_3 were assayed by the gas chromatographic technique previously described by Wolin et al (1963). Methyl-B_{12} was prepared by the method of Müller and Müller (1962) and N^5-methyltetra-

hydrofolate was prepared by the method outlined by Wood et al (1965).

When crude extract was pre-incubated with propyl iodide complete inhibition of CH_4 formation was observed when methyl-B_{12}, N^5-methyltetrahydrofolate or pyruvate were added to reaction mixtures. However, if identical reaction mixtures were exposed to visible light for 20 minutes, prior to the addition of the above substrates, quite significant restoration of enzyme activity was observed (Figure 1).

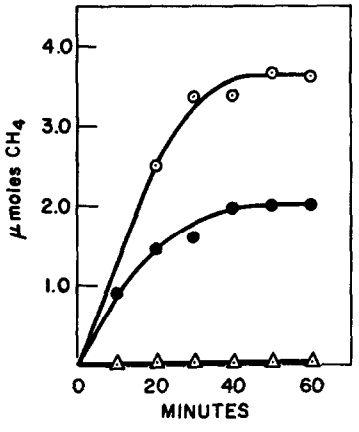


Fig. 1. Effect of propyl iodide on the formation of CH_4 from methylcobalamin. O–O, in the absence of propyl iodide; Δ–Δ, in the presence of 0.5 μmole of propyl iodide pre-incubated for 20 min prior to the addition of substrate; ●–●, in the presence of 0.5 μmole of propyl iodide pre-incubated for 20 minutes followed by 20 minute illumination from a 200 watt tungsten filament lamp (distance 15 cm) before the substrate was added to the reaction mixture. The reaction mixtures contained: crude extract (84.0 mg protein), 5.0 μmoles of methyl-B_{12}, 760 μmoles of potassium phosphate buffer pH 7.0, and 10.0 μmoles of ATP. Total reaction volume of 1.75 ml was incubated at 40°C under H_2.

The reaction rates were never as good as those recorded when no propyl iodide was added; it may be expected that a competition between the small amount of unreacted propyl iodide and the large excess of methyl-donating substrate for the active site of the enzyme could affect the reaction rate.

The anaerobic photolysis product of the propyl-enzyme complex was identified as propane (Figure 2).

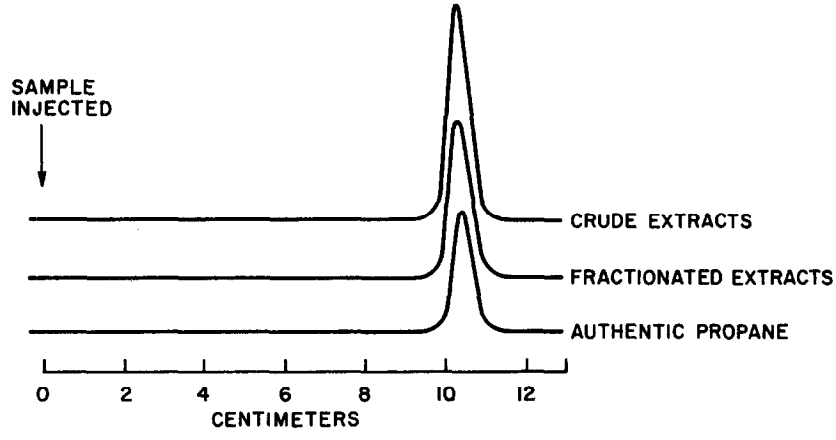


Fig. 2. Identification of propane as the anaerobic photolysis product of the propyl-enzyme complex. Each reaction mixture contained 50 µmoles of propyl iodide, crude extract (84.0 mg of protein) or extract fraction obtained at 60 to 100% saturation with ammonium sulfate (21.2 mg of protein). Gas phase H_2; temperature 25°C; illumination time seven hours. From each reaction vial (total gas volume 18.5 ml) a 0.4 ml sample was injected into the gas chromatograph. The peak for authentic propane represents 0.028 µmole; propane from the reaction mixture containing fractionated extract represents 0.035 µmole, and that from the reaction mixture containing crude extract 0.049 µmole.

The site of alkylation was shown to be protein-bound after fractionation of the cell-free extract with a saturated solution of ammonium sulfate which had been adjusted to pH 7.0 with ammonium hydroxide (Table 1).

Table 1

Ability of various fractions of cell extract to produce propane in the presence of propyl iodide and light.

Fraction	µmoles of propane/mg of protein/hr
Crude extract	0.035
0-50% $(NH_4)_2SO_4$	0.0195
50-70% $(NH_4)_2SO_4$	0.0187
70-100% $(NH_4)_2SO_4$	0.061

Propane formation was shown to be directly proportional to the illumination time (Figure 3).

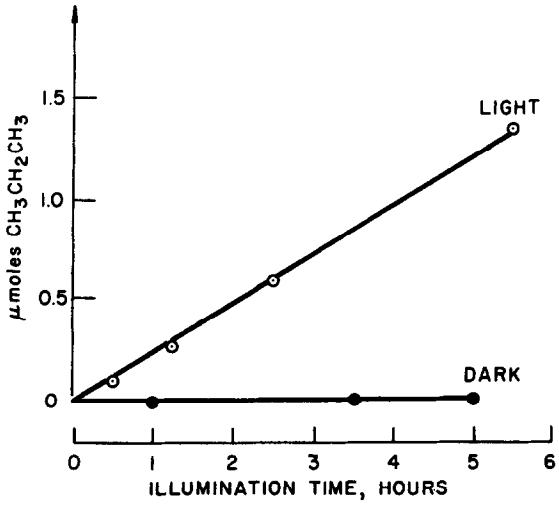
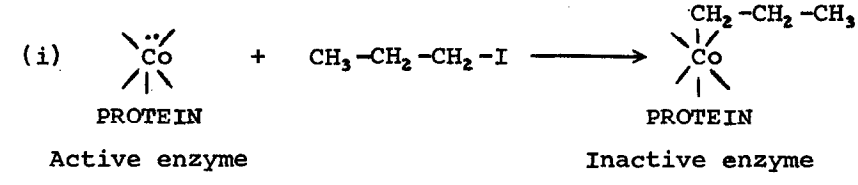
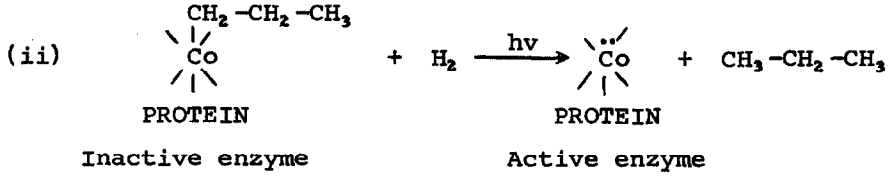


Fig. 3. Light catalyzed formation of propane from 50 μmoles of propyl iodide in the presence of crude extract of M. omelianskii (73.6 mg of protein). Gas phase H_2, temperature 25°C.

The identification of propane as the anaerobic photolysis product of the propyl-enzyme complex, prepared from extracts of M. omelianskii, is consistent with the concept that a cobamide moiety of the methane-forming enzyme exists in the fully reduced hydride configuration.

Current concepts of the propylation of the enzyme and its reactivation by light are summarized in the following scheme.





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