A NEW ACTIVATOR IN THE VITAMIN B_{12}-DEPENDENT METHIONINE BIOSYNTHESIS OF *ESCHERICHIA COLI*

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1. Introduction

The vitamin B_{12}-dependent methionine synthetase (N^5-methyltetrahydrofolate-homocysteine-methyltransferase) from *Escherichia coli* and other sources is known to be activated by *S*-adenosylmethionine [1–4]. Since the methyl group of $^{14}CH_3$-*S*-adenosylmethionine may be bound to the enzyme [5–7] and released again by visible light [5,8], the enzyme appears to be activated by methylation at the cofactor B_{12} site, thereby enabling the enzyme to enter the transmethylation cycle [8]. Enzymes usually are not activated by their own products or sequential derivatives; it is difficult to imagine the physiological meaning of an activation of this kind. Mangum and North [9] and Taylor and Weissbach [10] report that occasionally their enzyme preparations were able to work in absence of *S*-adenosylmethionine; Taylor and Weissbach [10] explain this phenomenon by an unspecific adsorption of *S*-adenosylmethionine to the enzyme which they believe to persist through the purification procedure.

Recently we found that there exists an activating agent different from *S*-adenosylmethionine which is derived from one of the substrates of the enzymic reaction, namely N^5-methyltetrahydrofolate.

2. Experimental

Methionine synthetase was purified to a specific activity of 5.1 μmoles hr^{-1} mg^{-1} from *E. coli* cells which had been grown in presence of cyanocobalamin [11]. N^5-Methyltetrahydrofolate was prepared by a procedure similar to that described by Blair and Saunders [12]. The activation of the enzyme was determined by a modification of the method given in [13]: 125 μl of 20 mM N^5-methyltetrahydrofolate were added to 1 ml of a solution containing 25 μmoles DL-homocysteine, 12.5 μmoles dithiothreitol, 0.125 μmoles FMN, 0.0125 μmoles aquocobalamin, and 83 μmoles sodium phosphate buffer pH 7.2. The mixture was incubated at room temperature for different time intervals and under the aeration conditions indicated. 175 μl of the preincubated mixture were added to 60 μg of enzyme protein in 50 μl 0.1 M Tris-HCl buffer, pH 8.0, or to 50 μl Tris buffer as blank, respectively, in triplicate. Incubations and analyses were performed as described earlier [13], the concentration of methinyltetrahydrofolic acid was assayed at 366 nm in cuvettes of 40 mm pathlength.

3. Results and discussion

During our investigations on the high molecular weight demethylating factor from *E. coli* [14] we observed that occasionally methionine synthetase displayed an unusually high activity in absence of *S*-adenosylmethionine. This activation does not depend on the enzyme preparation, but is due to the pretreatment of the incubation mixture we use in order to determine the enzyme activity. This mixture consists of the substrates (N^5-methyltetrahydrofolate and homocysteine) and a reducing system (dithiothreitol, FMN, and aquocobalamin). Working anaerobically and very quickly, we observed a low activity of the enzyme in the absence of *S*-adenosylmethionine; if, however, air is allowed to enter the incubation mixture prior to the activity assay proper, the enzyme is activated in a

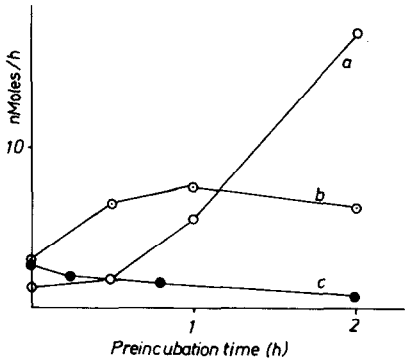

Fig. 1. Activation of methionine synthetase by an oxidation product of N^5-methyltetrahydrofolate. Aerobic incubation of the mixture from the substrates and the reducing system, filled into test tubes to a height of 10 mm (a, open circles), 3 mm (b, dotted circles), and 3 mm with stirring (c, closed circles). For details see experimental section.

manner reproducible with difficulty. The appearance and disappearance of the activating effect of the incubation mixture is illustrated in fig. 1. The activating ability of the mixture rises steadily if the aeration is limited by a relatively high level and small surface of the solution in the test tube (a). At a medium level and a more extended surface (b), the activating substance is formed, but the ability to activate the enzyme passes through a maximum, either because on prolonged oxidation the activating factor is destroyed or an inhibitor is formed which antagonizes the activating effect. The same holds true if the aeration is optimal by stirring the solution (c); no activation at all can be observed, but the enzyme activity declines slightly.

Apparently one of the components of the incubation mixture on limited oxidation forms a substance which can activate methionine synthetase. By testing all combinations of the single components, we stated that N^5-methyltetrahydrofolate must be the source of the activator. In preliminary experiments, carefully controlled oxidation of N^5-methyltetrahydrofolate yielded a product which was able to activate methionine synthetase, though its activating ability was very labile. Oxidation products of N^5-methyltetrahydrofolate which have been described [15,16] are not identical with the activator of methionine synthetase since they do not exert any influence on the enzyme [17].

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