RESOLUTION OF THE METHIONINE SYNTHETASE SYSTEM FROM ESCHERICHIA COLI K-12*

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Summary. Methionine synthetase from Escherichia coli K-12 is resolved during purification into two protein components, both of which are required for optimal enzymatic activity. The two proteins, designated M and S, have molecular weights of about 125,000 and 3,000, as judged by gel filtration. The reconstructed system involving M and S shows an absolute requirement for both S-adenosyl methionine and a suitable B_{12} compound (B_{12a} or methyl-B_{12}), as well as a partial requirement for a reducing agent such as dithioerythritol. The latter can be replaced by a DPNH-dependent reductase.

The enzymatic synthesis of methionine (reaction 1) requires, in addition

5-Methyl tetrahydrofolate + homocysteine ⟶ tetrahydrofolate + methionine (1)

to the substrates, a reducing system and catalytic amounts of S-adenosyl methionine[1] and a B_{12} coenzyme. Methionine synthetase, the enzyme responsible for reaction (1), has been purified from several sources, including various Escherichia coli auxotrophs (1-6); the most highly purified of the latter enzyme preparations have consisted of a single protein containing a tightly-bound B_{12} moiety. During our previous study of the methionine

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1. Abbreviations used: AM, S-adenosyl methionine; DTE, dithioerythritol.

synthetase from E. coli K-12, it was observed that purification of the enzyme was accompanied by an extensive loss of total units of activity (cf. Table I in ref. 6). Further investigation of this problem has now revealed that the fractionation procedure gives rise to two protein components (referred to as "M" and "S"), both of which are necessary for optimal methionine synthetase activity.

The M and S proteins are separated and partially purified by the following procedure. Cells of E. coli K-12, grown on a B_{12}-supplemented medium (Grain Processing Co.), are disrupted by sonication and the extract recovered by centrifugation. Following treatment with protamine sulfate to remove nucleic acids, the supernatant solution is fractionated with solid ammonium sulfate. The S component and a DPNH-dependent reductase (whose participation in methionine synthesis will be discussed subsequently) are present in the

Table I

Purification of M and S Proteins

Step	M		M + S	
	Specific Activity	% Recovery	Specific Activity	% Recovery
Cell-free extract	0.23	100	0.23	100
Protamine sulfate supernatant	0.27	103	0.27	103
0-50% $(NH_4)_2SO_4$ precipitate	0.13	25	0.55	106
DEAE-cellulose	0.37	16	2.60	98
DEAE-Sephadex	1.00	10	12.0	108

The assay system contained the following components in a total volume of 0.5 ml: 0.3 mM 5-methyl tetrahydrofolate (labeled with ^{14}C in the methyl group); 10 mM homocysteine, 4 μM AM; 40 μM methyl-B_{12}; 20 mM DTE; 40 mM potassium phosphate, pH 7.0; and suitable amount of M and S. The reaction mixture was incubated for 10-20 min under argon at 37° and ^{14}C-methionine was assayed by the method of Weissbach, et al. (7). Specific activity is defined as μmoles methionine synthesized/hr/mg protein.

50-90% fraction; these proteins are separated by passage of the mixture through Sephadex G-50 that had been equilibrated with 1 M KCl and 10 mM mercaptoethanol. The M component, which is present in the 0-50% ammonium sulfate fraction, is further purified by batchwise chromatography on DEAE-cellulose and gradient chromatography on DEAE-Sephadex (potassium phosphate, pH 7.0, 0.15 M→0.45 M). Prior to each of these chromatographic steps, methyl-B_{12} (final concentration, 0.1 mM) is added in order to stabilize the M component. During the above purification procedure, there is a progressive loss of methionine synthetase activity in the M fraction alone, but this activity is recovered when the M and S fractions are tested together (Table I). The S fraction alone shows no synthetase activity at any stage of its purification.

Cofactor requirements for the reconstructed system (M plus S) are shown in Table II. There is an absolute requirement for a suitable B_{12} com-

Table II

Component Study for Methionine Synthesis in E. coli K-12

Conditions	Methionine
	mμmoles
Complete system	288
M omitted	0
S omitted	15
Methyl-B_{12} omitted	0
Methyl-B_{12} replaced by B_{12a}	300
AM omitted	14
DTE omitted	60
DTE replaced by DPNH + reductase	236

Assay system as in Table I except that 0.1 mg of M and 0.085 mg of S were used. Time of incubation, 10 min. Where indicated, methyl-B_{12} was replaced by the same amount of B_{12a} and DTE was replaced by 1 mM DPNH and 2 mg of reductase.

pound. Methyl-B_{12} and B_{12a} will each suffice, provided that AM and DTE are also present. DTE can be replaced by a DPNH-dependent reductase.[2] The latter protein has properties similar to the "methionine holoenzyme synthetase" which Brot and Weissbach (8) found in the 50-90% ammonium sulfate fraction of E. coli K-12. Stravrianopoulos and Jaenicke (5) have also described a DPNH-dependent flavoprotein with similar activity from E. coli.

Gel filtration experiments suggest that the M component has a molecular weight of about 125, 000. Somewhat surprising, however, was the low molecular weight (ca. 3, 000) of the S component when estimated by this technique (Fig. 1). The S component is relatively heat-stable. For example, there was

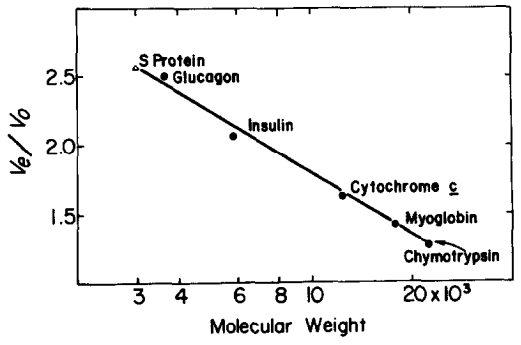

Fig. 1. Molecular weight determination of S component. Procedure of Whitaker (9) using a 2 x 120 cm column of Sephadex G-50 and indicated standards. V_e, elution volume; V_o, void volume. Molecular weights are plotted on a log scale.

no loss in activity when it was held at 60° for 5 min, and when this treatment was extended to 20 min, only 30% of the activity was lost. Exposure to pH 1

2. This enzyme, which appears to be a flavoprotein (MW ≅ 60, 000) having dihydrolipoate dehydrogenase activity, will be described in a subsequent communication.

at 0° for 3 min, however, completely destroyed the S activity. Complete inactivation was also obtained by treatment of S with Nagarse (4 hrs at 37° under argon). In the absence of added thiols, S tends to form higher molecular weight aggregates, as evidenced by its altered behavior on Sephadex columns.

Several other B_{12}-dependent enzyme systems, notably glutamate mutase (10), glycerol mutase (11), lysine deaminase (12), methane synthetase (13), acetate synthetase (14, 15) and ribonucleotide reductase (if considered along with thioredoxin (16)), have been shown to consist of two protein components. As shown by the present data, methionine synthetase from E. coli K-12 also fits this pattern. There is still considerable uncertainty, however, about the role of each of the proteins in the above systems. With regard to the methionine synthetase, it seems likely that the M protein contains the binding sites for 5-methyl tetrahydrofolate, homocysteine and the B_{12} coenzyme. The S component may modulate the activity of M or, alternatively, it may stabilize a reduced form of B_{12}. It is of interest that neither E. coli B thioredoxin (17), nor the S component from glutamate mutase (10), can substitute for the S component in the methionine-synthesizing system.

Although the overall reaction (as written in equation 1) involves the non-oxidative transfer of a methyl group, the well-established requirement for a reducing system in methionine synthesis remains to be explained. Either a DPNH-dependent reductase[2] or dithiols will suffice for this purpose (cf. Table II). Among the more likely targets for reduction are the S component or the B_{12} moiety. The latter possibility gains some support from the observations that dithiols can reduce both B_{12a} and B_{12r} nonenzymatically and that DPNH-dependent B_{12a} and B_{12r} reducing systems occur in Clostridium tetanomorphum (18).

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