Vol. 85, No. 3, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
December 14, 1978 Pages 1165-1172

BIOSYNTHESIS OF METHIONINE IN MOUSE CELLS CULTURED IN VITRO

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Received October 6, 1978

SUMMARY: In the mouse cell-lines cultured in vitro, viz. L-cells and mouse embryo fibroblasts, the methylation of homocysteine to methionine is carried out by vitamin B12-dependent 5-methyltetrahydrofolate:L-homocysteine methyltransferase only. In these cells grown in the standard Eagle medium, the activity of another methyltransferase, which utilizes betaine as the methyl donor, was not detected. The high activity of the vitamin B12-dependent methionine synthetase is typical for mouse cells from the logarithmic phase of growth. In L-cells 60%, and in the mouse fibroblasts 30% of the enzyme exist in the holo-form; the ratio between the holo- and apoenzyme activity remains stable in cells from logarithmic and stationary cultures. The level of the activity of methionine synthetase strongly depends on the presence of vitamin B12, folate and methionine in the culture medium and is greater after prolonged contact of the cells with these agents.

Mammalian cells in culture differ among themselves in the intensity of the methionine synthesis and in the requirement for exogenous methionine for growth. The aim of this work was first to find out which enzymes in mouse cells cultured in vitro catalyse the last step in methionine biosynthesis i.e. the methylation of homocysteine to methionine and secondly, whether the methionine biosynthesis in mouse cells undergoes regulation mechanisms known to operate in cultured baby hamster kidney cells (1) or Chinese hamster ovary cells (2).

MATERIALS AND METHODS

Growth of cells and preparation of extracts: Mouse L-cells and mouse embryo fibroblasts (MF), up to 4-7 passages, were grown as monolayers in Eagle's medium as already described (3). In experimental series this medium was also used with added vitamin B12 (cyano-form), folate or methionine. The removal of cells from glass and preparation of enzyme extract was done as already described (3).

0006-291X/78/0853-1165$01.00/0

Enzyme assays: The activity of betaine:L-homocysteine methyltransferase (2.1.1.5) and that of 5-methyltetrahydropteroyl-L-glutamate:L-homocysteine 5-methyltransferase (2.1.1.13) was estimated by the standard methods (4, 1, 3) and expressed as nmoles of methionine per mg protein per hour. Formation of labelled methionine was verified by autoradiography (5) on thin-layer chromatograms.

Protein content was determined by the method of Lowry et al. (6). For estimation of vitamin B12 content in cell extracts, the microbiological method with the use of Lactobacillus leichmani 7830 was applied (7). The estimations were done by Dr. Jadwiga Tautt from the Institute of Drug Research and Control, Warsaw.

RESULTS AND DISCUSSION

1. Enzymes synthesizing methionine in mouse cells cultured in vitro

The activity of methyltransferase betaine:homocysteine and methyltransferase N^5-methyltetrahydrofolate: homocysteine, two basic enzymes involved in methionine biosynthesis in mammalian tissues, was determined in extracts of different mouse cells cultured in vitro and for comparison reasons in liver extracts of some mammals (Table 1). The activity of the enzyme utilizing betaine as the methyl group-donor was demonstrated in liver, exclusively. In contrast, vitamin B12-dependent methyltransferase utilizing methyl group from methyltetrahydrofolate was found to be active both in the livers and in the mouse cells tested, and the levels of their activity were comparable. We conclude therefore, that in the mouse cells cultured in vitro, the only enzyme involved in the last step of methionine formation is vitamin B12-dependent methyltetrahydrofolate: homocysteine methyltransferase, designated further as methionine synthetase. The activity of the third enzyme, vitamin B12-independent 5-methyltetrahydrofolatetriglutamate:homocysteine methyltransferase (2.1.1.14), known to catalyse in bacteria and plant tissues the last step in methionine biosynthesis (9) was not checked in the mouse cells, because of the lack of triglutamate derivative of methyltetrahydrofolate.

2. Characteristics of methionine synthetase from L-cells and mouse embryo fibroblasts

Cofactor requirements. Methionine synthetase in tested cells shows similar cofactor requirements (vitamin B12,

Table 1. Activity of betaine:homocysteine methyltransferase and 5-methyltetrahydrofolate:homocysteine methyltransferase in liver of several mammals and different mouse cells cultured in vitro

Source of enzyme	Specific activity	
	Betaine:homocysteine methyltransferase	5-methyltetrahydrofolate:homocysteine methyltransferase
	(nmoles methionine/mg protein/h)	
L i v e r		
mouse	2,000	3.8
rat	2,400	3.2
rabbit	2,500	2.9
calf	3,000	3.6
bovine	1,200	1.7
C e l l s		
MF	0	3.5
L	0	2.0
L5178Y	0	2.5

The incubation mixture for betaine:homocysteine methyltransferase contained in total volume of 0.2 ml : 175 mM potassium phosphate buffer pH 7.4, 125 mM dithiotreitol, 32.5 mM homocysteine (prepared just before use from thiolactone form) 32.5 mM (10 uCi) of [$^{14}CH_3$]betaine and 100-500 ug of protein. The incubation mixture for 5-methyltetrahydrofolate:homocysteine methyltransferase contained in total volume of 0.2 ml : 100 mM potassium phosphate buffer pH 7.4, 125 mM 2-mercaptoethanol, 5.0 uM vitamin B12, 25 uM SAM, 250 uM homocysteine (prepared just before use from thiolactone form), 52 uM (0.25 uCi) 5-methyltetrahydrofolate and 100-400 ug of protein.

SAM and reducing agents) as the enzyme of E.coli (8,9). When propyl iodide, which inactivates vitamin B12 in transmethylations reactions was present in the incubation mixture, the activity of methionine synthetase practically could be detected neither in L-cells nor in MF. In the absence of SAM in the incubation mixture, only 60% of the enzyme activity found in the complete incubation mixture could be demonstrated. Replacement of SAM by S-adenosyl-homocysteine, the inhibitor of the transmethylations reactions from SAM results in negligible enzyme activity (about 15%). The omission from the incubation mixture of 2-mercaptoethanol or dithiotreitol has shown their indispensability for the expression of methionine synthetase activity.

Holo and apoenzyme activity. When tested in cells collected 24 hours after subculturing, the holoenzyme activity, estimated in the absence of vitamin B12 in the incubation mixture, was in MF about 30% and in L-cells about 60% of the total. Although the total activity of the enzyme in cells from 72 hour cultures was considerably lower than in cells from 24 hour cultures, the ratio between the holo- and apoenzyme activity remained the same. A similar relationship was found for L1210 cells which evidently contained less holoenzyme than MF and L-cells, although all these cells used folate as the only source of the vitamin (10). It is possible to relate the differences in the holoenzyme content in L-cells and MF to the higher vitamin B12 content in the extracts from L-cells than from MF, which were 8.6 and 0.5 pmoles/mg protein, respectively. It should be noted that the only source of vitamin B12 for the tested cells grown in the standard Eagle medium was the bovine serum, which contains traces of cyano derivative of vitamin B12 (10^{-10}M).

The amount of methionine synthesized by the action of methionine synthetase, was found to be twice as high in MF as in L-cells. Nevertheless, their values of specific activity correspond to those reported for normal human fibroblasts and several of their malignant and transformed counterparts (11,12). Our results therefore support the opinion of Hoffman and Erbe (11), that there is no substantial difference between normal and transformed cells in their ability to methylate homocysteine to methionine, which was postulated by Ashe et al. (13) and Halpern et al. (14).

3. The activity of methionine synthetase in L-cells and MF cultured in the standard medium supplemented with vitamin B12, folate or methionine

The effect of vitamin B12, folate or methionine on methionine synthetase activity was determined in parallel sets of experiments, is which compounds under investigation were added either to the incubation mixture (Figs 1A, 2A, 3A) or to the culture medium (1B, 2B, 3B). When vitamin B12 was present in the incubation mixture (Fig. 1A), enzyme activity was found to be higher, whereas in the presence of folate (Fig. 2A) or methionine (Fig. 3A) enzyme activity was always found to be lower.

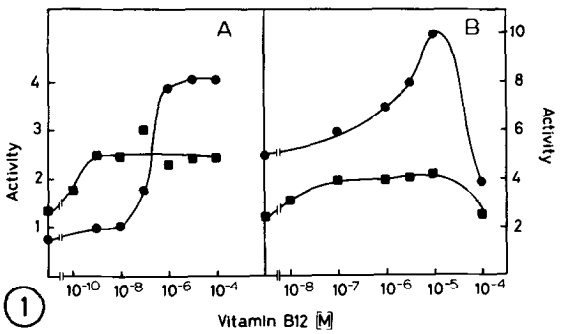


Fig. 1. Concentration effects of vitamin B12 on the activity of methionine synthetase in L-cells (■) and MF (●) as added into the incubation mixture (A) or into the culture medium (B).

In the experiments presented in part A the cells were cultivated in the standard Eagle medium for 48 hours after subculturing. The incubation mixture was as described in the legend to Table 1, except of vitamin B12. In the experiments presented in part B the cells were grown for 18 hours either in the control medium or in that supplemented with vitamin B12. The incubation mixture as described in the legend to Table 1.

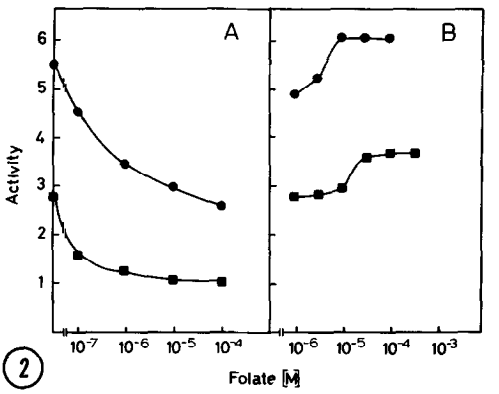
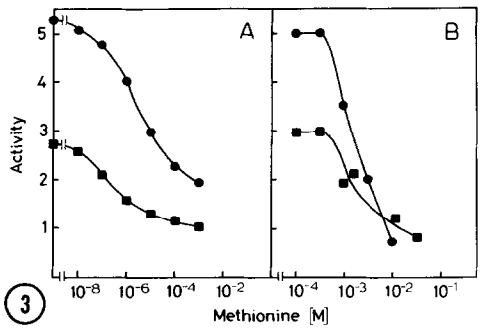


Fig. 2. Concentration effects of folate on the activity of methionine synthetase in L-cells (■) and MF (●) as added into the incubation mixture (A) or into the culture medium (B).

In the experiments presented in part A the cells were cultivated in the standard Eagle medium for 48 hrs after subculturing. The incubation mixture was as described in the legend to Table 1 except of folate. In the experiments presented in part B the cells were grown for 18 hours either in the control medium already containing folate at 10^{-6}M or in that supplemented in extra amounts of folate. The incubation mixture as described in the legend to Table 1.

In experiments in which methionine was added to the medium, the decrease in this enzyme activity was also found, even in cells cultivated only 18 hours in such a medium (Fig. 3B). In contrast, in the media with an extra amount of folate (Fig. 2B) or vitamin B12 especially (Fig. 1B), a considerable increase in the activity of methionine synthetase in the tested cells was found. The maximum increase in the activity of methionine synthetase from L-cells and MF was observed if 10^{-8}M or 10^{-5}M vitamin B12 was present in the medium. Further elevation of the vitamin B12 content depressed this enzyme activity in L-cells and MF as well.



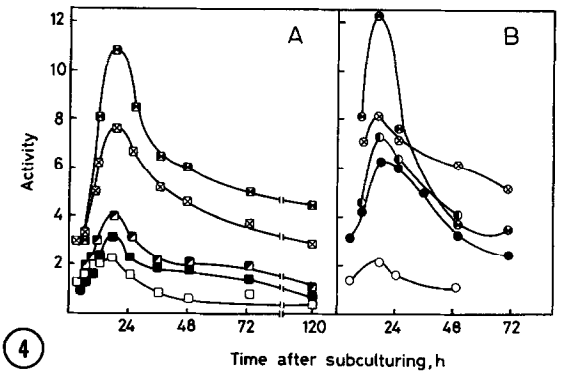


Fig. 3. Concentration effects of methionine on the activity of methionine synthetase in L-cells (■) and MF (●) as added into the incubation mixture (A) or into the culture medium (B).

In the experiments presented in part A the cells were cultivated in the standard Eagle medium for 48 hrs after subculturing. The incubation mixture was as described in the legend to Table 1 except of methionine. In the experiments presented in part B the cells were grown for 18 hours either in the control medium already containing methionine at 10^{-4}M or in that supplemented with extra amounts of methionine. The incubation mixture as described in the legend to Table 1.

Fig. 4. Activity of methionine synthetase in L-cells (A) and mouse embryo fibroblasts (B) cultured for several passages in the standard Eagle medium (■ , ●) or in the following experimental media with added vitamin B12 (7×10^{-6}M - ⊠ or 1.4×10^{-5}M ⊗), folate (5×10^{-6}M - ◪ , ◐); vitamin B12 plus folate (7×10^{-6}M, 5×10^{-6}M, respectively - ◪ , ◐) or methionine (2.2×10^{-3}M - □ , ○). Standard Eagle medium contains folate at 10^{-6}M and methionine at 10^{-4}M concentrations. The cells were grown without renewing of the culture medium up to 120 hrs. The incubation mixture as described in the legend to Table 1.

The effect of prolonged contact (for at least 3 weeks) of the mouse cells with the compounds under examination is shown in Fig. 4. The typical pattern of methionine synthetase activity in cells grown up to 120 hr in the control medium or in the experimental ones, was very similar. In both cases the highest activity of this enzyme was observed about 12-24 hours after subculturing, which indicates that methionine synthetase in the tested mouse cells is a log-phase enzyme, as in L1210 cells (10), human lymphoblasts (15) and lymphocytes (16) cultured in vitro. The highest

methionine synthetase activity characterized L-cells as well as mouse fibroblasts grown in the simultaneous presence of vitamin B12 and folate. Folate alone, only slightly influenced the level of enzyme activity. In the presence of vitamin B12 in the medium, in L-cells a quite high level of enzyme activity accurred. On the other hand, in the presence of methionine, a more pronounced decrease of enzyme activity was found for MF than for L-cells. This lower methionine synthetase activity persisted in the cells transfered from the methionine-rich to the standard Eagle medium, even up to 3 days. In contrast, when L-cells characterized by high level of activity of methionine synthetase, as the result of growing in the presence of vitamin B12, were transferred to a standard medium, the enzyme activity dropped to the level of the control cells, already after 18 hours. Thus, it is evident that the activity of methionine synthetase in L-cells and mouse fibroblasts is strongly dependent on the level of vitamin B12, folate and methionine in the culture medium as it was found for Chinese hamster ovary cells (2) or for baby hamster kidney cells (1). Although the effect of these compounds was visible even after short-time contact with the mouse cells, prolonged cultivation in the presence of vitamin B12, folate or methionine was required for causing the maximum effects. Increase in the enzyme activity, as the result of growth in vitamin B12 enriched medium, could be attributed to the increase in its apoenzyme content, whereas the decrease resulting from the growth in the excess of methionine, seems to be connected both with lowering of the holoenzyme content, and with the changes in the activity of some other folate-related enzymes, especially that of methylenetetrahydrofolate reductase (17).

Acknowledgment:

This work was supported in part by the Polish National Cancer Program PR-6 Grant No. 1322.

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