THE FORMATION OF METHIONINE FROM THE MONOGLUTAMATE FORM OF METHYLTETRAHYDROFOLATE BY HIGHER PLANTS*

E. G. Burton and W. Sakami

Department of Biochemistry
Case Western Reserve University
School of Medicine
Cleveland, Ohio 44106

Received May 29, 1969

SUMMARY

A dialyzed extract of green string beans has been found to catalyze the transmethylation of homocysteine with CH_3-H_4PteGlu$_3$[1] and, at a slower rate, with CH_3-H_4PteGlu. The transmethylation of these substrates neither required nor was increased by the addition of S-adenosylmethionine and a reducing system to the incubation mixtures and therefore could not have been due to a B_{12}-dependent methyltransferase. Transmethylase activities for both CH_3-H_4-folates were eluted from Sephadex G-100 in the same fractions.

The results are interpreted as indicating that green string beans possess a methyltransferase similar to the B_{12}-independent enzyme of microorganisms but capable of utilizing the monoglutamate as well as polyglutamate forms of CH_3-H_4 folate. Preliminary studies suggest that this type of enzyme may be widely distributed in higher plants.

INTRODUCTION

It has been reported that higher plants synthesize methionine by a B_{12}-independent mechanism similar to that of microorganisms (1, 2) but experimental evidence concerning this process has not been available.

The present communication describes our studies of methionine biosynthesis by extracts of higher plants. The results are consistent with the presence in these organisms of a methyltransferase differing from both the B_{12}-dependent and independent enzymes of microorganisms. It resembles the latter enzyme in its ability

*This work was supported by Grant 5R01 AM08089-06 from the U. S. Public Health Service and Training Grant 5-T01-GM0035 from the National Institutes of Health. This paper is taken from a thesis to be presented by Earl Burton for the degree of Doctor of Philosophy. A preliminary report of some of this work has been presented at the 51st annual meeting of the Federation of American Societies for Experimental Biology, Chicago, Illinois, April, 1967.

[1]Abbreviations for members of the folic acid group: PteGlu, pteroylmonoglutamate; PteGlu$_3$, pteroyl-γ-glutamyl-γ-glutamyl-glutamate; folate, all members of the group. CH_3-H_4-, the N^5-methyl-tetrahydroderivative.

to form methionine from CH_3-H_4PteGlu$_3$ and homocysteine in the presence of added Mg^{2+} but is different from this methyltransferase in that it is able to utilize the monoglutamate, CH_3-H_4PteGlu, as substrate. The B_{12}-dependent methyltransferase found in animals (3-5) and some microorganisms (1) was not detected in the higher plants studied.

MATERIALS AND METHODS

CH_3-H_4PteGlu and CH_3-H_4PteGlu$_3$ were prepared as described previously (6) and the ^{14}C-methyl derivative was synthesized in a similar manner using ^{14}C-formaldehyde. Green string beans and spinach were obtained commercially. Barley seedlings were obtained by germination of seeds for one week on sheets of moist filter paper. Escherichia coli PA-15, a serine-glycine auxotroph, was grown on the minimal media of Vogel and Bonner (7) supplemented with 0.4% glucose and 0.08% glycine. The cells were harvested with a Sharples centrifuge and washed with 0.1 M potassium phosphate buffer pH 7.8. In the preparation of

Table I

Methionine Synthesis by Cell-free Extracts of Plants

	Methionine formed (nmoles/hr/mg protein)			
	CH_3-H_4PteGlu		CH_3-H_4PteGlu$_3$	
Source of Extract	Incubation Mixture 1	Incubation Mixture 2	Incubation Mixture 1	Incubation Mixture 2
Green beans	8.3	4.8	58.5	64.3
Spinach	3.5	2.1	37.5	36.0
Barley sprouts	4.2	3.6	20.8	21.4
E. coli	0	101	113	195

Incubation mixture 1 contained in 0.5 ml, L-homocysteine, 5 μmoles; β-mercaptoethanol, 10 μmoles; potassium phosphate, pH 7.8, 25 μmoles; $MgCl_2$, 5 μmoles; extract 0.3 ml; and N^5-methyl-H_4PteGlu$_1$, 1.2 μmoles, or N^5-methyl-H_4PteGlu$_3$, 0.75 μmoles. Incubation was aerobic for 60 min at 37°. Mixture 2 contained, in 0.5 ml, the contents of mixture 1, and also S-adenosylmethionine, 0.2 μmoles; NADH, 5 μmoles; FAD, 0.05 μmoles; hydroxocobalamin, 5×10^{-4} μmoles. Incubation was under N_2 for 60 min at 37° in the dark. Methionine was determined microbiologically with Leuconostoc mesenteroides P60 (ATCC 8042)(10). All figures have been corrected for methionine formation in the absence of added folate.

extracts used as shown in Table I the plant materials were ground in a chilled mortar with 1 volume of 0.1 M potassium phosphate buffer, pH 7.8. The homogenates were filtered through cheesecloth, and the filtrates were centrifuged for 30 min at 27,000 X g. The resulting extracts were dialyzed for 12 hrs against 100 volumes of 0.1 M potassium phosphate buffer, pH 7.8. In the preparation of the extract of E. coli, a suspension of the cells in 2.3 volumes of the 0.1 M potassium phosphate pH 7.8 buffer was passed through a Hughes press. The resulting preparation was treated with DNAase by the procedure of Hatch et al. (8) to reduce viscosity and was centrifuged and dialyzed as described above.

The extracts of green beans used in the experiments shown in Table II and Fig. 1 were prepared by homogenizing the beans in a Waring blendor without buffer. The homogenates were filtered through cheesecloth, and the filtrates were centrifuged as described above. For the experiment shown in Table II small molecules were removed from the protein by chromatography of the extract on Sephadex G-25 equilibrated with 0.1 M potassium phosphate buffer, pH 7.0.

Protein was determined by the biuret method as described by Layne (9).

Table II

Methionine Synthesis by a Cell-free Extract of Green Beans

Reaction mixture	Methionine formed (nmoles)	
	CH_3-H_4PteGlu	CH_3-H_4PteGlu$_3$
Complete	39	195
No homocysteine	6	7
No enzyme	0	0

The complete reaction mixture included, in a volume of 1.0 ml., L-homocysteine, 2.5 μmoles; potassium phosphate, pH 7, 100 μmoles; β-mercaptoethanol, 200 μmoles; $MgCl_2$, 5 μmoles; extract of green beans, 4.5 mg protein; and $^{14}CH_3$-H_4PteGlu or $^{14}CH_3$-H_4PteGlu$_3$, 1.0 μmoles (360,000cpm). The mixture was incubated aerobically for 60 min at 37°. 170 μmoles of L-methionine were then added and reaction was termined by heating for 5 min at 100°. After protein was removed by centrifugation and folate with Dowex 1 (Cl^-) as described by Weissbach et al. (3) methionine was isolated by thin layer chromatography on preparative thin layer silica gel plates with butanol:formic acid:water (12:5:3). The methionine-containing spot was eluted with water, and the radioactivity of the eluate was determined by liquid scintillation counting (18).

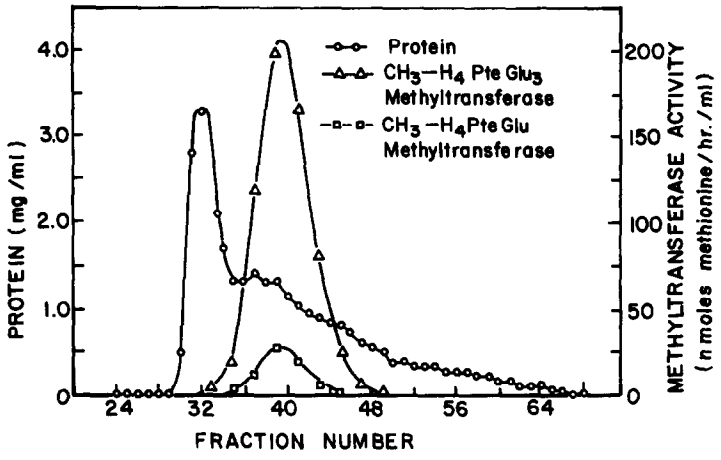

Figure 1. Chromatography on Sephadex G-100 of an Extract of Green Beans.

The crude extract of green beans was concentrated 6-fold by centrifugal filtration with Sephadex G-25 and then chromatographed (5 ml) on a column of Sephadex G-100 (2.5 X 95 cm) equilibrated with 0.1 M potassium phosphate, pH 7. The column was eluted with the same buffer. Fractions (6 ml) were assayed for methyltransferase activity by a procedure similar to that described in Table II.

RESULTS AND DISCUSSION

The mechanism of methionine biosynthesis in higher plants was studied by determining whether dialyzed extracts formed methionine under conditions suitable for the formation of methionine by similar microbial extracts containing the B_{12}-independent and dependent methyltransferases. Methionine formation was determined microbiologically with L. mesenteroides P60 (10). The results, shown in Table I, were different from those expected for either microbial enzyme which were as follows: (a) The B_{12}-independent methyltransferase would have formed methionine from homocysteine and CH_3-$H_4PteGlu_3$ in both incubation Mixture 1 and Mixture 2, but not from CH_3-$H_4PteGlu$ in either mixture. Only polyglutamate forms of CH_3-H_4 folate are utilized by this enzyme (1, 6). The only other required* substance, Mg^{2+}, was present in both types of incubation mixture. (b) The B_{12}-dependent apoenzyme or holoenzyme would have formed methionine from both

*A Mg^{2+} requirement of the B_{12}-independent methyltransferase of E. coli has been demonstrated by Guest et al. (11).

CH_3-H_4PteGlu and CH_3-H_4PteGlu$_3$ in Mixture 2, but not from either folate in Mixture 1. The B_{12}-enzyme transmethylates both forms of CH_3-H_4-folate, but requires for activity S-adenosylmethionine and a reducing system as well as homocysteine (1, 5, 12-14). A cobamide, such as hydroxocobalamin, is also required when the B_{12} enzyme is present in apoenzyme form (15-17). These additional factors are provided by Mixture 2 but are absent from Mixture 1.

The results obtained with the plant extracts (Table I) were those expected for the microbial-type B_{12}-independent methyltransferase except that in Mixture 1 homocysteine appeared to be methylated by CH_3-H_4PteGlu which is not a substrate of the microbial enzyme. To determine unequivocally whether methionine formed by plant extracts on incubation with the monoglutamate form of CH_3-H_4 folate, homocysteine and Mg^{2+} is derived from the methyl group of the folate, extracts of green beans were incubated with $^{14}CH_3$-H_4PteGlu or $^{14}CH_3$-H_4PteGlu$_3$, homocysteine and Mg^{2+} and the radioactivity of the methionine that was formed was determined. As shown in Table II, methionine incorporated methyl-^{14}C from both folates. These results clearly demonstrate that the monoglutamate as well as the triglutamate form of CH_3-H_4-folate are transmethylated with homocysteine by the extract of green beans. Whether the two transmethylations are catalyzed by the same enzyme has not yet been established. Our results are consistent with this possibility. When an extract of green beans was chromatographed on Sephadex G-100 a single peak of transmethylase activity was obtained for each folate, and the peaks of both activities appeared in the same fraction (Fig. 1).

No evidence for the presence of a B_{12}-dependent methyltransferase in higher plants has been obtained (Table I). This is consistent with reports that these organisms contain little or no vitamin B_{12} or related compounds (19). The ability of our incubation conditions to detect this enzyme is indicated by the analysis of E. coli PA15 grown without vitamin B_{12} (Table I). The extract of this organism contains the B_{12}-dependent apoenzyme together with the B_{12}-independent methyltransferase (1). In our experiment the extract formed methionine under conditions similar to those that have been reported to be suitable for the action

of each of these enzymes (11, 17, 20, 21).

The occurrence in higher plants of the transmethylation of the monoglutamate form of methyltetrahydrofolate is of some interest. It has been suggested that the presence of more than one glutamic acid residue in methyltetrahydrofolate is a structural requirement for methyl transfer in the absence of a cobamide prosthetic group (1). The present study demonstrates that this process can occur with the monoglutamate. This observation is consistent with the hypothesis that extra glutamic acid groups affect primarily the binding of CH_3-H_4 folate to the enzyme (6). The transmethylation of the monoglutamate also may be of physiological significance. Cossins and his associates have reported that CH_3-H_4PteGlu is the principal folate of germinating pea seedlings (22) and recently have observed the formation of methionine from this substance by extracts of the plants.*

REFERENCES

1. Woods, D. D., Foster, M. A., and Guest, J. R., in S. K. Shapiro and F. Schlenk (Eds.), Transmethylation and Methionine Biosynthesis, University of Chicago Press, Chicago, 1965, p.138.

2. Elford, H. L., Katzen, H. M., Rosenthal, S., Smith, L. C., and Buchanan, J. M., in S. K. Shapiro and F. Schlenk (Eds.), Transmethylation and Methionine Biosynthesis, University of Chicago Press, Chicago, 1965, p. 157.

3. Weissbach, H., Peterkofsky, A., Redfield, B. C., and Dickerman, H. J., J. Biol. Chem. 238, 3318 (1963).

4. Loughlin, R. E., Elford, H., and Buchanan, J. M., J. Biol. Chem. 239, 2888 (1964).

5. Kerwar, S. S., Mangum, J. H., Scrimgeour, K. G., Brodie, J. D., and Huennekins, F. M., Arch. Biochem. Biophys. 116, 305 (1966).

6. Burton, E. G., Selhub, J., and Sakami, W., Biochem. J. 111, 793 (1969).

7. Vogel, H. J., and Bonner, D. M., J. Biol. Chem. 218, 97 (1956).

8. Hatch, F. T., Larrabee, A. R., Cathou, R. E., and Buchanan, J. M., J. Biol. Chem. 236, 1095 (1961).

*Private communication during the preparation of this paper.

9. Layne, E., in S. P. Colowick and N. O. Kaplan (Eds.), Methods in Enzymology, Vol. III, Academic Press, Inc., New York, 1957, p. 447.

10. Steel, B. F., Sauberlich, H. E., Reynolds, M. S., and Baumann, C. A. J. Biol. Chem. 177, 533 (1949).

11. Guest, J. R., Friedman, S., Foster, M. A., Tejerina, G., and Woods, D. D., Biochem. J. 92, 497 (1964).

12. Larrabee, A. R., Rosenthal, S., Cathou, R. E., and Buchanan, J. M., J. Biol. Chem. 238, 1025 (1963).

13. Taylor, R. T., and Weissbach, H., J. Biol. Chem. 242, 1502 (1967).

14. Stavrianopoulos, J., and Jaenicke, L., European J. Biochem. 3, 95 (1967).

15. Takeyama, S., Hatch, F. T., and Buchanan, J. M., J. Biol. Chem. 236, 1102 (1961).

16. Guest, J. R., Friedman, S., Dilworth, M. J., and Woods, D. D., Ann. N. Y. Acad. Sci. 112, 774 (1964).

17. Brot, N., and Weissbach, H., J. Biol. Chem. 241, 2024 (1966).

18. Bray, G. A., Anal. Biochem. 1, 279 (1960).

19. Evans, H. J., and Kliewer, M., Ann. New York Acad. Sci. 112, 735 (1964).

20. Cauthen, S. E., Foster, M. A., and Woods, D. D., Biochem. J. 98, 630 (1966).

21. Buchanan, J. M., Elford, H. L., Loughlin, R. E., McDougall, B. M., and Rosenthal, S., Ann. New York Acad. Sci. 112, 756 (1965).

22. Roos, A. J., Spronk, A. M., and Cossins, E. A., Can. J. Biochem. 46, 1533 (1968).