Effect of Vitamin B₁₂ Deficiency on *S*-Adenosylmethionine Metabolism in Rats

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Summary The effect of vitamin B₁₂ (B₁₂) deficiency on the levels of *S*-adenosylmethionine (SAM) in tissues and the activities of hepatic methionine synthase, methionine adenosyltransferase and glycine *N*-methyltransferase were investigated. The striking depression of methionine synthase activity was observed in all rats fed the B₁₂-deficient diets with or without methionine supplementation for 150 days. The SAM level in liver was decreased by B₁₂ deficiency. However, brain SAM level was not affected. The activities of hepatic methionine adenosyltransferase isozymes, α -form and β -form, were decreased by B₁₂ deficiency. Hepatic glycine *N*-methyltransferase activity in rats fed the low methionine-B₁₂-deficient diet showed a tendency to lower, although the change the activity was not statistically significant, compared with B₁₂-supplemented rats. It is proposed that the fall in the activity of hepatic methionine adenosyltransferase may be one of the causes of the decreased hepatic SAM level in B₁₂-deficient rats.

Key Words vitamin B₁₂-deficient rats, *S*-adenosylmethionine, methionine adenosyltransferase, glycine *N*-methyltransferase

The methylfolate trap hypothesis was first proposed about twenty-five years ago (1, 2). Since then, this hypothesis has provided one of the explanations for the derangement of folate metabolism in vitamin B₁₂ (B₁₂) deficiency. Many investigators postulated that under conditions of B₁₂ deficiency the activity of B₁₂-dependent methionine synthase [EC 2.1.1.13] is significantly decreased, and, as the result, folate is trapped as the 5-methyltetrahydrofolate which is produced by essentially irreversible 5,10-methylenetetrahydrofolate reductase [EC 1.1.99.15] reaction (3, 4). Kutzbach and Stokstad (5) reported that *S*-adenosylmethionine (SAM) was a potent inhibitor of 5,10-methylenetetrahydrofolate reductase.

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Inhibition of this enzyme by SAM may result in an increase of tetrahydrofolate. Thus, SAM seems to play a regulatory role in the folate metabolism. And SAM seems to be responsible for the action of methionine on the folate metabolism in B₁₂-deficient state.

The authors previously observed that the activities of hepatic methionine synthase and methylmalonyl-CoA mutase [EC 5.4.99.2] markedly decreased in B₁₂-deficient rats (6). It is considered that the dietary B₁₂-deficient rats are useful for biochemical investigations of B₁₂ deficiency as an experimental animal model.

In the present paper we report changes in the activity of methionine adenosyltransferase [EC 2.5.1.6] which catalyzes the conversion of methionine to SAM and the level of SAM in B₁₂-deficient rats. Furthermore, the activity of hepatic glycine *N*-methyltransferase [EC 2.1.1.20], which catalyzes the transmethylation reaction between glycine and SAM, was determined.

METHODS

Animal and diets. Wistar strain male rats, weighing about 30 g, which were born and weaned from dams fed the B₁₂-deficient diet during pregnancy and lactation, were randomly divided into four experimental groups: basal soybean protein diet/B₁₂-deficient (−B₁₂ −Met) or B₁₂-supplemented (+B₁₂ −Met), and 0.5% DL-methionine-added soybean protein diet/B₁₂-deficient (−B₁₂ +Met) or B₁₂-supplemented (+B₁₂ +Met). Rats were individually housed in stainless steel screen bottom cages under the conditions of constant temperature (22 ± 2°C) and humidity (60 ± 5%). Each group was fed its respective diet *ad libitum* for 150 days. B₁₂-supplemented rats received 1 µg of CN-B₁₂ per day by oral administration. The composition of experimental diets has been previously described (6, 7).

Preparation of liver extract for enzyme assays. Rats were killed by exsanguination from cardiac puncture under anesthetizing with ether. Liver and brain were rapidly removed and homogenized with 4 vol of 40 mM potassium phosphate buffer, pH 7.5, per gram of tissue. The homogenate was centrifuged at 105,000 × *g* for 60 min at 4°C. The supernatant was collected and used for enzyme assays.

Methionine synthase activity. Measurement of methionine synthase activity was carried out following the method described by Matsuno *et al.* (8), except that the authors used NADH instead of FMNH₂.

S-Adenosylmethionine levels in liver and brain. Extraction of SAM from tissues and partial purification were carried out by adapting the method of Eloranta *et al.* (9). One gram of tissue was homogenized with 5 vol of 10% trichloroacetic acid. The homogenate was then centrifuged at 10,000 × *g* for 10 min at 4°C. The supernatant was pretreated 3 times with an equal volume of ether to remove trichloroacetic acid, then applied to Cellex-P (H⁺-form) column. The column was washed with 50 ml of 1 mM HCl, 40 ml of 10 mM HCl, 40 ml of 50 mM HCl and then SAM was eluted with 10 ml of 500 mM HCl. The eluate was evaporated to dryness at 30°C using a rotary evaporator, and dissolved in distilled water. This sample was

subjected to high-performance liquid chromatography (10).

Methionine adenosyltransferase activity and separation of methionine adenosyltransferase isozymes. Determination of methionine adenosyltransferase activity and separation of methionine adenosyltransferase isozymes in liver were carried out by the method of Liao *et al.* (11) and that of Suma *et al.* (12), respectively. Two methionine adenosyltransferase isozymes, α -form and β -form, were present in adult rat liver. The β -form has much higher K_m for methionine and ATP than the α -form (13), and is stimulated by dimethylsulfoxide (DMSO) which causes K_m to decrease (14). Accordingly, the activity was determined with or without DMSO. Moreover, to compare the isozyme pattern among each experimental group, the α -form and β -form of methionine adenosyltransferase were fractionated by Phenyl-Sepharose chromatography as follows: The soluble fraction saturated with 25% (NH₄)₂SO₄ was applied onto a Phenyl-Sepharose column (1 $\times$ 5 cm) equilibrated with Buffer A (50 mM Tris-HCl buffer (pH 7.8), 0.2 mM dithiothreitol, 0.1 mM EDTA, 10 mM MgCl₂, 20% glycerol)/25% saturation (NH₄)₂SO₄. After the column was washed with Buffer A/25% saturation (NH₄)₂SO₄, the α -form isozyme was eluted with Buffer A, and then the β -form isozyme was eluted with Buffer A/50% DMSO. Fractions of 2 ml were collected and an aliquot of each fraction was taken to determine the activity.

Glycine N-methyltransferase activity. Glycine N-methyltransferase activity in liver was determined by the method of Cook and Wagner (15).

Determination of protein. Protein was determined by the method of Lowry *et al.* (16) with bovine serum albumin as the standard.

RESULTS

Methionine synthase activity

Methionine synthase activities are shown in Table 1. Methionine synthase activities in liver and brain of rats fed the B₁₂-deficient diet for 150 days were

Table 1. Methionine synthase activities in liver and brain of vitamin B₁₂-supplemented and -deficient rats with or without dietary methionine supplementation.

Dietary treatment	Methionine synthase activity (pmol/min/mg protein)	
	Liver	Brain
+ B ₁₂ - Met	170.1 $\pm$ 24.9*	140.2 $\pm$ 14.2
- B ₁₂ - Met	8.1 $\pm$ 1.0 ^a	13.1 $\pm$ 3.8 ^a
+ B ₁₂ + Met	161.7 $\pm$ 44.8	110.5 $\pm$ 25.4
- B ₁₂ + Met	7.7 $\pm$ 0.8 ^a	11.7 $\pm$ 3.9 ^a

* Data presented as means $\pm$ SD of five rats fed the experimental diets for 150 days.

^a Significantly different from mean of vitamin B₁₂-supplemented group, $p < 0.01$.

Table 2. *S*-Adenosylmethionine levels in liver and brain of vitamin B₁₂-supplemented and -deficient rats with or without dietary methionine supplementation.

Dietary treatment	<i>S</i> -Adenosylmethionine (nmol/g tissue)	
	Liver	Brain
+ B ₁₂ – Met	58.10 ± 5.72*	23.38 ± 1.87
– B ₁₂ – Met	19.66 ± 5.43 ^a	25.67 ± 2.36
+ B ₁₂ + Met	68.43 ± 7.95 ^b	26.57 ± 1.16
– B ₁₂ + Met	28.34 ± 3.96 ^{a,b}	26.38 ± 1.22

* Data presented as means ± SD of five rats fed the experimental diets for 150 days.

^a Significantly different from mean of vitamin B₁₂-supplemented group, *p* < 0.01.

^b Significantly different from mean of – Met group, *p* < 0.05.

Table 3. Hepatic methionine adenosyltransferase activities of vitamin B₁₂-supplemented and -deficient rats with or without dietary methionine supplementation.

Dietary treatment	Methionine adenosyltransferase activity (pmol/min/mg protein)	
	– DMSO	+ DMSO
+ B ₁₂ – Met	119.4 ± 19.0*	1,178.3 ± 19.0
– B ₁₂ – Met	79.3 ± 8.7 ^a	473.7 ± 99.8 ^a
+ B ₁₂ + Met	142.9 ± 12.6 ^b	1,158.0 ± 157.0
– B ₁₂ + Met	93.0 ± 12.5 ^a	794.6 ± 103.0 ^a

* Data presented as means ± SD of five rats fed the experimental diets for 150 days.

^a Significantly different from mean of vitamin B₁₂-supplemented group, *p* < 0.01.

^b Significantly different from mean of + B₁₂ – Met group, *p* < 0.05.

markedly decreased to 5% and 10% of the B₁₂-supplemented control, respectively. But the supplementation of methionine in the diet did not influence methionine synthase activity in both tissues.

S-Adenosylmethionine level

Table 2 shows the results of determination of SAM level in liver and brain. The hepatic SAM level was decreased by B₁₂ deficiency. Also, dietary methionine supplementation elevated the hepatic SAM level, and in increasing order were – Met – B₁₂, + Met – B₁₂, – Met + B₁₂, and + Met + B₁₂. However, in brain there was little effect of B₁₂ deficiency and methionine supplementation on the SAM level.

Methionine adenosyltransferase activity

Hepatic methionine adenosyltransferase activity was determined with or without DMSO in reaction mixture. The results are shown in Table 3. When

Fig. 1. Phenyl-Sepharose chromatography of methionine adenosyltransferase from liver of vitamin B₁₂-supplemented and -deficient rats with or without dietary methionine supplementation, Buffer A: 50 mM Tris-HCl, 0.2 mM dithiothreitol, 0.1 mM EDTA, 10 mM MgCl₂, 20% (v/v) glycerol; pH 7.8. Buffer A/DMSO: Buffer A/50% (v/v) dimethylsulfoxide.

Table 4. Hepatic glycine *N*-methyltransferase activities of vitamin B₁₂-supplemented and -deficient rats with or without dietary methionine supplementation.

Dietary treatment	Glycine <i>N</i> -methyltransferase activity (nmol/min/mg protein)
+ B ₁₂ - Met	2.51 ± 0.24*
- B ₁₂ - Met	2.09 ± 0.31
+ B ₁₂ + Met	2.54 ± 0.16
- B ₁₂ + Met	2.34 ± 0.22

* Data presented as means ± SD of five rats fed the experimental diets for 150 days.

methionine adenosyltransferase activity was determined with DMSO, lower activities were observed in the B₁₂-deficient groups compared with those of B₁₂-supplemented groups. Moreover, the activities without DMSO were also decreased by B₁₂ deficiency. The decrease of the activity produced by B₁₂ deficiency showed a tendency to be alleviated by dietary methionine supplementation.

Hepatic methionine adenosyltransferase isozymes, α -form and β -form, were completely separated by Phenyl-Sepharose chromatography (Fig. 1). Each fraction was collected and dialyzed to remove DMSO, and then the effect of DMSO on the activity of each fraction was examined. From these results, the first peak which

eluted with low ion strength buffer and the second peak which eluted with buffer containing DMSO were identified as α -form and β -form, respectively (12). As shown in Fig. 1, the level of both isozymes were decreased by B₁₂ deficiency.

Hepatic glycine N-methyltransferase activity

Hepatic glycine *N*-methyltransferase activity is shown in Table 4. Hepatic glycine *N*-methyltransferase activity in the —Met —B₁₂ group showed a tendency to lower, although the change in the activity was not statistically significant, compared with the B₁₂-supplemented group.

DISCUSSION

In addition to the role as the constituent of protein and the precursor of sulfur in cysteine, methionine is converted to SAM which is a methyl group donor for transmethylation reaction. The decrease of hepatic SAM has been reported in B₁₂-deficient rats (17), nitrous oxide-exposed rats (18), and B₁₂-deficient sheep (19). We also observed that both hepatic methionine synthase activity and hepatic SAM level were decreased by B₁₂ deficiency. Moreover, the low activity of hepatic methionine adenosyltransferase was observed in B₁₂-deficient rats.

The decrease of SAM may be due to the defect of methionine *de novo* synthesis; however, it is still not clear whether or not the tissue methionine level decreases in dietary B₁₂-deficient rats. Hitherto, such a decrease was not directly demonstrated. In nitrous oxide-exposed rats, Lumb *et al.* (18) reported that there was a transient fall in liver and plasma methionine levels both of which recovered in 4 to 6 days; however, SAM level in liver decreased to 40% of its initial level after 11 days of nitrous oxide exposure in spite of restoration of methionine level. The data we obtained in this study suggests the possibility that the decrease of hepatic methionine adenosyltransferase activity is one of the causes for the low hepatic SAM level in B₁₂-deficient rats.

In spite of the absence of betaine-homocysteine methyltransferase [EC 2.1.1.5] activity in the brain, there was no effect of B₁₂ deficiency on the brain SAM level. Lumb *et al.* (18) reported that the levels of methionine and SAM in the brain of nitrous oxide-exposed rats were not changed. They considered that this is due to either increased uptake of methionine from plasma or unidentified alternative pathways in the brain for methionine synthesis. In addition to these considerations, we proposed that the activity of methionine adenosyltransferase in the brain must be examined. It is reported that methionine adenosyltransferase isozyme found in nonhepatic tissues including the brain is different from hepatic α -form and β -form isozymes (20). It is named γ -form isozyme. Abe *et al.* (21) showed that α -form and β -form isozymes were immunologically very similar, and that there was no cross-reaction between γ -form and antisera of β -form. Moreover, in hepatic methionine adenosyltransferase-deficient patients, a normal level of the enzyme was found in erythrocytes, cultured fibroblasts, and lymphoid cells (20). These findings suggest

that there is different genetic control between hepatic and nonhepatic isozymes. Further experiments need to explore methionine and SAM metabolism including tissue methionine level and methionine adenosyltransferase activity in nonhepatic tissues in B₁₂ deficiency.

Glycine *N*-methyltransferase catalyzes the SAM-dependent methylation of glycine. This enzyme has the highest activity of known methyltransferase in rat liver extract (22). Ogawa and Fujioka (23) suggested that glycine *N*-methyltransferase played an important role in methionine metabolism, since the hepatic activity of this enzyme increased in the rats fed a high methionine diet. It seems that the decrease of glycine *N*-methyltransferase activity may conserve SAM and restrict *S*-adenosylhomocysteine formation. *S*-Adenosylhomocysteine is known to be a potent inhibitor of SAM-dependent methyltransferase, therefore the ratio of SAM and *S*-adenosylhomocysteine in the cell seems to be a regulatory factor of transmethylation reactions (24, 25).

Recently, Wagner *et al.* (26) found that 5-methyltetrahydropteroylpentaglutamate is a highly specific and potent inhibitor of glycine *N*-methyltransferase. On the other hand, it is known that SAM is an inhibitor of 5,10-methylenetetrahydrofolate reductase which catalyzes the formation of 5-methyltetrahydrofolate from 5,10-methylenetetrahydrofolate (5). In B₁₂ deficiency, it was postulated that the level of SAM is decreased by insufficient neogenesis of the methyl group, and the inhibition of 5,10-methylenetetrahydrofolate reductase by SAM is relieved. This seems to lead to the accumulation of 5-methyltetrahydropteroylpentaglutamate. When the amount of 5-methyltetrahydropteroylpentaglutamate increases, the inhibition of glycine *N*-methyltransferase should be intensified. However, such scheme was not verified by the data obtained in this study.

From the results of immunological titration, Ogawa and Fujioka (23) showed that the increase in activity of glycine *N*-methyltransferase in liver of rats fed a high methionine diet was due to enzyme induction. It is reported that in nitrous oxide-exposed rats, the relative level of 5-methyltetrahydropteroylpentaglutamate in liver was decreased from 18% to 2% of the total folate by methionine injection with expansion of tetrahydrofolate pool and restoration of histidine oxidation (27). Although it is suggested that the changes of molecular species in folate pentaglutamates which seems to be responsible for the restoration of C₁-unit metabolism are caused by methionine injection in nitrous oxide-exposed rats, net accumulation of methylfolate does not occur with nitrous oxide exposure (27, 28). However, it is possible that such modulatory system may act as a fine control system of SAM level.

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REFERENCES

- 1) Noronha, J. M., and Silverman, M. (1962): On folic acid, vitamin B₁₂, methionine and formiminoglutamic acid metabolism. Vitamin B₁₂ and Intrinsic Factor, ed. by Heinrich, H. C., Enke, Stuttgart, p. 728-736.
- 2) Herbert, V., and Zalusky, R. (1962): Interrelation of vitamin B₁₂ and folic acid metabolism: Folic acid clearance studies. *J. Clin. Invest.*, **41**, 1263-1276.
- 3) Scott, J. M., and Weir, D. G. (1981): The methylfolate trap. *Lancet*, **2**, 337-340.
- 4) Shane, B., and Stokstad, E. L. R. (1985): Vitamin B₁₂ folate interrelationships. *Annu. Rev. Nutr.*, **5**, 115-141.
- 5) Kutzbach, C., and Stokstad, E. L. R. (1967): Feed back inhibition of methylenetetrahydrofolate reductase in rat liver by S-adenosylmethionine. *Biochim. Biophys. Acta*, **139**, 217-220.
- 6) Doi, T., Kawata, T., and Maekawa, A. (1986): Changes of vitamin B₁₂-dependent enzyme activities in liver of vitamin B₁₂-deprived rats. *Vitamins (J. Vitam. Soc. Jpn.)*, **60**, 205-209.
- 7) Kawata, T., Maekawa, A., and Suzuki, T. (1981): The changes of urinary methylmalonic acid excretion and the effect of vitamin B₁₂-deprivation in the rats. *Vitamins (J. Vitam. Soc. Jpn.)*, **55**, 141-146.
- 8) Matsuno, R., Urakami, Y., Hibino, K., Sakata, T., and Kamikubo, T. (1984): Mode of action of various corrinoids on the reaction of vitamin B₁₂-dependent methionine synthase from *E. Coli* 215. *Vitamins (J. Vitam. Soc. Jpn.)*, **58**, 433-438.
- 9) Eloranta, T. O., Kajander, E., and Raina, A. (1976): A new method for the assay of tissue S-adenosylhomocysteine and S-adenosylmethionine. *Biochem. J.*, **160**, 287-297.
- 10) Iizawa, T., and Carson, D. A. (1985): Differential regulation of polyamine synthesis and transmethylation reaction in methylthioadenosine phosphorylase deficient mammalian cells. *Biochim. Biophys. Acta*, **844**, 280-287.
- 11) Liau, M. C., Chang, C. F., Belanger, L., and Grenier, A. (1979): Correlation of isozyme patterns of S-adenosylmethionine synthetase with fetal stages and pathological states of the rat liver. *Cancer Res.*, **39**, 162-169.
- 12) Suma, Y., Yamanaka, Y., and Tsukada, K. (1983): Synthesis of S-adenosylmethionine synthetase isozymes from rat and mouse liver. *Biochim. Biophys. Acta*, **755**, 287-292.
- 13) Okada, G., Teraoka, H., and Tsukada, K. (1981): Multiple species of mammalian S-adenosylmethionine synthetase. Partial purification and characterization. *Biochemistry*, **20**, 934-940.
- 14) Okada, G., Sawai, Y., Teraoka, H., and Tsukada, K. (1979): Differential effect of dimethylsulfoxide on mammalian S-adenosylmethionine synthetase from rat liver and hepatoma. *FEBS Lett.*, **106**, 25-28.
- 15) Cook, J. R., and Wagner, C. (1984): Glycine N-methyltransferase is a folate binding protein of rat liver cytosol. *Proc. Natl. Acad. Sci. USA*, **81**, 3611-3634.
- 16) Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951): Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193**, 266-275.
- 17) Vidal, A. J., and Stokstad, E. L. R. (1974): Urinary excretion of 5-methyltetrahydrofolate and liver S-adenosylmethionine levels of rats fed a vitamin B₁₂-deficient diet. *Biochim. Biophys. Acta*, **362**, 245-257.
- 18) Lumb, M., Sharver, N., Deacon, R., Jennings, P., Purkiss, P., Perry, J., and Chanarin, I. (1983): Effects of nitrous oxide-induced inactivation of cobalamin on methionine and S-adenosylmethionine metabolism in the rat. *Biochim. Biophys. Acta*, **756**, 354-359.

- 19) Gawthron, J. M., and Smith, R. M. (1974): Folic acid metabolism in vitamin B₁₂-deficient sheep. *Biochem. J.*, **142**, 119–126.
- 20) Tabor, C. W., and Tabor, H. (1984): Methionine adenosyltransferase (S-adenosylmethionine synthetase) and S-adenosylmethionine decarboxylase, in *Advances in Enzymology*, ed. by Meister, A., John Wiley & Sons Inc., New York, pp. 259–265.
- 21) Abe, T., Okada, G., Teraoka, H., and Tsukada, K. (1982): Immunological distinction of S-adenosylmethionine synthetase isozymes from rat liver and kidney. *J. Biochem.*, **91**, 1081–1084.
- 22) Heady, J. E., and Kerr, J. S. (1973): Purification and characterization of glycine methyltransferase. *J. Biol. Chem.*, **248**, 69–72.
- 23) Ogawa, H., and Fujioka, M. (1982): Induction of rat liver glycine methyltransferase by high methionine diet. *Biochem. Biophys. Res. Commun.*, **108**, 227–232.
- 24) Zappia, V., Zydek-Cwick, C. R., and Schlenk, F. (1969): The specificity of S-adenosylmethionine derivatives in methyltransfer reactions. *J. Biol. Chem.*, **244**, 4499–4509.
- 25) Deduchi, T., and Barchas, J. (1971): Inhibition of transmethylation of biogenic amines by S-adenosylhomocysteine. *J. Biol. Chem.*, **246**, 3175–3181.
- 26) Wagner, C., William, T. B., and Cook, R. J. (1985): Inhibition of glycine N-methyltransferase activity by folate derivatives. *Biochem. Biophys. Res. Commun.*, **127**, 746–752.
- 27) Brody, T., Watson, J. E., and Stokstad, E. L. R. (1982): Folate pentaglutamate and folate hexaglutamate mediated one-carbon metabolism. *Biochemistry*, **21**, 276–282.
- 28) Lumb, M., Perry, J., Deacon, R., and Chanarin, I. (1981): Changes in tissue folates accompanying nitrous oxide-induced inactivation of vitamin B₁₂ in the rat. *Am. J. Clin. Nutr.*, **34**, 2412–2417.