

Decarboxylation of *S*-Adenosyl-L-methionine in Vitamin B₆ Deficiency in Rat Liver

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The effect of a prolonged diet deficient in B₆-vitamin on the activity of adenosylmethionine decarboxylase from rat liver was investigated. In contrast to an earlier report,¹² adenosylmethionine decarboxylase activity was not found to be decreased under these restricted nutritional conditions. Moreover, the addition of pyridoxal phosphate into the standard incubation mixture did not stimulate the activity of adenosylmethionine decarboxylase from the livers of the rats fed the B₆-vitamin deficient diet. It is suggested that the contradictory results of Sturman and Kremzner¹² might be based on an artificial liberation of carbon dioxide from *S*-adenosyl-L-methionine in the presence of pyridoxal phosphate. The results of this communication do not support the view that pyridoxal phosphate acts as the prosthetic group of rat liver adenosylmethionine decarboxylase.

Adenosylmethionine decarboxylase (Ado-Met DC; EC 4.1.1.50) appears to play a regulatory role in the biosynthesis of spermidine and spermine in eucaryotic tissues.¹⁻³ The mechanism of the enzymic decarboxylation of *S*-adenosyl-L-methionine (Ado-Met) is not fully understood and there exists some controversy as to whether pyridoxal phosphate (PLP) or some other carbonyl compounds function as the prosthetic group of the putrescine-activated mammalian decarboxylase. Primarily based on experiments with 4-bromo-3-hydroxybenzoyloxyamine, a compound known to inhibit PLP requiring histidine decarboxylase (EC 4.1.1.22) from rat fetus,⁴ it has been suggested that PLP also acts as the coenzyme of mammalian Ado-Met DC.⁵⁻⁷ On the other hand, common inhibitors of enzymes requiring PLP inhibited the activity of Ado-Met DC

from different mammalian tissues only slightly, if at all, although some other carbonyl reagents rapidly inactivated the decarboxylase.^{1,8-10} Furthermore, an inclusion of PLP in the incubation mixture did not stimulate Ado-Met DC from rat liver purified to electrophoretic homogeneity.¹¹ Very recently Sturman and Kremzner¹² reported that undialyzed liver cytosol fraction from rats maintained on a diet deficient in B₆-vitamin exhibited decreased Ado-Met DC activity. Full activity was reportedly restored by the addition of PLP to the incubation mixture.¹²

Because of the apparent controversy concerning the possible participation of PLP in the enzymic decarboxylation of Ado-Met in mammalian tissues, the effect of a B₆-vitamin deficient diet on the activity of Ado-Met DC from rat liver was reinvestigated in more detail. In addition to Ado-Met DC, the activities of some other soluble enzymes, *i.e.* spermidine synthase (EC 2.5.1.16), tyrosine aminotransferase (EC 2.6.1.5) and lactate dehydrogenase (EC 1.1.1.27), were assayed using undialyzed liver cytosol fraction as the source of the enzymes.

The activity of Ado-Met DC was not shown to be influenced by the vitamin B₆ deficiency nor was it stimulated by an inclusion of PLP to the standard incubation mixture.

EXPERIMENTAL

1-¹⁴C-DL-Methionine (sp.act. 5.65 mC/mmol) and 1,4-¹⁴C-putrescine (sp.act. 19.5 mC/mmol) were purchased from the New England Nuclear Corp. PLP, pyridoxine hydrochloride, py-

ruvate, and α -ketoglutaric acid were obtained from E. Merck, Darmstadt. NADH was a product of Boehringer, Mannheim. Dithiothreitol (DTT) and unlabelled putrescine were supplied by Calbiochem. The synthesis of Ado-Met from either L-methionine or radioactive DL-methionine has been described elsewhere.⁵ Methylthioadenosylhomocysteamine (decarboxylated adenosylmethionine) was prepared as described earlier.¹³ All other reagents were of analytical grade of purity.

Animals and preparation of tissue extracts. Male albino rats of the Wistar strain weighing 45–60 g were divided in two groups of five animals. The first group was fed a vitamin B₆ deficient diet (Nutritional Biochemicals Corp.) and the control group was fed the same diet supplemented with 50 mg/kg pyridoxine hydrochloride.¹³ The animals were allowed to eat and drink *ad libitum*. After five weeks the rats were killed. The livers were rapidly removed and homogenized with two volumes of 25 mM phosphate buffer, pH 7.2, containing 0.1 mM EDTA and 0.5 mM DTT (Buffer A). The homogenates were centrifuged at 75 000 g_{max} for 60 min and the supernatant fractions were used for the enzyme assays. Solid ammonium sulfate (Mann/Schwarz special enzyme grade) was used for the fractionation of pooled cytosols. The proteins precipitated between 0.35 and 0.60 were collected, dialyzed overnight against Buffer A and used for the determination of Ado-Met DC activity.

Enzyme assays. The standard incubation mixture for Ado-Met DC contained 100 mM phosphate buffer, pH 7.4, 3.3 mM DTT, 0.2 mM carboxyl labelled Ado-Met (sp.act. 0.91 mC/mmol), 2.5 mM putrescine (if present) and the enzyme in a final incubation volume

of 0.15 ml. One unit is defined as the liberation of (1 pmol of ¹⁴CO₂ from Ado-Met)/(30 min) (mg protein) at 37 °C. The incubation conditions for spermidine synthase has been described earlier.³ One unit is expressed as the production of 1 pmol of spermidine from radioactive putrescine (sp.act. 5.0 mC/mmol) in the presence of (decarboxylated adenosylmethionine)/(30 min) (mg protein) at 37 °C. Each Ado-Met DC and spermidine synthase assay was accompanied by blanks where the active protein was replaced either by water or boiled enzyme. Lactate dehydrogenase activity was determined as described by Lindy.¹⁴ One unit is defined as the oxidation of (1 μ mol of NADH)/(1 min) (mg protein) at 25 °C. The activity of tyrosine aminotransferase was measured by the method of Diamondstone, as modified by Hayashi *et al.*¹⁵ One unit is expressed as the formation of (1 nmol of *p*-hydroxybenzaldehyde)/(1 min) (mg protein) at 37 °C. All enzyme assays were carried out in duplicate. The concentration of PLP (if present) in the incubation mixtures was 0.05 mM.

Protein was measured by the method of Lowry *et al.*¹⁶ using bovine serum albumin as the standard.

RESULTS AND DISCUSSION

The rats fed the B₆-vitamin deficient diet rapidly developed typical deficiency symptoms (see Ref. 17) including a clear retardation of growth ($p < 0.05$, Table 1). In contrast to the results obtained by Sturman and Kremzner,¹² liver Ado-Met DC activity was not decreased

Table 1. Effect of vitamin B₆ deficient diet on the activities of adenosylmethionine decarboxylase, spermidine synthase, tyrosine aminotransferase and lactate dehydrogenase from rat liver. Undialyzed cytosols were used as the source of the enzymes and the activities are expressed as units (\pm SEM for adenosylmethionine decarboxylase) for incubation conditions and definition of units see Experimental). The activity of adenosylmethionine decarboxylase was assayed separately from each liver, other enzyme activities are means of two separate pools, two and three livers in each, from either group. The concentration of pyridoxal phosphate (PLP), if present in the incubations, was 0.05 mM. Ado-MetDC, adenosylmethionine decarboxylase; SpdS, spermidine synthase; TAT, tyrosine aminotransferase; LDH, lactate dehydrogenase.

| Diet | - B ₆ - PLP | + PLP | + B ₆ - PLP | + PLP |
|---------------------------|---------------------------|----------------|---------------------------|----------------|
| Body wt. (g) | 121 \pm 6 | | 170 \pm 8 | |
| Liver wt. (g) | 4.8 \pm 0.3 | | 6.7 \pm 0.3 | |
| Enzyme activities (units) | | | | |
| Ado-Met DC | | | | |
| - Putrescine | 15.9 \pm 3.3 | 18.4 \pm 2.7 | 17.1 \pm 2.9 | 18.5 \pm 2.2 |
| + Putrescine | 150 \pm 7.5 | 161 \pm 8.8 | 193 \pm 19.5 | 171 \pm 13.0 |
| SpdS | 922 | 1049 | 1348 | 1148 |
| TAT | 0.6 | 23.9 | 1.5 | 17.5 |
| LDH | 5.2 | 5.2 | 5.7 | 5.7 |

Table 2. Adenosylmethionine decarboxylase activity (units) in dialyzed liver cytosols from rats kept on vitamin B₆ deficient or pyridoxine hydrochloride supplemented diets. Each value represents the mean determined from two separate pools from either group after dialyzing the cytosols against Buffer A (see Experimental). Each enzyme assay was carried out in duplicate accompanied by blanks where the enzyme was omitted. Pyridoxal phosphate (PLP) concentration, if present in the incubation mixture, was 0.05 mM.

| Diet | - putrescine | | + putrescine | |
|-----------------|--------------|-------|--------------|-------|
| | - PLP | + PLP | - PLP | + PLP |
| -B ₆ | 27.0 | 23.0 | 409 | 426 |
| +B ₆ | 21.6 | 16.9 | 320 | 318 |

in rats maintained on the vitamin B₆ deficient diet, nor was the activity stimulated by the addition of PLP to the incubation mixture. Only minor changes were found in the activities of spermidine synthase and lactate dehydrogenase (Table 1). As indicated in Table 1, the activity of tyrosine aminotransferase (when assayed in the presence of PLP) was somewhat increased in liver cytosols of rats fed the vitamin B₆ deficient diet supporting the suggestion that under these conditions the amount of the apoenzyme might be increased.¹⁸ The activity of Ado-Met DC was not stimulated by the addition of PLP to the standard incubation mixture when either dialyzed liver cytosols (Table 2) or dialyzed ammonium sulfate fractions (not tabulated) from either group was used as the enzyme source. The dialysis of the cytosol fraction, however, increased the activity of Ado-Met DC, especially when assayed in the presence of putrescine. In fact using partially purified Ado-Met DC from rat liver (DEAE step, Ref. 2, producing 5000 units ¹⁴CO₂ from Ado-Met), an addition of 25 μl of the pooled undialyzed cytosol (about 1 mg protein) into the routine incubation mixture reduced the activity of the purified decarboxylase by 50 % (vitamin B₆ deficient group) and by 35 % (control group). No inhibition was produced by a similar addition of dialyzed cytosol from either group, thus indicating the presence of small molecular weight inhibitor(s) in the undialyzed cytosols.

There is a strong disagreement between the results obtained by Sturman and Kremzner¹² and those tabulated in Table 1, with regard to the stimulation of Ado-Met DC by the exogenous addition of PLP to the incubation mixture. To study possible reasons for this inconsistency, some experiments were carried out in incubation conditions used by Sturman and Kremzner¹² (DTT was omitted from the incubation mixture). In agreement with these authors, when undialyzed cytosols were used as the enzyme, the addition of PLP to the incubation medium (in the absence of putrescine) increased the decarboxylation of Ado-Met from 14.2 units to 28.8 units in livers of B₆-vitamin deficient rats, but had no effect on the decarboxylation rate in the control group. However, when dialyzed cytosols (dialysis overnight against 25 mM phosphate buffer, pH 7.2; Buffer B) were used as the enzyme, the inclusion of PLP to the incubation mixture (in the absence of DTT and putrescine) resulted in increases in CO₂ production from carboxyl labelled Ado-Met in the vitamin B₆ deficient- and the control groups of 80 % and 40 %, respectively. Furthermore, when the decarboxylation of Ado-Met was catalyzed by the dialyzed ammonium sulfate fractions (redialysis overnight against Buffer B), in the absence of DTT and putrescine, the additional PLP in the incubation mixture increased the liberation of ¹⁴CO₂ by about 40 % in either group. These results suggest that the incubation conditions employed by Sturman and Kremzner¹² make Ado-Met susceptible to nonenzymic decarboxylation by PLP, as also indicated earlier.¹⁹ It should be noted that in the presence of putrescine in the incubation mixture (containing no DTT) the addition of PLP did not increase the liberation of carbon dioxide from Ado-Met by any of the preparations used as the enzyme. This is consistent with the report of Sturman and Kremzner¹² concerning Ado-Met decarboxylation in rat brain. It is suggested that the previous report of the decreased activity of Ado-Met DC from undialyzed liver cytosols of rats maintained on the vitamin B₆ deficient diet¹² cannot be considered by any means as a proof of the dependence of the enzymic decarboxylation of Ado-Met upon PLP. The B₆-vitamin deficiency appears to lead to the

accumulation of small molecular weight compound(s) (or the diet contains some impurities) which inhibit(s) the activity of Ado-Met DC in rat liver, complicating the interpretation of the results obtained with undialyzed liver cytosols. The results of this communication do not support the view that PLP acts as the coenzyme for Ado-Met DC from rat liver.

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