ADENOSYLMETHIONINE DECARBOXYLASE FROM VARIOUS ORGANISMS: RELATION OF THE PUTRESCINE ACTIVATION OF THE ENZYME TO THE ABILITY OF THE ORGANISM TO SYNTHESIZE SPERMINE

H. Pösö[1], P. Hannonen[2], J.-J. Himberg[3] and J. Jänne[1]

Departments of Biochemistry[1], Medical Chemistry[2] and Pharmacology[3], University of Helsinki, SF-00170, Helsinki 17, Finland

Received November 7, 1975

SUMMARY: S-adenosyl-L-methionine decarboxylase (EC 4.1.1.50) from most eukaryotic organisms is activated by putrescine whereas the corresponding enzyme from bacterial sources shows a stringent requirement for magnesium ions. Adenosylmethionine decarboxylase from lower eukaryotes such as protozoa, however, is not influenced by diamines, neither are any metals needed for its maximal activity. A common characteristic of those organisms containing putrescine-insensitive adenosylmethionine decarboxylase appeared to be either a total absence or very low intracellular content of spermine. While extracts of all organisms containing putrescine-activated adenosylmethionine decarboxylase (animal tissues and yeast) exhibited easily measurable spermine synthase activity, no such activity was detected in cells of *Tetrahymena pyriformis*, *Escherichia coli* or *Pseudomonas aeruginosa* all containing adenosylmethionine decarboxylase insensitive to putrescine and other diamines.
The activation of adenosylmethionine decarboxylase by putrescine, the immediate precursor of spermidine, may thus assure the availability of sufficient amounts of decarboxylated adenosylmethionine (S-methyladenosylcysteamine) for the synthesis of spermidine even in the presence of a spermine synthesizing system competing for the same precursor (decarboxylated adenosylmethionine).

The biosynthetic pathway of polyamines spermidine and spermine involves the decarboxylation of S-adenosyl-L-methionine, the resultant S-methyladenosylhomocysteamine (decarboxylated adenosylmethionine) serving as the propylamine moiety of the polyamine molecule (1). This decarboxylation of adenosylmethionine is accomplished by a spesific adenosylmethionine decarboxylase in all organisms studied so far.

Adenosylmethionine decarboxylase is an interesting enzyme at least in two respects: (i) in mammalian tissues the enzyme has an unusually short biological half-life (35 to 60 min) (2,3), and (ii) in most organisms its activity is profoundly influenced by low molecular weight effectors.

Adenosylmethionine decarboxylase from animal tissues as well from baker's yeast is intensively and also specifically activated by certain diamines, notably by putrescine (1,4-diaminobutane), cadaverine (1,5-diaminopentane)

and 1,3-diaminopropane (4,5,6,7), while the corresponding enzyme from all prokaryotes studied (*E.coli* and *Azotobacter vinelandii*) shows a stringent requirement for magnesium ions (5,8). In addition to these two types of adenosylmethionine decarboxylase, some lower eukaryotes (protozoa) contain adenosylmethionine decarboxylase activity that is closely connected with the synthesis of spermidine but is insensitive to both diamines and divalent cations (9,10).

The regulation pattern of adenosylmethionine decarboxylase activity in different organisms is further complicated by the observation indicating that some ammonium sulphate fractions from extracts of bean sprouts (eukaryotic plant) exhibited adenosylmethionine decarboxylase activity that was stimulated not by putrescine but by magnesium ions (5).

It thus appears that the stimulation of adenosylmethionine decarboxylase activity by diamines is not a general property of eukaryotes but might be related to some other characteristic of a given organism.

In the present communication we have analyzed a number of eukaryotic and prokaryotic organisms for their capacity to accumulate and synthesize of polyamines in attempts to relate the dependence of adenosylmethionine decarboxylase on diamines or metals to other characteristics of the biosynthesis of polyamines in these organisms. It will be shown that those organisms possessing a putrescine-insensitive adenosylmethionine decarboxylase (protozoa and bacteria) contained only traces of spermine (derived from the culture media ?), and no spermine synthase activity was detected in the extracts of these organisms.This was in marked contrast to those organisms containing putrescine-activated adenosylmethionine decarboxylase (animal tissues and yeast) which contained appreciable amounts of spermine and were also able to synthesize spermine from spermidine and decarboxylated adenosylmethionine.

MATERIALS AND METHODS

Chemicals

Unlabelled S-adenosyl-L- methionine was synthesized enzymically by the method of Pegg and Williams-Ashman (4). Decarboxylated adenosylmethionine was prepared with the aid of adenosylmethionine decarboxylase partially purified (until ammonium sulphate fractionation) from cells of *E.coli* (8). The product of the enzymic decarboxylation was isolated from the incubation mixture with the aid of a Dowex-50 H^+ column and finally separated from adenosylmethionine by a preparative paper electrophoresis (11).

(1,4-^{14}C)Putrescine (specific radioactivity 17.5 mCi/mmole), (1,4-^{14}C)spermidine (sp. act. 12.4 mCi/mmole) and (carboxyl-^{14}C)S-adenosyl-L-methionine (sp. act. 60 mCi/mmole) were purchased from the New England Nuclear Corp. (Dreieichenhain, West-Germany). Radioactive putrescine was purified before use on a Dowex-50 H^+ column.

Organisms and growth conditions

E.coli (strain ATCC 4157) was grown aerobically at pH 7.0 in a medium containing 10 g of NaCl, 10 g of tryptone (Difco, Detroit, Mich.), 10 g of powdered yeast extract (Difco), 10 g of glucose and 2.5 g of K_2HPO_4 per liter. The cells were harvested at early stationary phase.

The culture coditions for *Tetrahymena pyriformis* and *Pseudomonas aeruginosa* have been described earlier (10,12).

Cells of baker's yeast (*Saccharomyces cerevisiae*) were obtained from Oy Alko (Helsinki, Finland).

Preparation of cell and tissue extracts

Acetone-dried cell powder from *E.coli* and *Ps.aeruginosa* was prepared essentially as described by Ellfolk and Soininen (12). The acetone-treated cells were extracted with 6 volumes of 25 mM potassium phosphate buffer (pH 7.1) containing 0.1 mM EDTA and 1 mM dithiothreitol (standard buffer) at 4° C for 2 h with continuous stirring. After stirring the homogenates were centrifuged at 15000 x g for 15 min, dialyzed overnight and the dialyzed supernatant fractions were used as the source of the enzymes.

Cells of baker's yeast were disintegrated by the method of Pösö *et al.* (7), and those of *Tetrahymena pyriformis* ultrasonically. The resultant homogenates were centrifuged and dialyzed as above.

The livers of fish (pike perch, *Lucioperca sandra*, obtained from local grocery) and rat were homogenized with a Potter-Elvehjem homogenizer with two volumes of the standard buffer. Human prostates (obtained from forensic autopsies) were homogenized with an Ultra Turrax homogenizer. The tissue homogenates were centrifuged and dialyzed as above.

Analytical methods

The activity of adenosylmethionine decarboxylase was assayed in the absence or presence of 2.5 mM putrescine or 10 mM $MgCl_2$ essentially as described earlier (13). The activity of spermidine synthase (EC 2.5.1.16) was assayed in the presence of radioactive putrescine and unlabelled decarboxylated adenosylmethionine (14). The activity of spermine synthase was assayed in the presence of radioactive spermidine and unlabelled decarboxylated adenosylmethionine by the method of Hannonen *et al.* (15).

Protein was measured by the method of Lowry *et al.* (16) and polyamines directly from the homogenates by the method of Raina and Cohen (17). Before the electrophoresis the polyamine samples were hydrolyzed in 6 M HCl for 12 h at 105° C.

RESULTS AND DISCUSSION

As shown in Table 1, adenosylmethionine decarboxylase from various organisms could apparently be divided to three distinct types: (i) putrescine-activated enzymes which did not require any metals (animal tissue and yeast), (ii) putrescine-insensitive enzyme requiring no metals either (*Tetrahymena*) and (iii) putrescine-insensitive enzymes from bacteria showing stringent requirement for magnesium ions.

The stimulation by putrescine (Table 1) varied in different organisms, i.e. from about 2-fold in the livers to more than 26-fold in the yeast. There was no chance in the activity of the protozoan adenosylmethionine

TABLE 1. Effect of putrescine or $MgCl_2$ on the activity of adenosylmethionine decarboxylase from various organisms. The enzyme activity was assayed in the presence of putrescine (2.5 mM) or $MgCl_2$ (10 mM) using dialyzed extracts as the source of enzyme. The enzyme activities are expressed as pmoles of formed CO_2 per mg. protein per 30 min.

Source of the enzyme	Adenosylmethionine decarboxylase activity				
	No additions (a)	Putrescine (b)	b/a	$MgCl_2$ (c)	c/a
Mammalia					
Human prostate	72	270	3.8	61	0.84
Rat liver	52	94	1.8	39	0.75
Osteichthyes					
Fish liver (*Luciopérca sándra*)	13	26	2.0	10	0.76
Yeast					
Saccharomyces cerevisiae	220	5900	26.5	193	0.88
Protozoa					
Tetrahymena pyriformis	130	130	1.0	99	0.77
Bacteria					
E. coli	130	130	1.0	4170	32.6
Ps. aeruginosa	260	260	1.0	6300	23.8

decarboxylase in the presence of 2.5 mM putrescine, and, if anything, 10 mM $MgCl_2$ tended to inhibit the reaction (Table 1). In agreement with earlier reports (4,5,8) adenosylmethionine decarboxylase from *E. coli* was completely insensitive to putrescine whereas an addition of magnesium ions did stimulate the enzyme activity by more than 30-fold. As also

TABLE 2. The concentrations of putrescine, (Pu), spermidine, (Spd) and spermine, (Sp) in various organisms.

Organisms	Polyamine concentration (nmoles/g wet weight)			Molar ratios		
	Putrescine	Spermidine	Spermine	Pu/Spd	Pu/Sp	Spd/Sp
Mammalia						
Human prostate	200	100	3700	2.0	0.05	0.03
Rat liver	100	880	770	0.11	0.13	1.1
Osteichthyes						
Fish liver (*Luciopérca sándra*)	320	420	240	0.76	1.30	1.8
Yeast						
Saccharomyces cerevisiae	30	140	90	0.21	0.30	1.6
Protozoa						
Tetrahymena pyriformis	190	180	n.d.[1]	1.1	-	-
Bacteria						
E. coli	5200	680	50	7.7	104	13.6
Ps. aeruginosa	5000	180	traces[2]	28	-	-

[1]not detected

[2]A spot was seen in electropherograms, however, the concentration was not high enough to permit quantitative analyses.

seen in Table 1, another gram-negative bacterium *Pseudomonas aeruginosa* also exhibited putrescine-insensitive adenosylmethionine decarboxylase activity that was almost absolutely dependent on the presence of $MgCl_2$.

Regardless of the different properties of the adenosylmethionine decarboxylases they all appear to be closely connected with the synthesis of spermidine and spermine (1,5,10).

We next analyzed the same organisms for their polyamine contents. As

TABLE 3. The activity of spermidine and spermine synthase in extracts of various organisms. The enzyme activities were assayed as described in the text. The enzyme activities are expressed as nmoles of polyamine formed per mg. protein per 30 min.

Organism	Spermidine synthase	Spermine synthase
Mammalia		
Human prostate	0.047	22.4
Rat liver	0.73	0.33
Osteichthyes		
Fish liver (*Luciopérca sándra*)	0.47	0.58
Yeast		
Saccharomyces cerevisiae	1.74	10.9
Protozoa		
Tetrahymena pyriformis	0.56	0.00
Bacteria		
E.coli	0.99	0.00
Ps.aeruginosa	0.45	0.00

shown in Table 2, there were two different polyamine patterns among the organisms investigated: (i) animal tissues and yeast contained all three polyamines (putrescine, spermidine and spermine), and (ii) protozoan and bacteria containing putrescine and spermidine, but had either none or only traces of spermine. Even the small amount of spermine found, for instance in the cells of *E.coli*, might have been derived from the culture medium (containing yeast extract among other things) since an active transport system for spermine has been described in *E.coli* (18).

It thus appears that those organisms possessing a putrescine-insensitive adenosylmethionine decarboxylase do not contain or only contain traces of spermine. This idea is supported by a recent observation of Mitchell

and Rush (9) who reported that the lower eukaryote *Physarum polycephalum* exhibited adenosylmethionine decarboxylase activity that was not stimulated by putrescine and was clearly inhibited by metals. Interestingly, only traces of spermine were found in this organism (9).

Also in agreement with the present results of Weller *et al.* (19) found that the cells of *Tetrahymena pyriformis* contained very little spermine as compared with putrescine and spermidine. In fact, they reported that putrescine and spermidine together constituted 95% of the total polyamines bound to the ribosomes of this protozoan (19).

The synthesis of higher polyamines (spermidine and spermine) is accomplished by at least three different proteins: adenosylmethionine decarboxylase and two separable propylamine transferases transferring the propylamine moiety of decarboxylated adenosylmethionine to either putrescine to yield spermidine (spermidine synthase) or to spermidine to yield spermine (spermine synthase) (14,15,20,21). As shown in Table 3, all organisms analyzed contained spermidine synthase activity. However, a measurable spermine synthase activity was only found in mammalian tissues and yeast, i,e. in those organisms possessing a putrescine-activated adenosylmethionine decarboxylase. No spermine synthase activity whatsoever could be detected in extracts of the protozoa and bacteria.

The rationale of the connection of putrescine-activation of adenosylmethionine decarboxylase with the organism's capacity to accumulate and synthesize spermine is not clear. However, it is obvious that both propylamine transferases (spermidine and spermine synthases) are competing for the common substrate decarboxylated adenosylmethionine. The activation of adenosylmethionine decarboxylase by putrescine would simply assure that sufficient amounts of decarboxylated adenosylmethionine will be channeled to the synthesis of spermidine always when the concentration of putrescine increases. It also known that putrescine not only activates adenosylmethionine decarboxylase but also powerfully inhibits the synthesis of spermine by spermine synthase in mammalian tissues (15,22). This kind regulatory role of putrescine apparently prefers the synthesis of spermidine over that of spermine in organisms possessing both spermidine and spermine synthase activities. On the contrary, in those organisms which do not contain any spermine synthase activity, there would be no need for this kind of preferential channeling of decarboxylated adenosylmethionine formed since it is only (?) used for the synthesis of spermidine.

To strengthen this hypothesis some gram-positive bacteria (*Lactobacilli*), known to contain both spermidine and spermine (23), were also analyzed for their adenosylmethionine decarboxylase activity. However, in agreement with

the results obtained with *Micrococcus* (5) we found that these organisms contained such a low adenosylmethionine decarboxylase activity that did not allow any conclusions of the possible regulation of the enzyme activity by diamines or metals.

Realizing that the material presented is relatively small and apparently needs to be substantiated, we wish to suggest that the activation of adenosylmethionine decarboxylase by putrescine in higher organisms can conceivably be an evolutionary change associated with the appearance of a specific spermine synthase.

ACKNOWLEDGEMENT

The competent technical assistance of Mrs. Soja Antin is gratefully acknowledged.

REFERENCES

1. Tabor, H. and Tabor, C.W. (1972) Advances in Enzymology, 36, 203-268.
2. Russell, D.H. and Lombardini, J.B. (1971) Biochim. Biophys. Acta, 240, 273-285.
3. Hannonen, P., Raina, A. and Jänne, J. (1972) Biochim. Biophys. Acta, 273, 84-90.
4. Pegg, A.E. and Williams-Ashman, H.G. (1969) J. Biol. Chem, 244, 682-693.
5. Coppoc, G.L., Kallio, P. and Williams-Ashman, H.G. (1971) Int. J. Biochem, 2, 673-681.
6. Hannonen, P. (1975) Acta Chem. Scand, B 29, 295-299.
7. Pösö, H., Sinervirta, R. and Jänne, J. (1975) Biochem. J, 151, 67-73.
8. Wickner, R.B., Tabor, C.W. and Tabor, H. (1970) J. Biol. Chem, 245, 2132-2139.
9. Mitchell, J.L.A. and Rusch, H.P. (1973) Biochim. Biophys. Acta, 297, 503-516.
10. Pösö, H., Sinervirta, R., Himberg, J.-J. and Jänne, J. (1975) Acta Chem. Scand, in the press.
11. Jänne, J., Williams-Ashman, H.G. and Schenone, A. (1971) Biochem. Biophys. Res. Commun, 43, 1362-1370.
12. Ellfolk, N. and Soininen, R. (1970) Acta Chem. Scand, 24, 2126-2136.
13. Jänne, J. and Williams-Ashman, H.G. (1971) Biochem. Biophys. Res. Commun, 42, 222-229.
14. Jänne, J., Schenone, A. and Williams-Ashman, H.G. (1971) Biochem. Biophys. Res. Commun, 42, 758-764.
15. Hannonen, P., Jänne, J. and Raina, A. (1972) Biochim. Biophys. Acta, 289, 225-231.
16. Lowry, O.H., Rosebrough, H.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem, 193, 265-275.
17. Raina, A. and Cohen, S.S. (1966) Proc. Natl. Acad. Sci. U.S, 55, 1587-1593.
18. Tabor, C.W. and Tabor, H. (1966) J. Biol. Chem, 241, 3714-3727.
19. Weller, D.L., Raina, A. and Johnstone, D.B. (1968) Biochim. Biophys. Acta, 157, 558-565.
20. Tabor, C.W. (1962) Methods in Enzymology, 5, pp. 761-765, Academic Press, New York.
21. Raina, A. and Hannonen, P. (1971) FEBS Lett, 16, 1-4.
22. Pegg, A.E. and Williams-Ashman, H.G. (1970) Arch. Biochem. Biophys, 137, 156-165.
23. Guirard, B.M. and Snell, E.E. (1964) J. Bacteriol, 88, 72-80.