carrier in the course of the transition between B and C are always in an ATP (or ADP) bound form is consistent with the concept that, in the exchange of ADP or ATP across the mitochondrial membrane, the AdN carrier is always bound with either ADP or ATP.

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Acetylation of Decarboxylated *S*-Adenosylmethionine by Mammalian Cells†

Anthony E. Pegg,* Rita S. Wechter, Richard S. Clark, Laurie Wiest, and Bradley G. Erwin

Department of Physiology and Specialized Cancer Research Center, The Milton S. Hershey Medical Center, The Pennsylvania State University, Hershey, Pennsylvania 17033

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ABSTRACT: Decarboxylated *S*-adenosylmethionine was found to be a substrate for the nuclear acetyltransferases that act on polyamines and on histones. The rate of acetylation of decarboxylated *S*-adenosylmethionine was more than twice that of spermidine at saturating substrate concentrations, and decarboxylated *S*-adenosylmethionine was an active inhibitor of the acetylation of histones by nuclear extracts from rat liver. The acetylation of decarboxylated *S*-adenosylmethionine occurred in vivo in SV-3T3 cells exposed to the ornithine decarboxylase inhibitor 2-(difluoromethyl)ornithine. The decline in putrescine and spermidine brought about by exposure to 2-(difluoromethyl)ornithine was found to be accompanied by a large rise in the content of both decarboxylated *S*-adenosylmethionine and acetylated decarboxylated *S*-adenosylmethionine. These results indicate that decarboxylated *S*-adenosylmethionine is metabolized not only in the well-known reactions in which it serves as an aminopropyl donor for polyamine biosynthesis but also by acetylation in reaction with acetyl coenzyme A. Furthermore, the inhibition of histone acetylation by decarboxylated *S*-adenosylmethionine could contribute to the biological effects brought about by inhibitors of ornithine decarboxylase.

An important role of *S*-adenosylmethionine is to serve as a precursor for polyamine biosynthesis [see reviews by Pegg & McCann (1982) and Tabor & Tabor (1984a) and references cited therein]. In order to serve as a donor of the aminopropyl groups in spermidine and spermine, *S*-adenosylmethionine must first be decarboxylated. Decarboxylated *S*-adenosylmethionine appears to be irreversibly committed to polyamine biosynthesis since it is very poorly if at all active as a substrate for methyltransferases (Zappia et al., 1969; Borchardt et al., 1976; Pegg, 1984a). *S*-Adenosylmethionine decarboxylase is a highly regulated enzyme and is a key step in polyamine biosynthesis (Pegg & Hibasami, 1979; Pegg, 1984a; Tabor & Tabor, 1984b). The cellular content of decarboxylated *S*-adenosylmethionine is normally very low, amounting to only 1 or 2% of the level of *S*-adenosylmethionine itself (Hibasami et al., 1980; Wagner et al., 1982). However, when the synthesis of putrescine and spermidine (which act as aminopropyl acceptors in the polyamine biosynthetic reactions) is prevented by the use of inhibitors of ornithine decarboxylase, there is a several hundred fold rise in the content of decarboxylated *S*-adenosylmethionine (Pegg et al., 1982; Mamont et al., 1982; Wagner et al., 1982; Pegg, 1984b). This rise strongly suggests that polyamine biosynthesis is indeed a major pathway of further metabolism of decarboxylated

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* Correspondence should be addressed to this author.

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S-adenosylmethionine; but it is not ruled out by these experiments that other metabolic routes exist. Such other routes could play important roles in the regulation of polyamine synthesis and cellular growth by limiting the availability of decarboxylated *S*-adenosylmethionine for the aminopropyltransferases.

Our laboratory has recently purified and characterized a cytosolic spermidine/spermine N^1-acetyltransferase (Matsui et al., 1981; Della Ragione & Pegg, 1982; Erwin et al., 1984). This enzyme is very highly inducible by a variety of stimuli including excess polyamines (Matsui et al., 1981; Persson & Pegg, 1984). It appears to catalyze the first step in polyamine degradation, and its induction brings about a decline in polyamine content (Matsui et al., 1981; Seiler et al., 1981, 1985). The inducible spermidine/spermine N^1-acetyltransferase acts on a variety of synthetic and naturally occurring amines but has an absolute requirement for aminopropyl groups (Della Ragione & Pegg, 1982, 1983). These observations raised the possibility that this enzyme might utilize decarboxylated *S*-adenosylmethionine as a substrate and in this way reinforce its actions in degrading polyamines by blocking their biosynthesis.

Experiments were carried out to test this hypothesis, but it was found that the inducible cytosolic acetyltransferase did not act at all on decarboxylated *S*-adenosylmethionine. However, it was found that the nuclear acetyltransferase, which is known to acetylate both histones and polyamines (Blankenship & Walle, 1977; Libby, 1978, 1980; Belikoff et al., 1980; Sures & Gallwitz, 1980; Garcea & Alberts, 1980; Wiktorowicz et al., 1981; Cullis et al., 1982; Wong & Wong, 1983), was active on decarboxylated *S*-adenosylmethionine. The resulting acetylated derivative was also found to accumulate in cells that had a high content of decarboxylated *S*-adenosylmethionine owing to the presence of the ornithine decarboxylase inhibitor 2-(difluoromethyl)ornithine.

Materials and Methods

Materials. [1-^{14}C]Acetyl-CoA (56.5 Ci/mol) was purchased from New England Nuclear, Boston, MA. ^{35}S-Labeled decarboxylated *S*-adenosylmethionine was prepared from [^{35}S]methionine (1050 Ci/mol, purchased from Amersham/Searle, Arlington Heights, IL) with *Escherichia coli* *S*-adenosylmethionine synthetase (Markham et al., 1983) and *E. coli* *S*-adenosylmethionine decarboxylase (Pegg, 1983). The *S*-adenosylmethionine synthetase was a generous gift from Dr. G. D. Markham, Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, PA. Unlabeled *S*-adenosylmethionine was prepared as previously described (Pegg, 1983), and a second preparation was generously provided by Dr. K. Samejima, Faculty of Pharmaceutical Sciences, Josai University, Saitama, Japan. *N*-[2-(*S*-Coenzyme A)acetyl]-*sym*-norspermidine amide (CNSA) was synthesized as previously described (Erwin et al., 1984). All other biochemicals including calf thymus histones (Sigma preparation IIA) were obtained from Sigma Chemical Co., St. Louis, MO.

Cell Culture and Determination of Nucleoside Content. Mouse SV-3T3 cells were grown as previously described (Pegg, 1984b) in the presence or absence of 5 mM 2-(difluoromethyl)ornithine for 96 h. The cells were harvested and extracted with 0.2 N perchloric acid in a total of 0.3 mL/mg of protein in the cell pellets. After centrifugation to remove protein, aliquots of the supernatant fraction were used directly for the analysis of *S*-adenosylmethionine and derivatives by HPLC using an ion-pair reversed-phase separation essentially as described by Seiler (1983). The aliquots (0.25 mL) were mixed with 0.45 mL of buffer A (0.1 M sodium acetate, 0.01 M sodium octanesulfonate, pH 4.5), centrifuged, and applied to a column (Beckman Ultrasphere ODS, 5 μm; 4.6 mm × 25 cm protected by a 4.6 mm × 4 cm guard column of ODS-5S from Bio-Rad) equilibrated with a mixture of 90% buffer A and 10% buffer B (10 parts 0.2 M sodium acetate and 0.01 M sodium octanesulfonate, pH 4.5, and 3 parts acetonitrile). The column was then eluted with a linear gradient of 90% buffer A/10% buffer B to 100% buffer B over 40 min at a flow rate of 1 mL/min at 37 °C. The eluate was monitored at 254 nm, and the amounts of *S*-adenosylmethionine and decarboxylated *S*-adenosylmethionine were calculated from the peak heights with standard curves constructed with known amounts of the authentic compounds. No marker for acetylated decarboxylated *S*-adenosylmethionine was available so the content was calculated by using the standard curve for *S*-adenosylmethionine since the peak was quite close to *S*-adenosylmethionine and had an identical height to width ratio. The results were expressed as nanomoles of the nucleosides present per milligram of total protein in the cell extracts. The column was washed with buffer B for a further 10 min and returned to 90% buffer A/10% buffer B over 5 min and reequilibrated with this buffer for a further 10 min before the next sample.

Assay of Acetyltransferase Activity. The standard assay for measurement of the acetylation of spermidine, histones, or decarboxylated *S*-adenosylmethionine contained the relevant substrate at the concentration indicated, 10 μmol of Tris-HCl [tris(hydroxymethyl)aminomethane hydrochloride], pH 7.8, 0.8 nmol (40 nCi) of [1-^{14}C]acetyl-CoA, and the enzyme extract in a total volume of 0.1 mL. After incubation at 30 °C for 10 min or the time indicated, the incorporation of radioactivity into material that bound to cellulose phosphate disks was measured as previously described (Della Ragione et al., 1982; Erwin et al., 1983). In experiments where the acetylation of histones in the presence of decarboxylated *S*-adenosylmethionine was determined, an assay method based on the procedure of Fukushima et al. (1980) was used. The reaction was stopped by the addition of 1.8 mL of 20% (w/v) trichloroacetic acid. The tubes were heated in a boiling water bath for 3 min, and the radioactive precipitate was collected on 0.22 μm Millipore filters. The filters were washed 3 times with 20% (w/v) trichloroacetic acid and 2 times with 95% ethanol, dried, and counted for radioactivity (Fukushima et al., 1980). It was confirmed that the acetylated decarboxylated *S*-adenosylmethionine was not retained under these conditions. The acetylation of decarboxylated *S*-adenosylmethionine in the presence of histones was measured by counting the fractions corresponding to acetylated decarboxylated *S*-adenosylmethionine isolated by HPLC on Partisil SCX as described below.

Crude rat liver cytosolic extracts containing polyamine acetylating activity were prepared as described by Matsui et al. (1981) from control, untreated rat livers and from the livers of rats treated 6 h before death with 1.5 mL/kg doses of CCl_4. Homogeneous preparations of inducible spermidine/spermine N^1-acetyltransferase were isolated as described by Della Ragione & Pegg (1982). Rat liver nuclear histone/polyamine acetyltransferase was prepared as described by Erwin et al. (1984). Antibodies to inducible spermidine/spermine N^1-acetyltransferase were prepared and used for immunoprecipitation of this enzyme as described by Persson & Pegg (1984). Protein was determined by the method of Bradford (1976).

Chromatographic Characterization of Acetylated Decarboxylated S-Adenosylmethionine. The reaction product was isolated by two different HPLC methods after three different

Table I: Acetylation of Decarboxylated *S*-Adenosylmethionine by Rat Liver Extracts[a]

source of acetyltransferase	addition to assay	acetylation of substrate shown (cpm × 10^{-3})	
		spermidine	decarboxylated *S*-adenosylmethionine
liver cytosol after CCl_4 induction of acetylase	none	2.37 (100%)	0.21 (100%)
liver cytosol after CCl_4 induction of acetylase	+ antiserum to inducible spermidine/spermine N^1-acetyltransferase	0.18 (8%)	0.20 (95%)
liver cytosol after CCl_4 induction of acetylase	0.5 μM CNSA	0.24 (10%)	0.11 (55%)
homogeneous spermidine/spermine N^1-acetyltransferase	none	2.26	0
liver chromatin extract	none	0.52	1.13
liver cytosol	none	0.12	0.23

[a] Spermidine or decarboxylated *S*-adenosylmethionine were added at 1 mM and the samples incubated for 10 min at 30 °C in the presence of 8 μM [1-^{14}C]acetyl-CoA, 100 mM Tris-HCl, pH 7.8, and the extract shown (approximately 0.1 mg of protein except for the homogeneous enzyme). Acetylation was then measured by the cellulose phosphate disk binding assay.

assays in which the labeled substrate was varied. In the first assay, the assay mixtures contained 40 μM [1-^{14}C]acetyl-CoA and 1 mM unlabeled decarboxylated *S*-adenosylmethionine. The reaction was halted and deproteinized by the addition of 70% (w/v) perchloric acid to give a final concentration of 4%. The protein was removed by centrifugation, and the reaction products were analyzed either by reversed-phase ion-pair HPLC as described above or by HPLC on a Partisil 10 SCX column (4.6 mm × 25 cm) that was equilibrated and eluted isocratically with 0.3 M ammonium formate, pH 4.0, containing 10% (v/v) acetonitrile. The column was operated at 40 °C and a flow rate of 2 mL/min. Fractions of 4 mL were collected and assayed for radioactivity after the addition of 10 mL of scintillation fluid ACS II (Amersham/Searle). The second assay mixtures contained unlabeled 40 μM acetyl-CoA and 0.1 mM ^{35}S-labeled decarboxylated *S*-adenosylmethionine and was analyzed by chromatography on Partisil SCX as above. Finally, the reaction mixture was scaled up 20-fold and unlabeled acetyl-CoA and decarboxylated *S*-adenosylmethionine were used. In this case, the product was detected after HPLC by monitoring the absorbance of the column effluent at 254 nm.

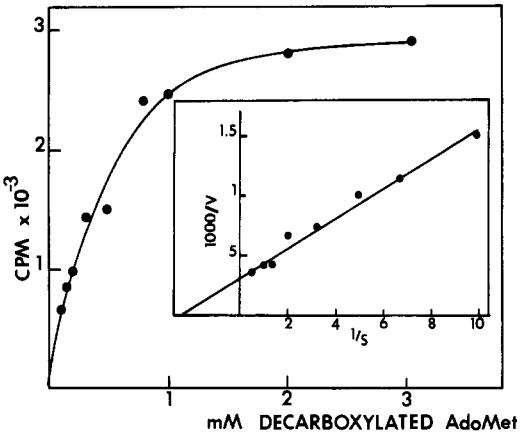

FIGURE 1: Determination of K_m for decarboxylated *S*-adenosylmethionine. The reaction was carried out with the rat liver nuclear acetyltransferase preparation and an incubation time of 10 min at 30 °C. The concentration of decarboxylated *S*-adenosylmethionine was varied as shown, and the amount of radioactivity incorporated from [1-^{14}C]acetyl-CoA into material that binds to cellulose phosphate disks was measured.

Results

When crude cytosolic rat liver extracts that are known to contain spermidine acetylating activity (Matsui et al., 1981) were prepared and incubated with [1-^{14}C]acetyl-CoA and spermidine, radioactivity was incorporated into material that binds to cellulose phosphate disks and was previously shown to be monoacetylspermidine. When decarboxylated *S*-adenosylmethionine was used as a potential acetyl acceptor instead of spermidine, it was found that acetylation also occurred with this substrate (Table I). However, this incorporation was not due to the CCl_4-inducible spermidine/spermine N^1-acetyltransferase because (a) treatment of the extracts with a specific antiserum to this protein inhibited the acetylation of spermidine by more than 90% but had no effect on the acetylation of decarboxylated *S*-adenosylmethionine, (b) addition of CNSA, which is a potent inhibitor of inducible spermidine/spermine N^1-acetyltransferase (Erwin et al., 1984), inhibited acetylation of spermidine by 90% but inhibited the acetylation of decarboxylated *S*-adenosylmethionine by only 45%, and (c), when homogeneous spermidine/spermine N^1-acetyltransferase was substituted for the liver extract, there was no reaction with decarboxylated *S*-adenosylmethionine (Table I). Furthermore, the capacity of crude rat liver extracts to acetylate decarboxylated *S*-adenosylmethionine was not changed by prior treatment of the rats with CCl_4 whereas the capacity to acetylate spermidine was greatly increased (Table I). These results suggested that decarboxylated *S*-adenosylmethionine might be a substrate for the histone acetylase which, although predominantly nuclear, is present in the crude cytosolic rat liver extracts and is known to also act on polyamines including spermidine (Blankenship & Walle, 1977; Libby, 1978, 1980; Cullis et al., 1982; Erwin et al., 1984). Therefore, a chromatin extract containing this activity was prepared and tested. As shown in Table I, this extract was approximately twice as active with decarboxylated *S*-adenosylmethionine as a substrate than with spermidine.

More detailed studies of the acetylation of decarboxylated *S*-adenosylmethionine were then carried out with the chromatin extract. Under the standard assay conditions, the acetylation was proportional to the amount of protein added within the range 0.1–0.5 mg of protein and to the time of incubation for up to 20 min (results not shown). The apparent K_m for decarboxylated *S*-adenosylmethionine was approximately 0.4 mM when assayed in the presence of 8 μM acetyl-CoA (Figure 1).

The experiments described above were carried out by using the capacity to bind to cellulose phosphate disks as a means to demonstrate the acetylation. In order to characterize the acetylation products more fully, the reaction mixture was deproteinized and separated by HPLC on a cation-exchange medium (Figure 2, lower panel). A new radioactive peak was present in the samples in which decarboxylated *S*-adenosylmethionine was added. This peak eluted at about 6 min and

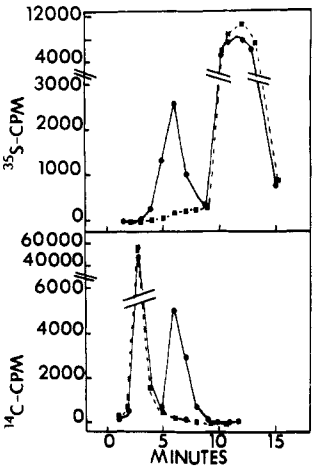

FIGURE 2: Isolation of acetylated decarboxylated *S*-adenosylmethionine by HPLC on Partisil SCX. In all cases, the assay mixture contained the rat liver nuclear acetyltransferase and was incubated for 20 min at 30 °C. At this time, the reaction mixture was halted by the addition of perchloric acid, protein removed by centrifugation, and the supernatant subjected to HPLC analysis on the cation-exchanger Partisil SCX as described under Materials and Methods. The elution time of acetyl-CoA was about 3 min, and the elution time of decarboxylated *S*-adenosylmethionine was 11 min. In the experiment shown in the upper panel, the assay mixture contained 0.1 mM ^{35}S-labeled decarboxylated *S*-adenosylmethionine plus 40 μM acetyl-CoA (●) or minus acetyl-CoA (■). In the experiment shown in the lower panel, the assay mixture contained 40 μM [1-^{14}C]acetyl-CoA plus 1 mM decarboxylated *S*-adenosylmethionine (●) or minus decarboxylated *S*-adenosylmethionine (■).

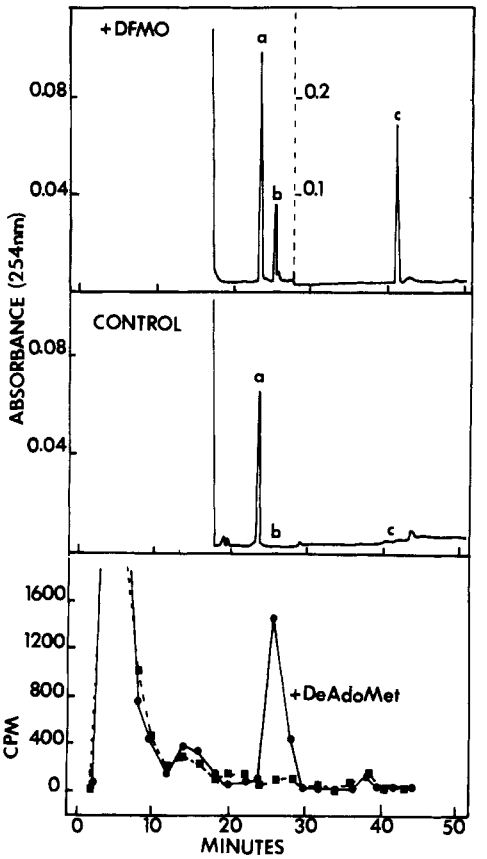

FIGURE 3: Isolation of acetylated decarboxylated *S*-adenosylmethionine by reversed-phase ion-paired HPLC. Samples were isolated from SV-3T3 cells grown in the absence (middle panel; CONTROL) or presence of 2-(difluoromethyl)ornithine (upper panel; +DFMO) and deproteinized and subjected to HPLC on a Beckman Ultrasphere ODS 5-μm column in the presence of sodium octanesulfonate as described under Experimental Procedures. The eluate was monitored for absorbance at 254 nm as shown. Note that the scale was altered from the full range of 0.2 to 0.5 in the upper panel at the time shown by the dotted line. This change was needed to prevent the large peak of decarboxylated *S*-adenosylmethionine from being off scale. The retention times were 23.2 min for *S*-adenosylmethionine, 25.8 min for acetylated decarboxylated *S*-adenosylmethionine, and 41 min for decarboxylated *S*-adenosylmethionine. In the experiment shown in the lower panel, aliquots of the same assay mixture as used for the lower panel of Figure 2 were used, and the eluate was assayed for radioactivity as shown. The assay mixture contained 40 μM [1-^{14}C]acetyl-CoA plus 1 mM decarboxylated *S*-adenosylmethionine (●) or minus decarboxylated *S*-adenosylmethionine (■).

was well separated from acetyl-CoA, which was eluted at 3 min, and from decarboxylated S-adenosylmethionine, which eluted at about 11 min. When the reaction was carried out with unlabeled acetyl-CoA and ^{35}S-labeled decarboxylated *S*-adenosylmethionine as substrates, there was a product peak eluting in the same region as found for the [^{14}C]acetylated product corresponding to 6 min that was well resolved and ahead of the decarboxylated *S*-adenosylmethionine (Figure 2, lower panel). Similarly, when the reaction mixture was scaled up with unlabeled substrates so that 0.8 nmol of product was expected, the product peak could be recognized from the UV absorbance at 254 nm (results not shown). The product produced in the standard assay was also readily separated by reversed-phase HPLC (Figure 3). In this case, the peak corresponded to a retention time of 25.8 min whereas the labeled acetyl-CoA was all eluted prior to 20 min and decarboxylated *S*-adenosylmethionine was eluted at 41 min (Figure 3, lower panel).

The most probable explanation for the results described above is that decarboxylated *S*-adenosylmethionine is acetylated by the nuclear histone acetylase, but it was also possible that a separate chromatin-bound enzyme was responsible. The following experiments suggest that the acetylation is carried out by the histone acetylase. First, as shown in Table II, decarboxylated *S*-adenosylmethionine was an effective inhibitor of the acetylation of histones. (This experiment was carried out with an assay for histone acetylation in which the proteins were precipitated with 20% trichloroacetic acid and acetylated decarboxylated *S*-adenosylmethionine remains soluble under these conditions.) When 2 mg/mL calf thymus histone was used as a substrate, 1 mM decarboxylated *S*-adenosylmethionine inhibited histone acetylation by 32%, and complete inhibition was observed with 6 mM decarboxylated *S*-adenosylmethionine (Table II). Second, histone inhibited the production of acetylated decarboxylated *S*-adenosylmethionine, which was measured by HPLC as in Figure 2

Table II: Inhibition of Histone Acetylation by Decarboxylated *S*-Adenosylmethionine[a]

concn of decarboxylated *S*-adenosylmethionine (mM)	histone acetylation (cpm × 10^{-3})
none	1.47 ± 0.26
0.5	1.11 ± 0.07
1.0	0.99 ± 0.10
3.0	0.57 ± 0.10
6.0	0.01

[a] In this experiment only the acetylation of added histone (2 mg/mL) was measured with precipitation with 20% trichloroacetic acid for precipitation of the [^{14}C]acetylhistones.

(results not shown). Finally, when an assay procedure was used in which both histone acetylation and acetylation of decarboxylated *S*-adenosylmethionine could be determined, the total incorporation was not additive (Table III).

In order to test whether decarboxylated *S*-adenosylmethionine was acetylated under physiological conditions, transformed mouse fibroblasts (SV-3T3 cells) were grown for

Table III: Acetylation of Histones and Decarboxylated *S*-Adenosylmethionine by Chromatin Extracts[a]

substrate added	acetylation (cpm × 10^{-3})	expected (cpm × 10^{-3})
1 mM decarboxylated *S*-adenosylmethionine	2.2	
0.2 mg of histone	3.7	
1 mM decarboxylated *S*-adenosylmethionine + 0.2 mg of histone	4.0	5.8
0.1 mg of histone	1.5	
1 mM decarboxylated *S*-adenosylmethionine + 0.1 mg of histone	2.8	3.6

[a] Assays were carried out as described in Table I with a total assay volume of 0.1 mL containing the potential substrate indicated. The acetylation was measured by the cellulose phosphate disk binding assay omitting the centrifugation step to ensure that all histone acetylation was measured. The third column indicates the cpm expected if the acetylation of the two substrates was independent of each other and would, therefore, be additive.

Table IV: Accumulation of Acetylated Decarboxylated *S*-Adenosylmethionine in SV-3T3 Cells Treated with 2-(Difluoromethyl)ornithine for 96 h[a]

	content (nmol/mg of protein)	
nucleoside	control cells	2-(difluoromethyl)-ornithine-treated cells
S-adenosylmethionine	0.9 ± 0.3	1.5 ± 0.1
decarboxylated *S*-adenosylmethionine	<0.01	3.2 ± 0.2
acetylated decarboxylated *S*-adenosylmethionine	<0.01	0.5 ± 0.1

[a] The nucleoside content was determined by HPLC as shown in Figure 3. Results are shown as the mean ± SD for at least four measurements on each condition.

4 days in the presence of the ornithine decarboxylase inhibitor 2-(difluoromethyl)ornithine. Such treatment is known to greatly increase the cellular content of decarboxylated *S*-adenosylmethionine (Mamont et al., 1982; Pegg et al., 1982; Wagner et al., 1982; Pegg, 1984). The cells were then extracted with perchloric acid and aliquots of the deproteinized supernatants subjected to reversed-phase HPLC in the presence of the ion-pair sodium octanesulfonate. As shown in Figure 3, upper panel, there was a well-resolved peak (designated as b in Figure 3) that corresponded to acetylated decarboxylated *S*-adenosylmethionine and was well separated from *S*-adenosylmethionine itself (peak a) and decarboxylated *S*-adenosylmethionine (peak c). In extracts from the same amount of control cells, only the peak corresponding to *S*-adenosylmethionine was visible (Figure 3, middle panel). Quantitation of these results is shown in Table IV. There was a small increase in the content of *S*-adenosylmethionine in the cells treated with 2-(difluoromethyl)ornithine and a very large increase in decarboxylated *S*-adenosylmethionine and its acetylated derivative. The decarboxylated *S*-adenosylmethionine amounted to 214% of the *S*-adenosylmethionine, and the acetylated decarboxylated *S*-adenosylmethionine amounted to 31%.

Discussion

The material that accumulates in cells treated with 2-(difluoromethyl)ornithine that we have identified as acetylated decarboxylated *S*-adenosylmethionine is almost certainly the same as the unknown peak "X" that was reported by Wagner et al. (1982) to be present in HTC cells in a variety of mammalian tissues after exposure to this drug. The reversed-phase HPLC system that we have used is based on the method of Seiler (1983) and is very similar to that used by Wagner et al. (1982). Their unknown peak was eluted just after *S*-adenosylmethionine, which corresponds well with the peak that we have identified as acetylated decarboxylated *S*-adenosylmethionine. It is interesting that the total of *S*-adenosylmethionine, decarboxylated *S*-adenosylmethionine, and acetylated decarboxylated *S*-adenosylmethionine is increased by at least 5.5-fold in cells treated with 2-(difluoromethyl)ornithine. There is also an increase in the total adenine nucleotide pool in such cells (Heby et al., 1984) so there is no suggestion that the accumulation of the decarboxylated *S*-adenosylmethionine derivatives acts as an adenine trap. However, it is possible that a substantial amount of cellular methionine becomes tied up in this way.

Our results provide the first evidence for the metabolism of decarboxylated *S*-adenosylmethionine by reactions other than those involved in polyamine metabolism. Although Zappia et al. (1969) have observed that decarboxylated *S*-adenosylmethionine can take part in certain methyltransfer reactions in vitro, the K_m was much higher than that of the normal *S*-adenosylmethionine substrate, and there was no proof that this reaction actually occurred in vivo. In several other methyltransferase reactions decarboxylated *S*-adenosylmethionine was completely inactive (Zappia et al., 1969; Borchardt, 1976). Since we have observed a substantial buildup of acetylated decarboxylated *S*-adenosylmethionine in cells in which polyamine biosynthesis is blocked by 2-(difluoromethyl)ornithine (Table IV), it is clear that acetylation can take place in the cell.

It should be emphasized that such treatment with 2-(difluoromethyl)ornithine leads to a several hundred-fold increase in the concentration of decarboxylated *S*-adenosylmethionine and that in normal cells the concentration of this nucleoside is very low. For example, in rat liver the content of decarboxylated *S*-adenosylmethionine is about 1.6 nmol/g wet weight (Hibasami et al., 1980). It is therefore improbable that decarboxylated *S*-adenosylmethionine interferes with the acetylation of histones in normal cells. However, in cells that have been treated with an ornithine decarboxylase inhibitor the enormous increase in the content of decarboxylated *S*-adenosylmethionine results in concentrations that are quite likely to affect histone acetylation. To some extent this effect may be obviated by the decline in spermidine, which is also a substrate and inhibitor of histone acetylation by the nuclear acetylase (Blankenship & Walle, 1977; Libby, 1978, 1980; Cullis et al., 1982; Dod et al., 1982), but our results indicate that decarboxylated *S*-adenosylmethionine is a preferred substrate and its accumulation would therefore be expected to exert a predominant effect.

2-(Difluoromethyl)ornithine has striking effects on growth and in some cases differentiation of mammalian cells (Mamont et al., 1978, 1982; Heby, 1981; Pegg & McCann, 1982; Porter & Bergeron, 1983; Pegg, 1984). It has already been pointed out that some of these effects might be influenced not only by the depletion of putrescine and spermidine in the treated cells but also by virtue of the accumulation of decarboxylated *S*-adenosylmethionine (Pegg et al., 1982; Pegg, 1984b). This accumulation could result in the inhibition of certain methylation reactions, and the present results raise the possibility that it could also influence the acetylation of histones. Factors influencing histone acetylation are known to have profound effects on gene expression (Ruiz-Carrillo et al., 1975; Yamamoto & Alberts, 1976; Chalkley & Shires, 1985).

At present, it is not easy to ascertain whether the acetylation of decarboxylated *S*-adenosylmethionine also occurs in normal

cells. The concentration of decarboxylated *S*-adenosylmethionine is so low in these cells that its measurement is quite difficult and the same difficulties apply to the detection of the acetylated derivative. Although the normal intracellular level of decarboxylated *S*-adenosylmethionine is well below the K_m for the acetyltransferase, it is still possible that this reaction occurs and removes some of the cellular decarboxylated *S*-adenosylmethionine. If this is the case, any changes in the activity of the chromatin acetyltransferase activity could influence polyamine biosynthesis since the supply of decarboxylated *S*-adenosylmethionine is the limiting factor in this process (Pegg & Hibasami, 1979). The further metabolic fate of the acetylated decarboxylated *S*-adenosylmethionine is not known and deserves further study particularly with reference to the possibility that it could be used as an aminopropyltransferase substrate to yield acetylated polyamine directly.

Registry No. Decarboxylated *S*-adenosylmethionine, 22365-13-5; 2-(difluoromethyl)ornithine, 70052-12-9; acetyltransferase, 9012-30-0; spermidine, 124-20-9; histone acetylase, 9054-51-7.

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