Biochimica et Biophysica Acta, 971 (1988) 38–45
Elsevier

BBA 12303

S-Adenosylmethionine metabolism in HL-60 cells: effect of cell cycle and differentiation

Peter Chiba, Christina Wallner and Erich Kaiser

Department of Medical Chemistry, University of Vienna, Vienna (Austria)

(Received 29 December 1987)

Key words: *S*-Adenosyl-L-methionine; Cell cycle; Differentiation; (HL-60 cells)

The effect of the cell cycle and differentiation on *S*-adenosylmethionine (SAM) metabolism in HL-60 cells has been investigated. Synthesis and pool sizes of SAM and *S*-adenosylhomocysteine (SAH) were cell-cycle-independent (SAM, 315 μM; SAH, 4.6 μM). The SAM-synthase (ATP:L-methionine *S*-adenosyltransferase) of HL-60 cells has a K_m for methionine of 12.8 ± 2.0 μM and thus appears to be of the intermediate K_m type found in other malignant tissues. The enzyme does not show cell-cycle regulation. Treatment of cells with DMSO resulted in a rapid and marked decrease of SAM and SAH levels without affecting pool turnover or the SAM/SAH ratio. A decrease in SAM concentration could also be observed in a variant cell line resistant to differentiation with DMSO. DMSO inhibited SAM-synthase in cell-free extracts. This inhibition was noncompetitive with respect to L-methionine. Inhibition of SAM-synthase by cycloleucine lowered SAM levels in intact cells, but resulted in differentiation of only a minor percentage of cells. These data indicate that changes in SAM and SAH levels in HL-60 cells seem to be a consequence rather than a cause of differentiation.

Introduction

S-adenosylmethionine (SAM) is a key metabolite for cellular methylation reactions and polyamine synthesis, which have been implicated in the control of cell proliferation and differentiation.

Alterations in SAM biosynthesis have been found in most tumor cell lines, which, unlike their normal counterparts, show methionine-dependent growth [1–3]. As a result, these cells cannot be grown in culture media in which methionine is replaced by its immediate precursor, L-homocysteine. This metabolic peculiarity is found despite a normal methionine synthesis in cancer cells, and seems to correlate with the cellular *S*-adenosylmethionine/*S*-adenosylhomocysteine (SAM/SAH) ratio, whereby cells with a ratio of not greater than 1 are almost always found to be methionine dependent. It has been postulated that this difference in methionine metabolism might provide a basis for selective chemotherapy [4].

Changes in the pool sizes and especially in the ratio of SAM/SAH have been reported to influence cellular methylation reactions. Substances that interfere with these reactions could be shown to evoke differentiation in several tumor cell lines, including the HL-60 line [5–9].

Besides its direct effects on cellular methylation reactions, the ratio of SAM/SAH could also be

Abbreviations: SAM, *S*-adenosylmethionine; SAH, *S*-adenosylhomocysteine; SAM-synthase, *S*-adenosylmethionine synthase (EC 2.5.1.6); 1) DMSO, dimethylsulfoxide; Tes, 2-{[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]amino}ethanesulfonic acid.

Correspondence: P. Chiba, Department of Medical Chemistry, University of Vienna, Waehringerstraße 10, A-1090, Vienna, Austria.

shown to play a role in protein phosphorylation [10].

In addition, an altered polyamine metabolism has been found in several human leukemia cell lines [11], and a role of polyamines in HL-60 differentiation has been established [12]. Taken together, these data indicate that SAM and SAH concentrations and especially the SAM/SAH ratio might have a variety of biological effects that ultimately lead to cell differentiation.

The regulation of SAM and SAH metabolism during cell growth is still not completely understood. Furthermore, a clear role of *S*-adenosylamino acid metabolism in differentiation has not been established.

This paper presents data characterizing SAM metabolism with respect to the cell cycle and cell differentiation. Cells used in these studies were the human promyelocytic cell line, HL-60, as well as a variant, HL-60 R. Unlike the wild type, this variant is incapable of granulocytoid differentiation when treated with dimethylsulfoxide (DMSO) or retinoic acid.

Materials and Methods

Cell lines and culture. The HL-60 cell line was obtained from Dr. R.C. Gallo (NCI, NIH, Bethesda, MD.). The HL-60 R line was established as a subclone that spontaneously lost its potential to differentiate in the presence of DMSO or retinoic acid [13–15]. Cells were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum and gentamycin (25 μg/ml) in a humidified atmosphere containing 5% CO_2. The average generation time of both lines was about 30 h. Cultures were regularly checked for mycoplasma infection (Mycotect, Gibco, Grand Island, NY) and found to be uninfected.

Induction and assessment of differentiation. Cells were either seeded in normal medium or medium containing 1.5% DMSO (tissue culture grade, Sigma, St. Louis, MO). Functional maturity of the cells was assessed by their ability to reduce the dye Nitroblue tetrazolium as described by Yen and Albright [16].

Centrifugal elutriation. Separation of randomly growing cells into different cell-cycle phases was performed as previously described [14]. Flow cytometry was used to determine the cell-cycle distribution of each fraction [17].

Incorporation studies. Cells were seeded at a density of $0.5 \cdot 10^6$/ml. [^{35}S]Methionine (800 Ci/mmol, Amersham International, Amersham, U.K.) was added at 2 μCi/ml of culture medium. The concentration of unlabeled methionine in the culture medium was 100 μM. Cells were incubated at 37°C for the times indicated, acid-extracted with 0.5 M perchloric acid ($(1.0–1.5) \cdot 10^7$ cells/100 μl) and centrifuged to pellet acid-precipitable material. Acidic supernatants were stored in liquid nitrogen, since neutralization resulted in considerable degradation of *S*-adenosylamino acids. Acid-soluble metabolites were analyzed by means of an HPLC method described below. Incorporation of radiolabel into acid-precipitable material was determined according to the method of Schmidt and Thannhauser [18].

For pulse-chase experiments, cells were labeled with [^{35}S]methionine at 37°C for 1 h, by which time steady-state levels of labeling in SAM and SAH pools had been reached (Fig. 1). After 1 h, cells were chilled, spun down and resuspended in medium containing 100 μM unlabeled methionine. SAM and SAH levels were determined at various times after reseeding, as described below.

For determination of the effect of cycloleucine on SAM and SAH pools, cells were preincubated in medium containing various concentrations of cycloleucine. After 3 h, [^{35}S]methionine was added and cells were incubated for an additional 3 h. All incubations were carried out at 37°C. Sample processing was as described below.

DNA synthesis was measured by incubating cells with [6-^{3}H]thymidine (20 Ci/mmol, Amersham International), 2 μCi/ml of culture medium) for 30 min. Incorporation of radiolabel into acid-precipitable material was determined [18].

HPLC separation and quantitation of SAM and SAH. SAM and SAH were separated on a reversed-phase column (Varian MCH 10, C18). Buffer A was acetonitrile and buffer B, 50 mM Na_2HPO_4 (pH 3.2), containing 10 mM octanesulfonic acid as the ion-pair reagent. The column temperature was 35°C and the flow was 1.0 ml/min. Buffer A was increased from 5 to 13% in 20 min followed by an increase from 13 to 40%

buffer A in the next 15 min. Absorbance was detected at 254 nm. The retention time for SAH was 25 min, and for SAM, 29 min. The SAM peak could be completely digested under mild alkaline conditions and the ultraviolet spectrum was identical to that of authentic standard. The amounts of SAM and SAH were quantitated by peak integration of HPLC profiles and on basis of specific activity of extracellular methionine pools. Both methods yielded virtually identical values. Pools were quantitated routinely, from radioactivity associated with the SAM or SAH peak in HPLC separations.

Assay of ATP: L-methionine S-adenosyltransferase. The assay was performed according to the method of Kotb and Kredich [19]. Cells were lysed by three cycles of freezing and thawing and centrifuged at $100\,000 \times g$ for 1 h. The assay mix contained 80 mM Tes (pH 7.4), 50 mM KCl, 40 mM $MgCl_2$, 0.5 mM EDTA, 6 mM ATP, 10 μM L-[*methyl*-^{14}C]methionine (40–55 mCi/ml, Amersham International), varying concentrations of unlabeled methionine and 0.1–1.0 U of enzyme in a total volume of 50 μl. Methionine and SAM were separated by batch elution on Whatman P81 paper. Radiolabeled product was quantitated by liquid scintillation counting in a Packard CA 2000 LSC. The assay was linear up to 5% substrate conversion.

Determination of cell volume. Water-accessible space was determined as described by Wohlhueter et al. [20].

Results

In the first experiments, SAM and SAH levels as well as turnover of the SAM and SAH pools were determined. Steady-state levels of incorporation of radiolabel from [^{35}S]methionine into SAM pools were reached after approx. 40 min (Fig. 1). SAM levels were 189 ± 9 pmol/10^6 cells. Cell volume was determined to be 0.6 μl/10^6 cells. The SAM concentration could then be calculated to be 315 ± 15 μM.

SAH levels were 4.6 ± 0.3 pmol/10^6 cells, which corresponds with a concentration of 7.7 ± 0.5 μM. The SAM/SAH ratio in these cells is therefore approx. 40.

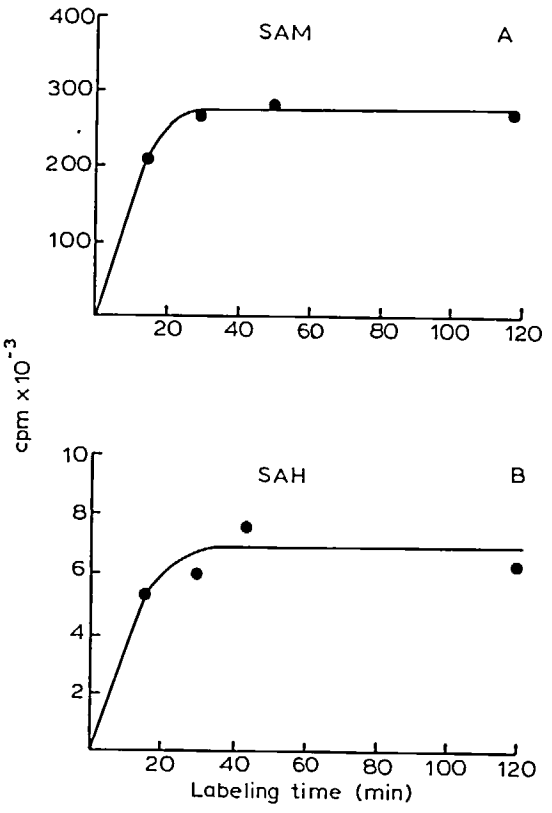

Fig. 1. Incorporation of radiolabel from [^{35}S]methionine into (A) SAM and (B) SAH pools. Experimental conditions were as described in Materials and Methods. For each time point, $1.6 \cdot 10^7$ cells were analyzed.

Pulse-chase experiments showed that the first-order rate constant for the consumption of SAM was 0.071. SAM was therefore utilized at a rate of 13.5 pmol/min per 10^6 cells (0.48 nmol/mg protein per h).

DMSO-induced changes in SAM and SAH levels in differentiating HL-60 cells

In some experiments, HL-60 cells were treated with DMSO, a potent terminal inducer of granulocytoid differentiation [21]. SAM and SAH pools were determined at early time points following addition of the inducer to the culture medium. Specific activity and turnover of SAM and SAH pools were the same in induced and uninduced cells. However, a gradual, time-dependent and coordinate decrease in SAM and SAH levels was observed. The SAM/SAH ratio stayed essentially constant (Table I). Concentrations of both

TABLE I

CHANGES IN SAM AND SAH LEVELS OF HL-60 CELLS AT DIFFERENT TIMES AFTER INDUCTION WITH 1.5% DMSO

Cells were incubated in the presence of 1.5% (v/v) DMSO for the times indicated. SAM and SAH level were determined by means of an HPLC method described in Materials and Methods. Concentrations at individual time points represent the average of triplicate determinations.

Time of induction (h):	0	3	6	9	12	18
SAM (pmol/10^6 cells)	180	170	164	144	142	113
SAH (pmol/10^6 cells)	4.6	4.5	3.4	3.5	3.5	2.8
SAM/SAH ratio	39	37	48	41	41	40

metabolites dropped by about 10% after 3 and by approx. 40% after 18 h. Since SAM and SAH levels dropped coordinately, the trivial explanation of a decreased methionine utilization from the medium had to be ruled out. A comparison of the first-order rate constants of [^{35}S]methionine uptake into induced and uninduced cultures was performed and could be shown to be identical (data not shown).

SAM is generated from ATP and L-methionine by SAM-synthase. Inhibition of this enzyme by DMSO could be responsible for the decrease in

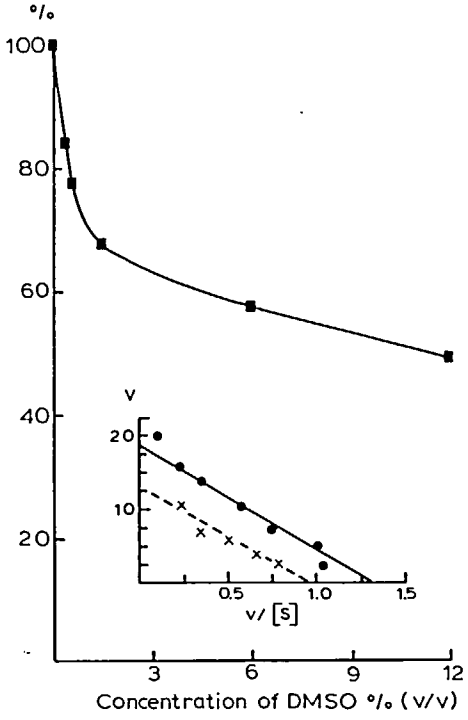

Fig. 2. Inhibition of ATP:L-methionine *S*-adenosyltransferase by various concentrations of DMSO. V_{max} values at different concentrations of DMSO are given as a percentage of the maximal velocity determined in the absence of inhibitor. Maximal velocities were determined in the V vs. $V/[S]$ plot by linear least-square fitting of data points obtained at different concentrations of L-methionine. A comparison of K_m values in the presence and absence of 1.5% DMSO is shown in the inset. The K_m for methionine was not altered by the inhibitor (noncompetitive inhibition). Velocity (V) is given in nmol/mg prot per h, substrate concentration in μmol/l. The K_m for methionine was 12.8 ± 2.0 μM.

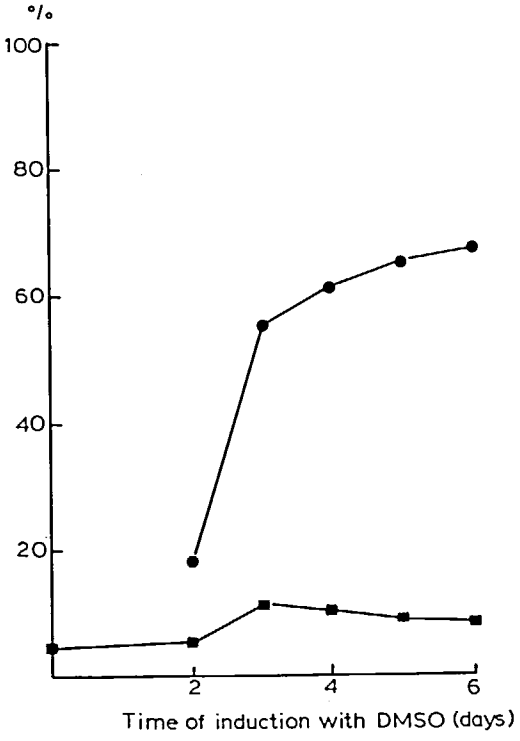

Fig. 3. Differentiation of HL-60 (●) and HL-60 R cells (■). Functional maturity was assessed as described in Materials and Methods.

TABLE II

SAM AND SAH LEVELS IN HL-60 AND HL-60 R CELLS BEFORE AND 18 h AFTER THE ADDITION OF DMSO

Data from two separate experiments are presented. Data represents counts per min recovered in the respective peak of HPLC separations, as described in detail in Materials and Methods.

	Expt. 1				Expt. 2			
	SAM		SAH		SAM		SAH	
	cpm	%	cpm	%	cpm	%	cpm	%
HL-60	173290	100	5730	100	172373	100	5153	100
HL-60 + DMSO	107440	62	3667	64	103423	60	3098	60
HL-60 R	141645	100	3544	100	144768	100	3233	100
HL-60 R + DMSO	96328	68	4785	135	102401	70	4697	145

SAM levels. Therefore, we investigated the effect of DMSO on this enzyme in cell-free extracts (Fig. 2). Concentration-dependent inhibition by DMSO could be shown. This inhibition was noncompetitive with respect to L-methionine (insert Fig. 2). At the DMSO concentration used for induction of differentiation, activity was decreased by approx. 30% (Fig. 2). 100% of control activity could be recovered in dialyzed extracts from cells treated with 1.5% DMSO for 18 h, showing that the decrease in activity was not due to an altered turnover of the enzyme protein.

DMSO-induced changes in SAM and SAH levels in a resistant cell line HL-60 R

If changes in *S*-adenosylamino acid metabolism were to play a major role in differentiation of HL-60 cells, these changes should not be observed in a cell line resistant to differentiation. Fig. 3 shows the percentage of differentiation of HL-60 cells and in a variant cell line resistant to DMSO-induced differentiation (HL-60 R). Less than 10% of the L-60 R cells showed maturation after being cultured in the presence of DMSO for 6 days, while approx. 65% of the cells scored positive in wild-type cultures.

DMSO was shown to decrease SAM levels in sensitive and resistant cells. SAH levels, however, increased in the resistant cell line, while they decreased in wild-type cells (Table II).

SAM and SAH levels in different cell-cycle phases

Cytokinetic effects of the inducer could be responsible for the observed effect of DMSO on

TABLE III

SAM AND SAH LEVELS IN DIFFERENT CELL-CYCLE PHASES

Randomly growing cells were separated into fractions enriched in different cell-cycle phases by means of centrifugal elutriation. Fraction 1 represents essentially pure G1-phase cells and fraction 7, mainly G2-phase cells. The intermediate fractions contain a varying percentage of S-phase cells. Cell-cycle distribution in fraction 4 was approx. 70% S-phase cells and 30% G2-phase cells. [6-^{3}H]Thymidine incorporation into DNA and [^{35}S]methionine incorporation into protein were determined as described in Materials and Methods.

Fraction No.	Incorporation					
	cpm/10^6 cells		pmol/10^6 cells		pmol/mg protein	
	[6-^{3}H]thd	[^{35}S]met	SAM	SAH	SAM	SAH
1	1670	29366	97	2.5	9.5	0.24
2	8124	38388	166	3.9	12.8	0.30
3	24066	46974	180	4.4	12.7	0.31
4	49800	51181	173	4.3	12.2	0.31
5	42819	53214	183	4.7	11.6	0.30
6	24929	54655	216	5.5	12.4	0.32
7	18267	57140	216	4.8	12.6	0.29

SAM and SAH pools if levels of these metabolites were regulated by the cell cycle. We therefore determined SAM and SAH levels in the different cell-cycle phases. Randomly growing cells were separated into fractions enriched in cells of different cell-cycle phase by means of centrifugal elutriation. Individual fractions were labeled with [^{35}S]methionine for 2 h or with [6-^{3}H]thymidine for 30 min. Results are summarized in Table III. Fraction 1 (98% G0/G1-phase cells) showed the lowest thymidine incorporation. The gradual increase in thymidine incorporation from fractions 1–4 was due to the increasing percentage of cells in S phase and a decreasing number of cells in G1 phase. Fraction 4 was essentially free of G1-phase cells and contained approx. 70% S-phase and 30% G2-phase cells. The opposite ratio was found in fraction 7. Incorporation of label from [^{35}S]methionine into protein increased from fraction 1–7 and paralleled the increase in cell volume and protein content per cell. SAM and SAH levels remained essentially constant when expressed on a per mg protein basis. Since cell volume and protein content increased, progressively higher levels were calculated when normalized to cell number. This experiment shows that SAM and SAH concentrations, and as a consequence the SAM/SAH ratio, are cell-cycle-independent. Therefore, the changes in pool sizes observed during differentiation could not be due to cytokinetic effects caused by the inducer.

Spontaneously differentiating cells constitute about 5% of randomly growing cells. Because of their small size, these cells are enriched in fraction 1. This explains the slightly lower SAM and SAH levels in this fraction.

SAM-synthase was shown to be expressed in a cell-cycle-independent manner. The V_{max} was 21 $\pm$ 1.5 nmol/mg protein per h. The k_m for methionine was 12.8 $\pm$ 2.0 μM (see inset, Fig. 2).

Differentiation of HL-60 cells by inhibitors of SAM-synthase

Cycloleucine, an inhibitor of SAM-synthase, was tested for its differentiating potential in HL-60 cells. The ID_{50} for inhibition of SAM-synthase in cell-free extracts was determined to be approx. 1.2 mM (Table IV). Correspondingly, a sustained, concentration-dependent decrease of SAM levels was observed when intact cells were treated with cycloleucine. This decrease was reflected by diminished SAM peak areas and by a reduction of [^{35}S]methionine incorporation into the SAM pool. Treatment of cells with 10 mM cycloleucine lead to a reduction of SAM levels equaling that observed after treatment of cells with 1.5% DMSO for 18 h. The effect on SAH levels was less pronounced. The percentage of differentiating cells at various concentrations of cycloleucine is shown in Table IV. Concentrations in excess of 10 mM led to a considerable decrease in cell viability after 6 days, and could therefore not be used in these

TABLE IV

EFFECT OF CYCLOLEUCINE ON HL-60 CELLS

Effects of cycloleucine on SAM-synthase activity in HL-60 extracts, and on SAM and SAH levels in intact cells after treatment for 6 h. SAM levels are given as relative area units (RAU) per 10^6 cells and as cpm per 10^6 cells incorporated into the SAM pool from [^{35}S]methionine. Differentiation was assessed as the ability of cells to reduce the dye Nitroblue tetrazolium (NBT) on day 6. Results are given as means $\pm$ S.D. of four determinations. Experimental procedures were as described in Materials and Methods. n.d., not determined.

Cycloleucine concn. (mM)	SAM-synthase (nmol/mg per h)	SAM (RAU/10^6 cells)	SAM (cpm/10^6 cells)	SAH (cpm/10^6 cells)	NBT-positive cells (%)
–	18.2 $\pm$ 1.0	894 $\pm$ 21	4605 $\pm$ 142	200 $\pm$ 17	4 $\pm$ 2
2.50	7.4 $\pm$ 0.4	709 $\pm$ 22	3100 $\pm$ 396	193 $\pm$ 14	8 $\pm$ 3
5.00	4.1 $\pm$ 0.8	647 $\pm$ 41	2868 $\pm$ 162	179 $\pm$ 13	10 $\pm$ 3
10.00	1.7 $\pm$ 0.7	576 $\pm$ 27	n.d.	n.d.	15 $\pm$ 4

experiments. Below 10 mM, cell viability was at least 90%. A small but dose-dependent increase in differentiating cells could be observed. The percentage of nitroblue-tetrazolium-positive cells, however, did not exceed 15%.

Discussion

SAM is a key metabolite for polyamine biosynthesis and represents the methyl donor for a great number of cellular methylation reactions. It has been implicated in the regulation of cell differentiation and in irregularities in methionine metabolism, which can be found in cancer cells, but not in normal tissue [1–3].

In HL-60 cells, SAM pools were not cell-cycle regulated. Cantoni proposed [22] that one way of regulating fluxes of SAM could be at the level of SAH. While SAH itself is not a branch-point metabolite, changes in intracellular SAH levels could determine the metabolic fate of SAM. Our data show that SAH levels are kept constant over the cell cycle. It thus appears that this mechanism of SAM-flux regulation is not operative during normal cell growth.

Different forms of SAM-synthase have been found to be present in normal extrahepatic tissues and in cancer cells, respectively [23]. SAM-synthase in HL-60 cells was found to have a K_m of 12.8 ± 2 μM for methionine, which is comparable to the K_m value found in other malignant tissues. The concentration of methionine in regular growth medium is 100 μM, and should therefore not be limiting for SAM synthesis.

An early and coordinate decrease in SAM and SAH pools could be detected in association with treatment of HL-60 cells with DMSO. The decrease in SAM levels could be explained by direct inhibition of SAM-synthase by DMSO. A decrease in the amount of enzyme protein was ruled out as the cause, since 100% of the enzyme activity was recovered in cultures treated with DMSO for 18 h.

SAM pools in human lymphoid cells have been found to be regulated in conjunction with turnover rate of the pools [24]. Increased turnover has thereby been found to be associated with enlargement of the SAM pool. In contrast, decreased turnover might have been the reason for decreased SAM pools in DMSO-treated cells. Our data, however, do not support this type of regulation in HL-60 cells.

Direct or indirect inhibitors of DNA methylation were shown to induce differentiation in these cells [6,8]. Since DMSO was found to interfere with SAM synthesis and, as a result, could affect methylation reactions, the influence of this inducer on SAM levels was assessed in a DMSO-sensitive and in a resistant cell line. Analogous changes were found in both lines. In addition, a specific inhibitor of SAM-synthase, cycloleucine, evoked differentiation in only a very small percentage of cells.

Furthermore, intracellular SAM levels (315 μM) are far in excess of the K_m values reported for DNA methyltransferase in human (HeLa cells, 3.25 μM) [25] and rodent cells (regenerating rat liver, 2.5 μM [26], mouse plasmocytoma cells, 1.2 μM [27]). A drop in SAM levels by at least an order of magnitude would be required to effect DNA methylation.

Since SAH acts as a potent inhibitor of cellular methylation reactions, effects of DMSO on SAH levels were also determined. Data showed a complete lack of correlation between SAH levels and HL-60 differentiation, since SAH levels decreased in the sensitive cell line, while they increased in the resistant cells, which is the exact opposite of what would have been expected if SAH levels played a role in DMSO-induced differentiation of HL-60 cells. One should, however, bear in mind, that the ratio of SAM/SAH in HL-60 cells is approx. 40 and a decrease to 2 was shown to be necessary to exert an influence on methylation reactions in human lymphoblastoid cells [28].

An alternative explanation for the differing metabolite pattern, found in resistant versus sensitive cells, would be that a low SAM/SAH ratio prevents differentiation. However, inhibitors of SAH-hydrolase, which cause an increase in SAH levels and thus a decrease in the SAM/SAH ratio, could be shown to lead to differentiation of HL-60 cells [29]. This means that an increase in SAH levels does not prevent differentiation of these cells. However, inhibition of SAH-hydrolase might not necessarily be the sole effect of these compounds and an alternative mode of action should be considered.

Taken together, our results strongly suggest that significant changes in SAM and SAH levels, or the SAM/SAH ratio, which can be detected in conjunction with DMSO-induced differentiation of HL-60 cells, are not causally related to the process of differentiation, but are of a secondary nature.

Acknowledgements

We thank Milos Nakicenovic for excellent technical assistance. We also want to thank J.G. Cory and R. Marz for helpful suggestions during the preparation of this manuscript. This investigation was supported by Research grant No. 396 from Medizinisch Wissenschaftlicher Fond des Bürgermeisters der Bundeshauptstadt Wien.

References

1 Coalson, D.W., Mecham, J.O., Stern, P.H. and Hoffman, R.M. (1982) Proc. Natl. Acad. Sci. USA 70, 4248–4251.
2 Kano, Y., Sakamoto, S., Kasahara, T., Kusumoto, K., Hida, K., Suda, K., Ozawa, K., Miura, Y. and Takaku, F. (1982) Cancer Res. 42, 3090–3092.
3 Stern, P.H., Wallace, C.D. and Hoffman, R.M. (1984) J. Cell Physiol. 119, 29–34.
4 Hoffman, R.M. (1984) Biochim. Biophys. Acta 738, 49–87.
5 Christman, J.K., Weich, N., Schoenbrun, B., Schneiderman, N. and Acs, G. (1980) J. Cell Biol. 86, 366–370.
6 Mendelsohn, N., Michl, J., Gilbert, H.S., Acs, G. and Christman, J.K. (1980) Cancer Res. 40, 3206–3210.
7 Creusot, F., Acs, G. and Christman, J.K. (1982) J. Biol. Chem. 257, 2041–2048.
8 Christman, J.K., Mendelsohn, N., Herzog, D. and Schneiderman, N. (1983) Cancer Res. 43, 763–768.
9 Sasaki, R. and Miura, Y. (1986) Int. J. Cell Cloning 4, 192–202.
10 Villalba, M., Varela, I., Merida, I., Pajares, M.A., Martinez del Poso, A. and Mato, J.M. (1985) Biochim. Biophys. Acta 847, 273–279.
11 Kamatani, N. and Carson, D.A. (1980) Cancer Res. 40, 4178–4182.
12 Kufe, D.W., Griffin, J., Mitchell, T. and Shafman, T. (1984) Cancer Res. 44, 4281–4284.
13 Leyva, A., Schwartsmann, G., Boeije, L.C.M., Pinedo, H.M. and de Waal, F. (1986) Biochem. Biophys. Res. Commun. 141, 629–635.
14 Schwartsmann, G., Pinedo, H.M. and Leyva, A. (1987) Eur. J. Cancer Clin. Oncol. 23, 739–743.
15 Schwartsmann, G., Madam, K., Heins, Y., Pinedo, H.M. and Leyva, A. (1987) Cell Biol. Int. Rep. 11, 651–655.
16 Yen, A. and Albright, K.L. (1984) Cancer Res. 44, 2511–2515.
17 Chiba, P., Plas, A., Wessels, J.M.C. and De Bruyn, C.H.M.M. (1984) Biosci. Rep. 4, 687–694.
18 Schmidt, G. and Thannhauser, S.J. (1945) J. Biol. Chem. 161, 83–89.
19 Kotb, M. and Kredich, N.M. (1985) J. Biol. Chem. 260, 3923–3930.
20 Wohlhueter, R.M., Marz, R., Graff, J.C. and Plageman, P.G.W. (1978) Methods Cell Biol. 20, 211–236.
21 Collins, S.J., Ruscetti, F.W., Gallagher, R.E. and Gallo, R.C. (1978) Proc. Natl. Acad. Sci. USA 75, 2458–2462.
22 Cantoni, G.L. (1986) in Biological Methylation and Drug Design (Borchardt, R.T., Creveling, C.R. and Ueland, P.M., eds.), pp. 227–238.
23 Liau, M.C., Chang, C.F. and Becker, F. (1979) Cancer Res. 39, 2113–2119.
24 German, D.C., Bloch, C.A. and Kredich, N.M. (1983) J. Biol. Chem. 258, 10997–11003.
25 Roy, P.H. and Weissbach, A. (1975) Nucleic Acids Res. 2, 1669–1684.
26 Simon, D., Grunert, F., V. Acken, U., Döring, H.P. and Kröger, H. (1978) Nucleic Acids Res. 5, 2153–2167.
27 Hitt, M.M., Tyzz-Liang, W., Chen, G. and Linn, S. (1988) J. Biol. Chem. 263, 4392–4399.
28 Hershfield, M.S., Kurtzberg, J., Chaffee, S. and Greenberg, M.L. (1985) in Biochemistry and Biology of DNA Methylation (Cantoni, G.L. and Razin, A., eds.), pp. 305–311, Alan R. Liss, New York.
29 Aarbakke, J., Miura, G.A., Prytz, P.S., Bessesen, A., Slordal, L., Gordon, R.K. and Chiang, P.K. (1986) Cancer Res. 46, 5469–5472.