COMPARATIVE EFFECT OF *S*-ADENOSYL-HOMOCYSTEINE (SAH) AND SINEFUNGIN ON tRNA-BASE METHYLATION IN WHOLE CELLS AND IN VITRO

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1. Introduction

S-Adenosyl-homocysteine (SAH) is one of the products and the physiological inhibitor of *S*-adenosyl-methionine (SAM) mediated transmethylases [1]. Sinefungin is an antifungal antibiotic isolated from cultures of *Streptomyces griseolus* [2,3]. The two molecules are closely related structurally, as Sinefungin differs from SAH only by the replacement of the sulfur atom by a CH–NH_2 group (fig.1). Both compounds inhibit various methylation reactions in vitro [4] but only Sinefungin inhibits Rous sarcoma virus (RSV) induced foci formation, SAH being inactive [5,6]. As we have observed, an increase in the concentration of methylated bases of tRNA parallels the transformation of chick embryo fibroblasts (CEF) infected by RSV [7]. Thus, this work was undertaken in order to find whether the two compounds have different effects on the methylation pattern of tRNA bases. Our results show that Sinefungin inhibits much stronger than SAH the monomethylation of guanine in position 2 and 7 and of adenine in position 1 and 6, which may suggest a role for these methylations in the transformation process.

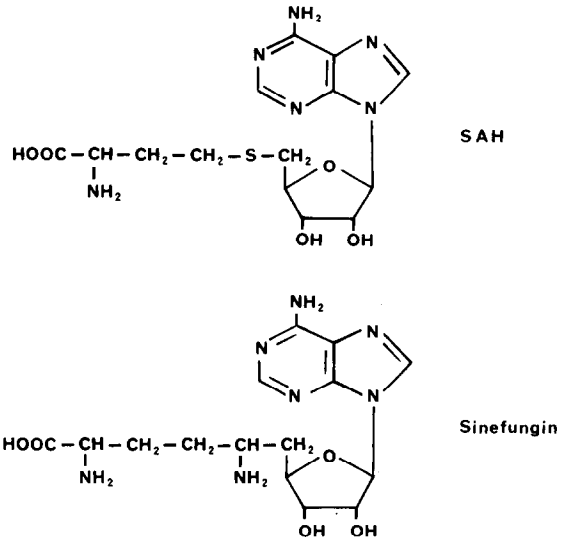

Fig.1. Structure of SAH and Sinefungin.

2. Materials and methods

Cells, viruses and media were as in [5]. For labelling experiments in whole cells, L-[*methyl*-^{14}C]methionine (30–50 mCi/mM) was purchased from the Commissariat à l'Energie Atomique, Saclay. The conditions were as in [7]. tRNAs were extracted from a 12 000 × *g* supernatant of labelled cells. SAH and Sinefungin were added to the medium at 500 μM.

The methylation of a mixture of *E. coli* K12 tRNAs (supplied by Mr Escaut, ICSN, CNRS, Gif-sur-Yvette) in vitro was performed as in [7] with *S*-adenosyl L-[*methyl*-^{14}C]methionine (10^{-4} M) (45 mCi/mM) purchased from the same source. tRNA was 0.5 mg/ml. To the incubation mixture were added 100 μM SAH and 30 μM Sinefungin, inhibiting by ~50% the overall methylation of the tRNAs.

In both cases, tRNAs were extracted by phenol–SDS and precipitated with ethanol. The quantity of tRNA was estimated by the orcinol method [8]. After hydrolysis in 80% formic acid at 170°C for 90 min, the methylated bases of tRNA were analyzed by bidimensional thin-layer chromatography on cellulose sheets (Eastman-Kodak) according to [9]. Standard methylated bases (Sigma, St Louis) were used as markers.

Protein concentration was determined in cell extracts as in [10] with crystalline bovine serum albumin as standard.

3. Results

Table 1 shows the distribution of the methyl group incorporation in the different methylated bases in whole cells and in vitro. Results are expressed as % of the total radioactivity.

3.1. *Effect of SAH and Sinefungin on the methylation of tRNAs in normal and in RSV-transformed CEF*

The percent of inhibition of the methyl group incorporation in whole cells by 0.5 mM SAH and Sinefungin are shown in table 2. The values have been calculated from the radioactivity recovered from different bases and are expressed in cpm/μg tRNA.

The inhibition by both compounds is somewhat stronger in normal than in transformed cells. The effect of SAH is generally weaker than that of Sinefungin as the inhibition produced by the former ranges from 20–40%, in both cell types. Sinefungin has a more selective effect. The strongest inhibition is observed on the formation of m^7G. The amounts of m^2G, m^1A and m^6A are diminished by 48–54% in tRNAs of transformed cells. Furthermore the methyl group incorporation in m^5C, m^5U, m^2G and m^3C is weakly or not reduced at all by this molecule.

3.2. *Effect of SAH and Sinefungin on methylation of* E. coli *K-12 tRNA in vitro*

A mixture of *E. coli* tRNA was methylated, respectively, by a normal and a RSV transformed cell-free extract to complete saturation. The same experiments under identical conditions were performed in the presence of either SAH or Sinefungin. The reaction was stopped after 120 min with buffer-saturated phenol.

Table 3 shows the % of inhibition by SAH and Sinefungin of methyl group incorporation into tRNA

Table 1
Repartition of the methyl group incorporation in the different bases in whole cells and in vitro

	In whole cells		In vitro	
	Normal cells	Transformed cells	Normal cells	Transformed cells
m^2G	16.4	17.9	53.2	57.4
m_2^2G	14.2	15.0	18.8	17.3
m^7G	8.9	8.2	4.0	2.0
m^1A	6.9	7.1	4.8	4.8
m^2A	0.1	0.1	0.6	0.3
m^6A	9.8	9.2	5.2	4.0
m_2^6A	0.5	0.2	0.6	0.3
m^5C	27.2	26.2	11.4	12.4
m^3C	4.1	4.0	0.4	0.3
m^5U	11.2	12.0	1.1	1.0

Results are expressed in % of total radioactivity

Table2
Effect of 0.5 mM SAH and Sinefungin on the methylation of tRNA in normal and RSV transformed CEF

	Normal cells		Transformed cells	
	% of inhibition		% of inhibition by	
	SAH	Sinefungin	SAH	Sinefungin
m^2G	38	62	22	48
m_2^2G	29	3	29	15
m^7G	42	95	23	94
m^1A	21	58	31	54
m^2A	–	–	–	–
m^6A	36	58	11	49
m_2^6A	0	37	36	26
m^5C	32	14	24	0
m^3C	37	+2	31	0
m^5U	39	8	4	9

Results are expressed as percent of inhibition of the methyl group incorporation in the different methylated bases. These values are calculated from 3 different experiments and the experimental error is estimated to ± 10%

Table 3
Methylation of *E. coli* K-12 tRNA by normal and RSV transformed CEF extracts

	Normal cells		Transformed cells	
	% of inhibition by		% of inhibition by	
	SAH	Sinefungin	SAH	Sinefungin
m^2G	26	78	10	84
m_2^2G	77	6	84	0
m^7G	+18	73	+30	72
m^1A	56	85	58	86
m^2A	33	66	50	67
m^6A	64	86	55	86
m_2^6A	66	33	66	66
m^5C	61	37	52	47
m^3C	0	0	66	33
m^5U	50	50	55	55

Results are given as percentage of inhibition of the methyl group incorporation in the presence of 100 μM and SAH and 30 μM Sinefungin. The values are the means of 3 different experiments and the experimental error is estimated to ± 10%

of *E. coli*. The results obtained with heterologue tRNAs are similar to those observed in whole cells. Sinefungin inhibits much more strongly than SAH the m^2G, m^7G and m^1A methyl transferases, while SAH but not Sinefungin is a very good inhibitor of m_2^2G methyl transferase. The extent of inhibition by the two substances is the same in the presence of the two different enzyme sources (normal and transformed cell-free extract).

4. Discussion

Most of the modified nucleosides have a specific location on the cloverleaf structure of tRNAs. In spite of the fact that the physiological role of these modified nucleosides is not yet fully understood, it seems that they confer to the tRNAs some structural characteristics essential for their function. Furthermore their presence brings about structural differences between isoaccepting tRNAs and by this means they may play a role in the regulation of the function of tRNAs [11,12].

A several-fold increase in the activity of tRNA methyl transferases in neoplastic tissues as compared to normal ones has been observed [13,14]. We have described that an increase in the concentration of methylated bases of tRNA parallels the transformation of CEF by RSV and also the fact that 5′-deoxy 5′-*S*-isobutyl thioadenosine (SIBA), an analogue which inhibits this increase, also inhibits cell transformation [7]. We showed also that SAH, the natural inhibitor of transmethylases does not affect cell transformation, whereas Sinefungin, a structurally closely related natural antibiotic, has very strong inhibitory effect on the same event [6]. The aim of this work was to compare the effect of the two compounds on tRNA methylation in whole cells and in vitro to understand whether their differential effect on cell transformation could be explained by their differential effect on tRNA–base methylation. The experiments show that Sinefungin but not SAH inhibits very strongly the methylation of G in position 2 and 7 and that of A in position 1 and 6. The modified nucleoside m^6A is generally found adjacent to the anticodon, for codons beginning with C and G. m^1A is located in the TψC loop and m^7G in the extra loop. m^2G is a minor modified base occurring only in tRNAs of eukaryotes. This base is located at the central part of the molecule which is involved in the interaction of tRNAs with the synthetases. According to Feldman's theory the role of these minor bases is to make temporary covalent bonds through methylene bridges with some components of the translation apparatus [12]. Further studies are needed to conclude in favour of a decisive role in the transformation process for the methylation of these four bases of tRNA.

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