SHORT COMMUNICATION

Differential Inhibitors of tRNA Methylases

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SUMMARY

The enzymes that methylate tRNA have qualitative and quantitative differences among different species. Inhibitors of these enzymes with three different specificities have been found: some restricted to bacterial enzymes, some to mammalian enzymes, and some with dual potencies.

Enzymes that methylate tRNA and complete the synthesis of this species of nucleic acid are ubiquitously distributed in all cells. The enzymes and their substrates are highly complex: in metazoa the enzymes are species-specific (1) and organ-specific (2); in bacterial extracts no fewer than six base specific enzymes have been identified (3).

While no definite function can as yet be assigned to the methyl groups present in tRNA, profound alterations in the methylating enzymes have been observed in several biological systems which are undergoing alterations in macromolecular synthesis. For example, we have observed *inter alia* that in lysogenic *Escherichia coli* K_{12}, when induced by ultraviolet light, an inhibitor of the tRNA methylases appears (4). The extracted product was found to be an especially effective inhibitor of the enzyme that methylates uracil at the polymer level in tRNA. However, the inhibitor proved inert against enzymes from mammalian sources. The root of this selectivity is known: while *E. coli* has a high capacity for the methylation of uracil, extracts of mammalian tissues have but marginal activity toward this base.[1] The finding of this specific inhibitor prompted a wider study because the differential inhibition of enzymes that alter the structure of a cardinal component of the protein-synthesizing apparatus of mammals and microorganisms may have both theoretical and practical relevance. In this pilot study we report that several products have such differential inhibitory potencies.

Adenosine has been reported by Hurwitz *et al.* to inhibit the guanine tRNA methylases of *E. coli* (5). We have confirmed and extended this observation (Tables 1 and 2).

[1] Analytical data on the content of methylated bases in tRNA are essentially in accord with *in vitro* enzyme activities. In *E. coli*, tRNA thymine is the major methylated component, and uracil tRNA methylase activity is very high. In extracts of mammalian tissues this enzyme activity is marginal and the level of thymine in the tRNA is very low. Dunn has found no thymine in tRNA of rat liver, and this base is the lowest of all methylated bases in tRNA of calf and pig liver. (D. B. Dunn, personal communication.)

TABLE 1
Effect of Adenine on the in Vitro Methylation of Guanine Residues in tRNA[a]

	Condition		
Log cells	cpm from ^{14}C in guanine (G)	cpm from ^{14}C in thymine (T)	G/T
1	2915	9490	0.30
2	1548	5280	0.29
3	1596	5150	0.31
4	1665	5740	0.29
+ Adenine, 415 μg/ml			
1	0	6715	0
2	126	4875	0.02

[a] Logarithmically growing cells of *E. coli* $K_{12}W_6$ were the source of the enzyme. The methylation was carried out with methyl-deficient tRNA as substrate and (methyl-^{14}C)*S*-adenosylmethionine as methyl donor. With each extract a pilot experiment was carried out to determine the saturation levels of enzyme extract (i.e., further addition of enzyme did not result in any significant change in total ^{14}C incorporated into RNA). The incubation mixture for the isolation of $^{14}CH_3$-tRNA contained the following per ml: 0.6 mg of methyl-deficient tRNA, 0.5 μC of (methyl-^{14}C)*S*-adenosylmethionine (specific activity 26–29.9 mC/mmole, Tris buffer (0.01 M, pH 8), $MgCl_2$ (0.01 M), mercaptoethanol (0.005 M), and sufficient enzyme extract to yield saturation level. For details of the isolation of the radioactive bases see (11). The reproducibility of replicate determinations at the same level of inhibitor is ±10%. The reproducibility of replicate analyses of base-specific enzyme activities in different batches of bacteria in logarithmic growth phase is also ±10%.

The data in Table 1 indicate that the methylation of guanine relative to that of uracil is substantially reduced in the presence of adenine in bacterial extracts. Table 2 shows that adenine, adenosine, and 7-deazaadenosine (tubercidin) are inhibitory to the tRNA methylases extracted from *E. coli*. There is some highly specific structural requirement for the inhibition. Neither adenylic acid nor the following purine and pyrimidine derivatives had significant inhibitory effects: inosine, 6-mercaptopurine, kinetin (6-furfurylamino adenine), N^6-methyladenosine, N^6-methyldeoxyadenosine, isopentenyladenosine, uridine, cytidine, thymidine, and guanosine.

Table 3 summarizes some of the studies with enzymes from mammalian sources. Adenine is just as effective an inhibitor of tRNA methylases of mammalian tissues as of the enzymes from bacterial origin. However, adenosine proved inert when added to mammalian extracts. The high specificity of the inhibitors is also apparent in the case of two analogues of adenosine, tubercidin and isopentenyl adenosine. The former is inhibitory toward both bacterial and mammalian enzymes, but the latter is active only in mammalian extracts. Thus we have at hand inhibitors with three different specificities: some restricted to bacterial enzymes, some restricted to mammalian enzymes, and some with dual potencies.

Partial inhibition of the tRNA methylases from mammalian sources by isopentenyl adenosine and by tubercidin is especially noteworthy since these compounds have been shown to be effective anti-tumor and cytotoxic agents (6, 7).

It has been reported (8) and confirmed (9) that the tRNA methylases from tumors of human and animal origin as well as from tumors in tissue culture (10) have altered specificities. Enzymes extracted from tumors have methylating capacities against a standard substrate several fold higher than extracts from normal tissue counterparts.

While nothing can be said at this time about the role the altered tRNA methylation may have in the etiology or the growth of tumors, the finding that two effective anti-tumor agents are inhibitors of the tRNA methylases invites a wider study of the inhibitors of these enzymes as possible anti-tumor agents.

Moreover, the existence of a specific inhibitor of the tRNA methylases of *E. coli* should encourage a search for other such agents that might selectivity interfere with the protein-synthesizing apparatus of pathogenic microorganisms. This is based on the assumption that the tRNA methylases of pathogenic organisms will prove species-specific. Experience with a wide

TABLE 2
Inhibition of Bacterial tRNA Methylases of E. coli by Various Purine and Pyrimidine Derivatives[a]

Expt.	Additions (μg/ml)		cpm incorporated into tRNA	% Inhibition
1	None		11,150	—
	Adenosine	50	9,425	15
		250	8,625	23
		500	6,840	39
2	None		8,350	—
	Adenine	140	6,200	26
		240	4,770	43
		340	4,360	48
3	None		2,900	—
	Adenine	500	1,550	47
	Adenosine	1120	1,705	42
	Adenylic acid	1400	2,828	3
4	None		1,395	—
	Adenosine	1120	935	33
	Inosine	1120	1,440	0
5	None		3,830	—
	Kinetin	800	3,725	3
6	None		2,900	—
	Isopentenyl adenosine	500	2,630	9
7	None		5,500	—
	6-Methyladenosine	500	6,070	0
	6-Methyldeoxyadenosine	500	5,510	0
8	None		10,310	—
	Adenine	240	6,600	36
	6-Mercaptopurine	250	10,300	0
9	None		8,200	—
	Uridine	750	7,950	3
	Cytidine	750	7,900	4
10	None		2,120	—
	Guanosine	300	2,250	0
	Cytidine	400	2,440	0
	Adenine	400	1,535	30
11	None		3,830	—
	Thymidine	500	4,010	0
12	None		4,410	—
	Tubercidin	390	3,380	24

[a] The incubation mixtures contained enzyme at half saturation level, 260 μg methyl-deficient tRNA, and the other components listed under Table 1 at proportionately reduced levels. After 30 minutes of incubation 1 mg of unlabeled tRNA and 1 mg of DL-methionine were added to each tube. Then 50% trichloroacetic acid was added to complete precipitation. The precipitate was dissolved in 0.2 M Tris and the RNA was precipitated with 10% trichloroacetic acid. The precipitation was repeated twice more. Finally, the washed precipitate was suspended in 2 M NH_4OH and was plancheted and counted. The values reported are the average of two incubations with a mean deviation of ±10%.

TABLE 3
Effect of Adenine and Some of Its Analogues on Mammalian tRNA Methylases

Expt.	Source of enzyme	Additions (μg/ml)		cpm incorporated into tRNA	% Inhibition
1	Liver	None		1535	—
		Adenine	500	900	40
2	Liver	None		965	—
		Isopentenyl adenosine	410	670	28
3	Liver	None		635	—
		Isopentenyl adenosine	410	445	30
4	Spleen	None		2280	—
		Tubercidin	340	1590	30
5	Liver	None		630	—
		Tubercidin	330	515	19
6	Spleen	None		2410	—
		Adenosine	460	2520	0
			930	2440	0
7	Liver	None		600	—
		6-Methyladenosine	500	570	5

[a] The organs originated from one calf. The incubation and isolation were carried out as in Table 2. Because of the high concentration of protein in mammalian extracts, no carrier tRNA was needed at the end of the incubation. The values reported are the average of two determinations with a mean deviation of ± 10%. The extent of inhibition is reproducible within ± 10% in incubations with the same organ; see Expts. 2 and 3 However, the extent of inhibition varies with different organs; see Expts. 4 and 5.

variety of organisms in several laboratories suggests that such species specificity can almost certainly be anticipated. The tRNA methylases may be components of host and parasite with qualitative differences on which therapy may be hinged.

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