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Evidence that the Inhibitory Effect of Adenosine, but not Cordycepin, on the Methylation of Nuclear RNA is Mediated by *S*-Adenosylhomocysteine Hydrolase

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SUMMARY

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The mechanism of action of adenosine, 2′-deoxyadenosine, and cordycepin (3′-deoxyadenosine) was explored in L1210 cells *in vitro* under conditions where their deamination was blocked by the adenosine deaminase inhibitor, 2′-deoxycoformycin. Cordycepin but not adenosine or 2′-deoxyadenosine inhibited the methylation of nuclear RNA in the presence of 2′-deoxycoformycin. Upon the addition of homocysteine, adenosine produced 65% inhibition of methylation of nuclear RNA, whereas 2′-deoxyadenosine was ineffective and the inhibitory effect of cordycepin was not potentiated. Under the latter conditions, RNA synthesis as measured by [^{14}C]uridine incorporation was marginally affected (30% inhibition) by adenosine plus homocysteine, but markedly inhibited by 70% by cordycepin. Cordycepin inhibited 2′-*O* methylation of nuclear RNA to a greater extent than base methylation, while the combination of adenosine and homocysteine inhibited these sites to equal degrees. Moreover, cordycepin inhibited >18 S nuclear RNA four times as extensively as 4 S nuclear RNA, in comparison to the equal extents of inhibition of these two classes of nuclear RNA by adenosine plus homocysteine. A positive correlation was observed between the generation of intracellular *S*-adenosylhomocysteine in L1210 cells and the inhibition of methylation of nuclear RNA by adenosine plus homocysteine, but not by cordycepin. These results indicate that a significant amount of *S*-adenosylhomocysteine can be generated in the presence of adenosine and homocysteine, presumably via *S*-adenosylhomocysteine hydrolase, leading to marked inhibition of methylation of nuclear RNA in mouse lymphoid leukemia cells.

INTRODUCTION

Elevation of adenosine and 2′-deoxyadenosine metabolites in reticuloendothelial tissues and blood of patients with severe combined immunodeficiency has been associated with the absence of the catabolic enzyme, adenosine deaminase (1–4). The "deletion" of this enzyme has also been accomplished pharmacologically by the use of adenosine deaminase inhibitors such as *erythro*-9-(2-hydroxy-3-nonyl)-adenine (EHNA) and the tight-binding stoichiometric inhibitor, 2′-deoxycoformycin (5, 6). Elevations of ATP, cyclic-3′,5′-AMP, and 2′-dATP all have been reported to be associated with the cytotoxicity produced by adenosine deaminase deficiency (7–10). However, recent investigations have suggested that the cytotoxic effects of adenosine and 2′-deoxyadenosine may be mediated by different mechanisms. Ullman *et al.* (11) reported that the sensitivity of mouse lymphoma cells to 2′-deoxyadenosine was greatly diminished in variants lacking 2′-deoxyadenosine kinase. In contrast, adenosine kinase-deficient lymphoblasts remained as sensitive as the parent cell line to growth inhibition produced by adenosine (12). This divergence in mechanism of action suggests that the cytotoxicity of adenosine may be mediated by an adenine nucleotide-independent effect. Evidence for such a proposal was recently reported by Kredich and Martin (13) and Kredich and Hershfield (14), where they demonstrated that the toxicity of adenosine to lymphoma cells was enhanced in the presence of homocysteine and lead to inhibition of methylation of DNA and RNA. This synergism was presumably mediated via reversal in the equilibrium of SAH[1] hydrolase to

[1] Abbreviations used: AR, adenosine; dAR, 2′-deoxyadenosine; HCY, L-homocysteine thiolactone; SAH, *S*-adenosylhomocysteine; SAM, *S*-adenosyl-L-methionine; nRNA, nuclear RNA; SDS, sodium dodecyl sulfate; HPLC, high-pressure liquid chromatography; SP Sephadex, sulfopropyl Sephadex; TCA, trichloroacetic acid.

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produce elevated levels of SAH (13, 14), a potent inhibitor of methyltransferase reactions (15, 16).

The present study was designed to determine whether the activity of SAH hydrolase was important for mediating the inhibition of methylation of nuclear RNA by adenosine, and possibly 2′-deoxyadenosine, in L1210 cells. In addition, the potent inhibitor of RNA methylation, cordycepin (3′-deoxyadenosine) (17, 18), was compared with the two naturally occurring adenosine metabolites in order to see if any similarities existed in their biochemical mechanisms of action.

MATERIALS AND METHODS

Materials. Cordycepin was kindly provided by the Drug Synthesis and Chemistry Branch, National Cancer Institute. Adenosine, 2′-deoxyadenosine, L-homocysteine thiolactone, SAH, and SAM were purchased from Sigma Chemical Co., St. Louis, Mo. Heat-inactivated fetal calf serum was obtained from Flow Laboratories, Rockville, Md. L-[*Methyl*-^{3}H]methionine (80 Ci/mmol) and [^{14}C(U)]uridine (462 mCi/mmol) were purchased from New England Nuclear Corp., Boston, Mass. SP Sephadex was obtained from Pharmacia Fine Chemicals, Piscataway, N.J.

Animals. L1210 cells were inoculated i.p. into BALB/c × DBA/2 F_1 mice at an inoculum of 10^5 cells/0.1 ml Hank's balanced salt solution. Cells were harvested 6 days after inoculation and were further diluted with incubation medium to 2×10^7 cells/ml.

Incubations. L1210 cells were incubated at 37°C in a shaking water bath at 100 rpm in 25 ml of RPMI-1630 containing 10% heat-inactivated fetal calf serum and 5 × 10^7 cells. Incubations were carried out in the following sequence: (1) preincubation with 1×10^{-6} M dCF for 15 min; (2) incubation with adenosine, 2′-deoxyadenosine, cordycepin, or homocysteine for 30 min; (3) incubation with either 500 μCi [*methyl*-^{3}H]methionine (80 Ci/mmol) alone, 500 μCi [*methyl*-^{3}H]methionine and 2 μCi [^{14}C]-uridine (468 mCi/mmol), or 2.5×10^{-7} M unlabeled L-methionine for 1 h.

RNA extraction. After incubation, cells were centrifuged at 400*g* for 20 min and washed once with incubation medium without serum. Nuclei were prepared as previously described (19), except that cells were swelled at 10 mM magnesium acetate (pH 5.1). This change in the swelling medium was found to markedly arrest the activity of intracellular RNase and thereby minimize the hydrolysis of nRNA before extraction. nRNA was extracted from nuclei with 3 ml of 0.1% SDS:0.14 M NaCl: 0.025 M sodium acetate (pH 5.1) and 3 ml of a phenol mixture (phenol:*m*-cresol:water (7:2:2, v/v) containing 0.1% 8-hydroxyquinoline) by vortexing vigorously for 2 min. The emulsion was clarified by centrifugation at 12,000*g* for 10 min and the upper aqueous phase was removed and precipitated with 3 vol of 2% potassium acetate in 95% ethanol at −20°C overnight.

Electrophoresis. nRNA was resolved by electrophoresis in cylindrical polyacrylamide gels (0.4 × 7 cm) containing 8% (w/v) acrylamide, 0.32% (w/v) diallytartardiamide, 6 M urea, 0.1% (w/v) SDS, 0.2% (w/v) ammonium persulfate, 0.04% (v/v) *N,N,N′,N′*-tetramethylenediamine, 0.4 M Tris–acetic acid (pH 7.2), 0.02 M sodium acetate, and 0.002 M EDTA. RNA samples containing 1 A_{260} unit were mixed with sampled buffer to give a final concentration of 0.04 M Tris–acetic acid (pH 7.2), 0.02 M sodium acetate, 0.002 M EDTA, 0.02% bromphenol blue, and 20% (w/v) sucrose (RNase free). Gels were electrophoresed at 4 mA per gel at 4°C. Gels were sectioned into 2-mm slices, dissolved in 2% (w/v) periodic acid at 37°C for 15 min, mixed with 10 ml of Aquasol (New England Nuclear, Boston, Mass.), and counted in a Searle Mark III liquid scintillation spectrometer. Gels were

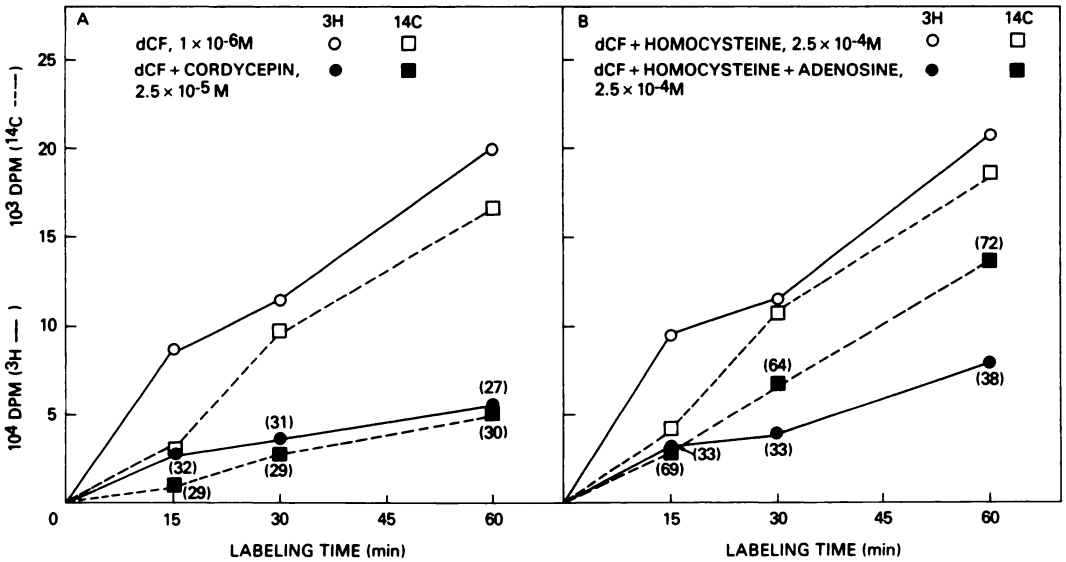

FIG. 1. *Inhibition of the synthesis and methylation of nRNA in L1210 cells by cordycepin and by adenosine plus homocysteine*

L1210 cells were incubated for 15 min with 1×10^{-6} M dCF, then for 30 min with or without 2.5×10^{-5} M cordycepin (A) or with 2.5×10^{-4} M homocysteine with or without 2.5×10^{-4} M adenosine (B), followed by pulse labeling for 15, 30, or 60 min with 500 μCi [*methyl*-^{3}H]methionine and 2 μCi [^{14}C]uridine. nRNA was isolated as described under Materials and Methods. Numbers in parentheses indicate percentages of the respective control values. Each value is the mean of two determinations.

stained for 1 h with 2% methylene blue dissolved in 15% acetic acid and destained by diffusion in 15% acetic acid (20).

DEAE-Sephadex chromatography. Alkaline hydrolysates of nuclear rRNA were prepared and chromatographed on DEAE-Sephadex equilibrated with 7 M urea: 20 mM Tris–HCl (pH 7.6) as previously described (17).

HPLC of SAM and SAH. Incubations were stopped by placing the flasks on ice and centrifuging at 400*g* for 10 min at 4°C. The cell pellet was vortexed with 1 ml of ice-cold 10% (w/v) TCA and centrifuged at 10,000*g* for 10 min at 4°C. The supernatant fluid was removed and extracted three times with ether to remove the TCA. One milliliter of 50 mM HCl was added to the sample and it was absorbed to a column (1 × 5 cm) of SP Sephadex equilibrated with 10 mM HCl (21). After washing the column with 30 ml of 50 mM HCl, SAM, SAH, and other metabolites were eluted with 10 ml of 2 M HCl and freeze-dried. Samples were dissolved in 100 μl of 10 mM potassium acetate (pH 5.5) containing 4% (v/v) methanol and centrifuged for 2 min at 16,000*g* in an Eppendorf centrifuge. A Waters Associates (Milford, Mass.) 204-W HPLC apparatus with a μBondapak C^{18} column (0.4 × 25 cm) was used for all analyses. Aliquots of 25 μl were injected into the column and SAM and SAH were eluted isocratically at 2 ml/min with 10 mM potassium acetate (pH 5.5) containing 4% (v/v) methanol. SAM and SAH were detected by uv absorption at 254 nm and identified by their retention times at 5 and 15 min, respectively, using purified standards as well as internal standardization with the cell extracts. SAM and SAH were quantitated by comparison with the area of known amounts of pure standards. For determination of the specific activity of SAM, fractions were collected based on their retention times by HPLC, and their radioactivity was determined as described above.

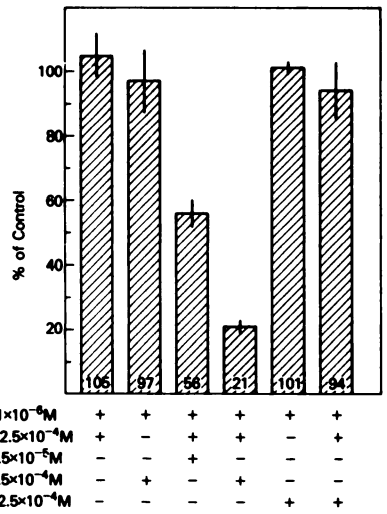

FIG. 2. *Inhibition of the methylation of nRNA by the combination of dCF, adenosine, and homocysteine*

L1210 cells were incubated as described in Fig. 1 except that labeling was carried out for 60 min with only [*methyl*-^{3}H]methionine. Numbers in each bar represent percentages of the respective controls. Each value is the mean ± SE of three to six determinations.

HPLC of CTP and UTP. Incubations were carried out as described above with [^{14}C]uridine, except that the washed cell pellet was vortexed with 1 ml of ice-cold 10% perchloric acid. After neutralization with KOH, the extracts were centrifuged in an Eppendorf centrifuge for 30 s, diluted with 4 vol of 0.02 M NH_4HCO_3, and adsorbed to a column (0.5 × 2 cm) of DEAE-cellulose equilibrated with 0.02 M NH_4HCO_3 and washed with 2 ml of 0.02 M

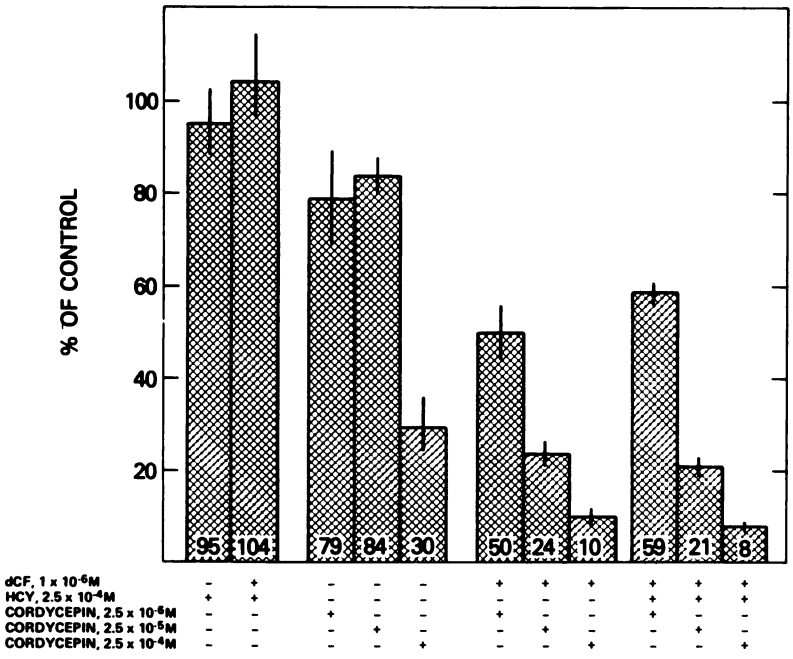

FIG. 3. *Inhibition of the methylation of nRNA by cordycepin in the presence and absence of dCF and homocysteine*

L1210 cells were incubated as described in Fig. 2 except that cordycepin replaced adenosine and 2′-deoxyadenosine. Numbers in each bar represent percentages of the respective controls. Each value is the mean ± SE of three determinations.

NH_4HCO_3. Nucleotides were then eluted with 1.5 ml of 0.6 M NH_4HCO_3, freeze-dried, and dissolved in 0.2 ml of 0.3 M KH_2PO_4, pH 3.85 (HPLC grade, Fisher Scientific). Aliquots of 25 μl were injected into a μBondapak NH_2 column (0.4 × 25 cm) and eluted isocratically at 1 ml/min with 0.3 M KH_2PO_4, pH 3.85. CTP and UTP were detected by uv absorption at 254 nm by their retention times of 8 and 9.7 min, respectively, then collected, and their radioactivities were determined as described above.

RESULTS

Methylation of nRNA. Initial experiments were designed to determine the relative effects of dCF plus cordycepin, and the combination of dCF, homocysteine, and adenosine on the incorporation of [*methyl*-^{3}H]methionine and [^{14}C]uridine into nRNA (Fig. 1). Cordycepin produced extensive inhibition of both methylation and synthesis of RNA throughout 1 h of labeling (Fig. 1A), while dCF, homocysteine, and adenosine inhibited meth-

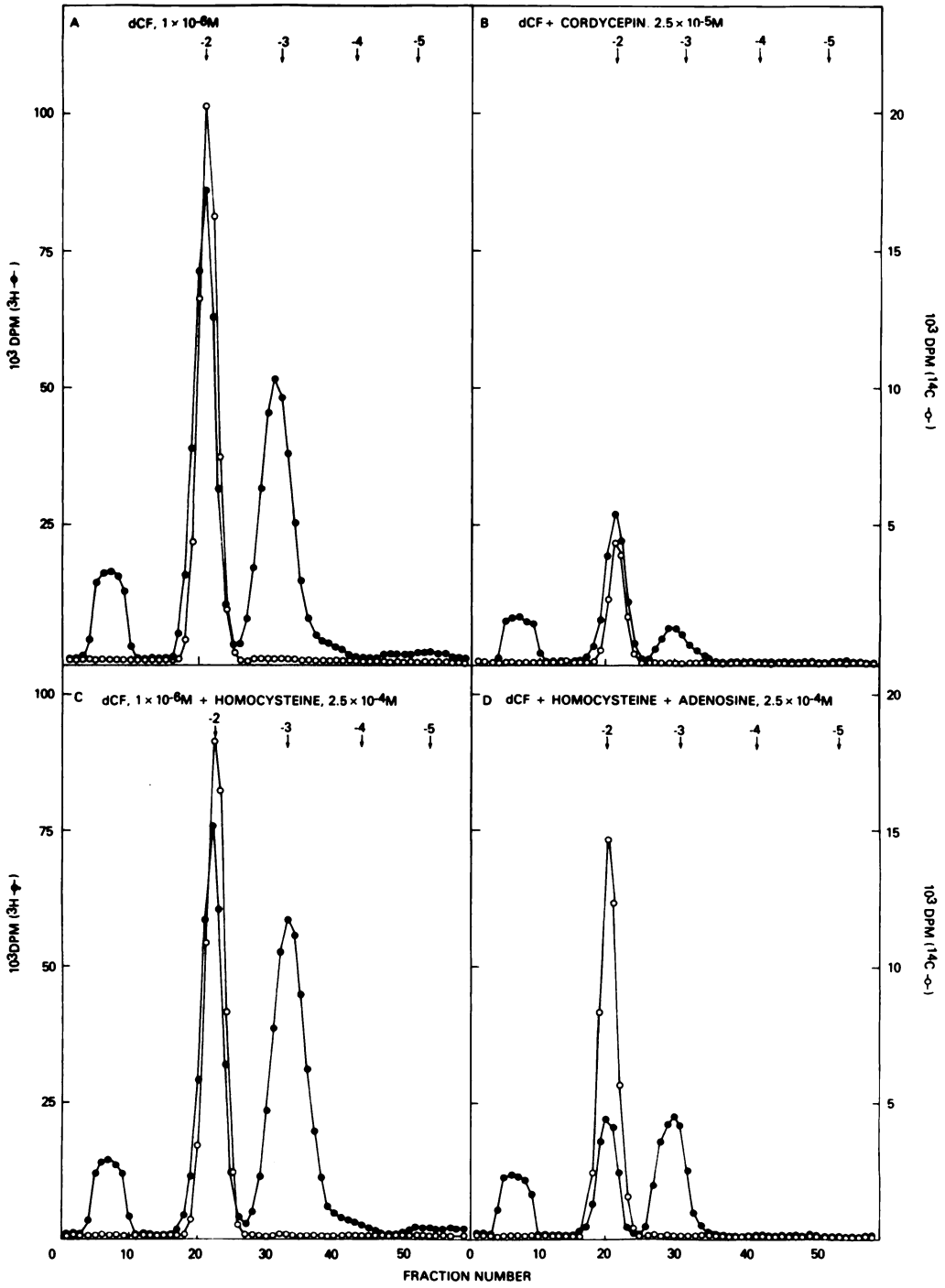

FIG. 4. *DEAE-Sephadex–urea chromatography of alkaline hydrolysates of nRNA*
L1210 cells were incubated as described in Fig. 1 except that labeling with [*methyl*-^{3}H]methionine and [^{14}C]uridine was carried out for 60 min.

ylation of nRNA to twice the extent of RNA synthesis (Fig. 1B).

Dose–response experiments comparing the effects of adenosine and 2′-deoxyadenosine on the methylation of nRNA in the presence of dCF and homocysteine are shown in Fig. 2. While 2.5×10^{-4} M adenosine and dCF were without effect on the methylation of RNA, significant inhibition was produced by 2.5×10^{-5} and 2.5×10^{-4} M adenosine in the presence of 2.5×10^{-4} M homocysteine and dCF. 2′-Deoxyadenosine was not inhibitory under the same conditions.

Similar experiments with cordycepin revealed that although the inhibitory effects of cordycepin on the methylation of RNA were potentiated approximately 10-fold by dCF, the addition of homocysteine to the incubation did not accentuate the effects of this adenosine analogue (Fig. 3).

Studies were next designed to see if the inhibitory effects of dCF plus cordycepin, and the combination of dCF, adenosine, and homocysteine on the methylation of RNA were associated with base methylation, 2′-*O* methylation, or both (Fig. 4). To carry out these experiments, nRNA was labeled with [*methyl*-^{3}H]methionine and [^{14}C]uridine, hydrolyzed with KOH, and chromatographed on DEAE-Sephadex (17). The combination of dCF and cordycepin produced 79% inhibition of [^{14}C]-uridine incorporation, 69% inhibition of base methylation (−2 charge peak), and 87% inhibition of 2′-*O* methylation (−3 charge peak) (Figs. 1A and B). In contrast, treatment of L1210 cells with dCF, homocysteine, and adenosine produced 25% inhibition of [^{14}C]uridine incorporation, 84% inhibition of base methylation, and 81% inhibition of 2′-*O* methylation (Figs. 1C and D). These results indicate that unlike cordycepin, adenosine plus homocysteine inhibits base and 2′-*O* methylation to equal degrees and far more extensively than nRNA synthesis.

Polyacrylamide gel electrophoresis of nRNA labeled with [*methyl*-^{3}H]methionine for 15, 30, and 60 min in the presence of dCF and cordycepin or dCF, homocysteine, and adenosine is presented in Fig. 5. Under denaturing conditions, the methylation of nRNA is observed in either >18 S nRNA or 4 S nRNA. While cordycepin inhibited the methylation of >18 S and 4 S nRNA by approximately 85 and 40%, respectively, homocysteine and adenosine produced 70–75% inhibition of both classes of nRNA. Thus, the general inhibitory effect of dCF, homocysteine, and adenosine on the methylation of nRNA pervaded all methylated species of RNA, in contrast to the more specific effect of cordycepin on >18 S nRNA.

HPLC of SAM and SAH. In order to confirm the proposed mechanism of action of the combination of dCF, homocysteine, and adenosine on the methylation of RNA via generation of SAH by SAH hydrolase, HPLC of cell extracts was performed. Typical elution profiles for several incubation conditions are shown in Fig. 6. The retention times of SAM and SAH were 5 and 15 min, respectively. Almost undectable levels of SAH were present under conditions where either dCF, dCF and adenosine, or dCF and homocysteine were present (Figs. 6A–E). When dCF, homocysteine, and either 2.5×10^{-5} or

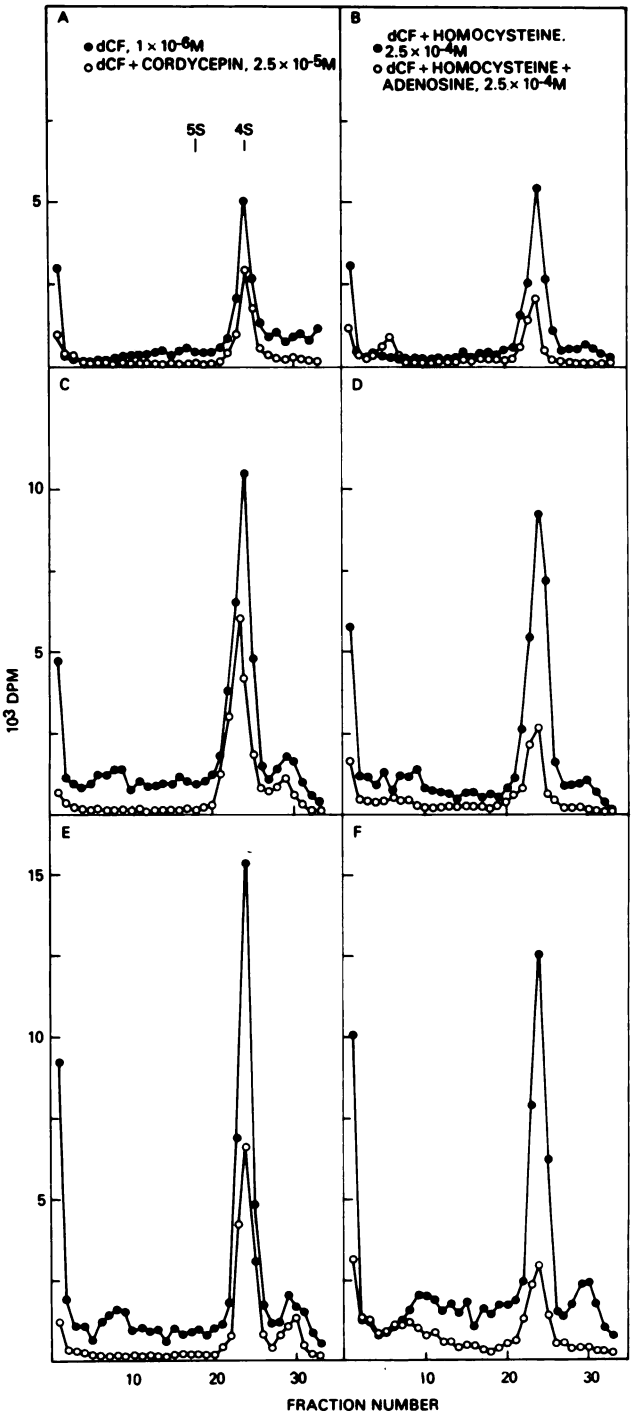

FIG. 5. *Polyacrylamide gel electrophoresis of nRNA labeled with [methyl-^{3}H]methionine*

L1210 cells were incubated as described in Fig. 1 except that labeling was carried out with [*methyl*-^{3}H]methionine for 15 min (A, B), 30 min (C, D), or 60 min (E, F).

2.5×10^{-4} M adenosine were present in the incubation, significant levels of SAH were generated (Figs. 6F and G). No elevated levels of SAH were formed when the concentration of adenosine was reduced to 2.5×10^{-6} M (Fig. 6H).

Table 1 presents the levels of SAM and SAH under a variety of incubation conditions. In the presence of either

dCF and cordycepin, dCF and 2′-deoxyadenosine, or dCF, homocysteine, and 2′-deoxyadenosine, minimal changes occurred in either the SAM or the SAH concentrations. Only in the presence of dCF, homocysteine, and adenosine did high elevations of SAH occur with a concomitant doubling in the concentration of SAM.

Since the elevation of SAH levels with coincident expansion in the concentration of SAM would alter the specific radioactivity of SAM, the latter activity was determined after HPLC of extracts from cells incubated with dCF, cordycepin, adenosine, and homocysteine (Table 2). A marked reduction in the specific radioactivity of SAM was produced by the combination of dCF, adenosine, and homocysteine which was comparable to the reduction in methylation of nRNA (Fig. 1). dCF and adenosine reduced the specific activity of SAM by 30%, while cordycepin reduced the specific radioactivity of SAM by approximately 45%. Homocysteine in the presence of dCF was without effect.

Analyses of the specific radioactivities of CTP and UTP following labeling of cells for 1 h with [^{14}C]uridine are presented in Table 2. The labeling of CTP was only 2% of the radioactivity of UTP and was affected only by cordycepin. The specific radioactivity of UTP was affected only by adenosine and adenosine plus homocysteine. In the latter instance, the reduction in specific activity of SAM was comparable to the reduction in labeling of nRNA by [^{14}C]uridine (Fig. 1).

Figure 7 presents the positive correlation between increased SAH concentrations in the cells and inhibition of methylation of nRNA. It is apparent that concentrations of SAH must exceed 1 nmol/5 × 10^7 cells (approximately 3 × 10^{-5} M SAH) for inhibition to occur. The concentration of SAH producing 50% inhibition of methylation of nRNA was approximately 11 nmol SAH/5 × 10^7 cells.

DISCUSSION

The present study has documented that under conditions where adenosine deaminase is inhibited and in the presence of homocysteine, adenosine but not 2′-deoxyadenosine inhibited the methylation of nRNA. This effect was directly related to the increased intracellular generation of SAH. Although the reduced specific radioactivity of SAM can account for most of the reduction in methylation of nRNA, it is unlikely that it is the cause of this phenomenon. The elevation in the levels of SAH by adenosine plus homocysteine was dose dependent (Table 1) and correlated with the inhibition of nRNA methylation (Fig. 2). Increased concentrations of SAM occurred only at the highest concentrations of adenosine and homocysteine and were evident secondary to the appearance of elevated concentrations of SAH (Table 1). The mechanism of the inhibition of methylation of nRNA most likely involves the reversal in equilibrium of SAH hydrolase toward formation of SAH with subsequent feedback inhibition of methyltransferase activity (Fig. 8). The feasibility of this reaction pathway has been demonstrated in mouse liver *in vitro* and *in vivo* with N^6-methyladenosine (22). This mechanism is also supported by our data showing an accumulation in the concentration of SAM with a resultant reduction of its specific radioactivity by the combination of dCF, adenosine, and homocysteine. Our data confirm, in part, the previous

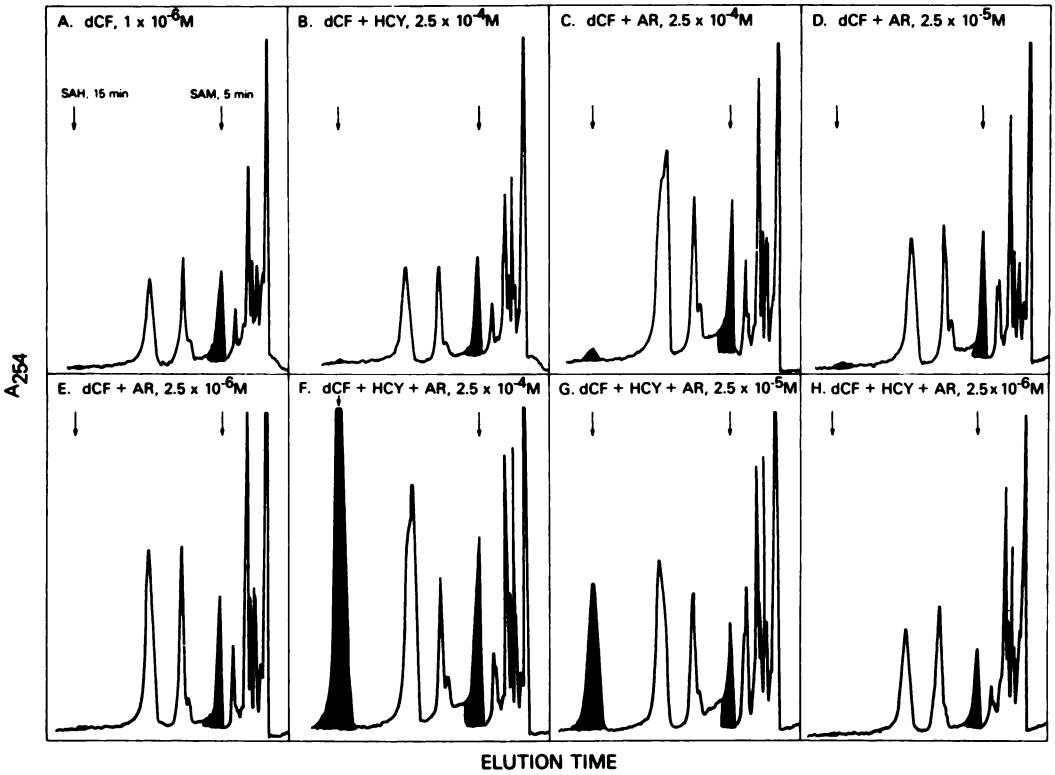

FIG. 6. *HPLC analysis of SAM and SAH in L1210 cells*

L1210 cells were incubated as described in Fig. 2 except that an equimolar (2.5 × 10^{-7} M) amount of unlabeled L-methionine replaced [^{3}H]methionine. The direction of elution is from right to left.

TABLE 1

SAM and SAH levels in L1210 cells incubated with dCF, homocysteine, adenosine, 2′-deoxyadenosine, and cordycepin

L1210 cells were incubated with dCF and various concentrations of homocysteine, adenosine, 2′-deoxyadenosine, and cordycepin as described under Materials and Methods. Incubations were carried out for 15 min with 1×10^{-6} M dCF, then for 30 min with cordycepin, adenosine, 2′-deoxyadenosine, and/or homocysteine, followed by 60 min with 2.5×10^{-7} M L-methionine. SAM and SAH levels were analyzed by HPLC as described in the text.

Addition	No. experiments	Concentration	
		SAM	SAH
		nmol/5 × 10⁷ cells	
dCF, 1×10^{-6} M	13	1.81 ± 0.16	0.15 ± 0.3
dCF + cordycepin, 2.5×10^{-5} M	11	1.66 ± 0.12	0.10 ± 0.002
dCF + homocysteine			
2.5×10^{-5} M	4	2.20 ± 0.23	0.10 ± 0.002
2.5×10^{-4} M	10	2.00 ± 0.17	0.15 ± 0.03
dCF + adenosine			
2.5×10^{-6} M	4	2.26 ± 0.17	0.10 ± 0.002
2.5×10^{-5} M	4	2.61 ± 0.36	0.43 ± 0.08
2.5×10^{-4} M	10	2.27 ± 0.32	0.65 ± 0.15
dCF + 2′-deoxyadenosine, 2.5×10^{-4} M	4	2.20 ± 0.29	1.17 ± 0.22
dCF + 2′-deoxyadenosine, 2.5×10^{-4} M, + homocysteine, 2.5×10^{-4} M	5	1.94 ± 0.11	0.71 ± 0.08
dCF + adenosine, 2.5×10^{-4} M, + homocysteine, 2.5×10^{-6} M	4	2.09 ± 0.40	1.27 ± 0.16
dCF + adenosine, 2.5×10^{-4} M, + homocysteine, 2.5×10^{-5} M	4	2.30 ± 0.30	5.70 ± 0.66
dCF + adenosine, 2.5×10^{-4} M, + homocysteine, 2.5×10^{-4} M	9	3.62 ± 0.41	23.87 ± 1.69
dCF + adenosine, 2.5×10^{-5} M, + homocysteine, 2.5×10^{-4} M	4	1.96 ± 0.20	8.86 ± 1.26
dCF + adenosine, 2.5×10^{-6} M, + homocysteine, 2.5×10^{-4} M	4	2.06 ± 0.17	0.70 ± 0.18

findings of Kredich and Martin (13) and Kredich and Hershfield (14) where SAH levels of 56 nmol/5 × 10^7 cells for mouse T lymphoma cells and 415 nmol/5 × 10^7 cells for human lymphoblasts were produced at the highest concentrations of exogenous adenosine plus homocysteine tested. In close agreement with their data, we have found that 50% inhibitory concentration of SAH for methylation of RNA to be 11 nmol/5 × 10^7 cells, in comparison to their values of 5 nmol/5 × 10^7 cells and 8 nmol/5 × 10^7 cells for inhibiting the methylation of DNA (13) and both DNA and total RNA (14) in lymphoma cells and lymphoblasts, respectively. In addition, we have established that 2′-deoxyadenosine does not produce inhibition of methylation of nRNA or inhibitory concentrations of SAH in leukemic cells. Therefore, its cytotoxicity undoubtedly involves an adenine nucleotide-dependent process as proposed from studies with deoxyadenosine kinase-deficient cells (11).

The mechanism of action of the combination of adenosine and homocysteine differs significantly in some respects from that of adenosine analogues such as cordycepin and xylosyladenine (17, 18). In the latter instance, these agents inhibited 2′-*O* methylation to a greater extent than base methylation of nRNA (17) in comparison to the equal inhibitory effects produced by adenosine plus homocysteine. In addition, cordycepin did not elevate the concentration of SAH, and thus, its mechanism of action differs greatly from that of adenosine plus homocysteine. Recently, evidence for the formation of an *S*-3′-deoxyadenosyl-L-methionine derivative of cordycepin was obtained in human erythrocytes (23). This hypothesis is also supported by our data showing a reduced specific radioactivity of SAM by cordycepin without an elevation in SAH levels. If *S*-3′-deoxyadenosyl-L-methionine is a less effective methyl donor than SAM, then reduced methylation of nRNA would be expected to occur. The reason for the unchanged level of "[^{3}H]SAM" previously demonstrated via SP Sephadex chromatography (18) was undoubtedly a mixture of [^{3}H]SAM plus [^{3}H]*S*-3′-deoxyadenosyl-L-methionine as shown by Zimmerman *et al.* (23) using HPLC. Thus, the mechanism of action of cordycepin on the methylation of nRNA appears to involve its conversion to *S*-3′-deoxyadenosyl-L-methionine.

TABLE 2

Specific radioactivities of SAM, CTP, and UTP in L1210 cells incubated with dCF, homocysteine, adenosine, and cordycepin

Cells were incubated as described in Table 1 and the specific radioactivities of SAM, CTP, and UTP were determined as described under Materials and Methods. Each value is the mean ± SE of four determinations. Numbers in parentheses represent percentages relative to dCF treatment alone (100%).

Treatment	SAM	CTP	UTP
	10^6 dpm/nmol	*dpm/nmol*	
dCF, 1×10^{-6} M	1.79 ± 0.07 (100)	94 ± 13 (100)	4970 ± 620 (100)
dCF + cordycepin, 2.5×10^{-5} M	1.00 ± 0.05 (56)	60 ± 13 (64)	5200 ± 610 (105)
dCF + homocysteine, 2.5×10^{-4} M	1.27 ± 0.07 (71)	96 ± 6 (102)	5470 ± 630 (110)
dCF + adenosine, 2.5×10^{-4} M	1.67 ± 0.42 (93)	90 ± 14 (96)	3780 ± 200 (76)
dCF + adenosine, 2.5×10^{-4} M, + homocysteine, 2.5×10^{-4} M	0.55 ± 0.17 (31)	89 ± 12 (95)	3510 ± 310 (71)

Cordycepin and related analogues are also potent in-

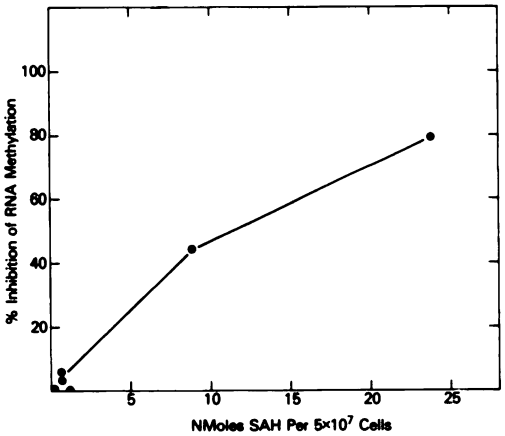

FIG. 7. *Correlation between the level of SAH and the inhibition of methylation of nRNA*

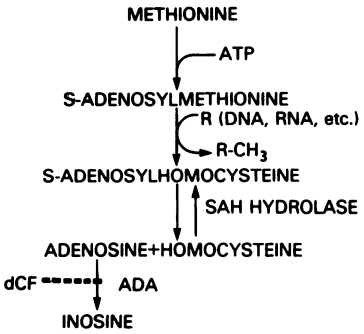

FIG. 8. *The metabolism of SAM and SAH*

The dashed line denotes inhibition of adenosine deaminase (ADA) by dCF.

hibitors of nRNA synthesis in the presence of dCF (17, 19, 24), in contradistinction to the weak inhibitory effect produced by the combination of dCF, adenosine, and homocysteine. The latter effect can be accounted for by the reduction in the specific radioactivity of UTP, in contrast to the lack of effect upon this parameter by cordycepin. The modest reduction in the labeling of CTP via [^{14}C]uridine by cordycepin, while not influencing the labeling of nRNA, confirms the previous findings of Plagemann (25).

The role of methylation in the functioning of various species of RNA is well documented. The maturation of rRNA from the 45 S precursor to 28 S and 18 S nRNA is highly dependent on 2′-*O* methylation, the absence of which results in extensive endonucleolytic cleavage (26, 27). The 5′-terminal "cap" structure of most eukaryotic mRNA also serves a protective function against exonucleolytic digestion (28–30). Reduced methylation of tRNA results in its decreased stability and underutilization in translation (31). Thus, hypomethylation of nRNA in cells exposed to adenosine plus homocysteine or adenosine analogues such as cordycepin would be expected to produce decreased cell viability through loss of translation activity.

In summary, our results establish that adenosine can interfere with base and 2′-*O* methylation in high molecular weight and 4 S nRNA in the presence of homocysteine and an adenosine deaminase inhibitor. This effect was not produced by 2′-deoxyadenosine and differed from the inhibitory effect on methylation produced by the anticancer drug, cordycepin.

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