Inhibition of Protein Synthesis by 5′-Sulfamoyladenosine*

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ABSTRACT: The biological and biochemical activity of a number of 5′-sulfamoyl nucleosides has been studied. Among these, only the 5′-sulfamate derivatives of adenosine or 2′-deoxyadenosine inhibited the growth of *Escherichia coli*, *Streptococcus faecium*, and leukemia L-1210, whereas 3′-*O*-sulfamoyl-2′-deoxyadenosine affected the growth of only the bacteria. The 5′-*O*-sulfamoyl derivatives of inosine, uridine, cytidine, 2′-deoxycytidine, and thymidine were inactive in all three test systems. The addition of 5×10^{-6} M 5′-*O*-sulfamoyladenosine to a growing culture of *E. coli* led to the prompt inhibition of growth and protein synthesis, while permitting the continued synthesis of RNA and DNA at a reduced rate. In a cell-free system from *E. coli*, the 5′-*O*-sulfamoyl derivative, at 4×10^{-4} M, inhibited the polyuridylate-directed synthesis of polyphenylalanine from free phenylalanine by approximately 90%. The interference was only minor (1–15%) when preformed L-phenylalanyl-RNA was used. The analog inhibited the formation of phenylalanyl-RNA to the same extent as it did the synthesis of polyphenylalanine from free phenylalanine. The formation of lysyl-RNA was inhibited by 90% at 1×10^{-5} M and that of prolyl-RNA by 80% at 1×10^{-6} M 5′-*O*-sulfamoyladenosine. Under conditions where 1 μmole of 5′-AMP was converted, within 1 min, to IMP by an AMP-deaminase from rabbit muscle, or cleaved to adenosine by snake venom (*Crotalus atrox*), or where 1 μmole of adenosine was deaminated to inosine by an adenosine deaminase from calf intestine, no effect of these enzymes on 5′-*O*-sulfamoyladenosine could be detected. When the incubation time was extended to 4 hr, approximately 7% of the analog was found deaminated in the presence of AMP-deaminase. As determined with extracts of *S. faecium*, sulfamoyladenosine, at 1×10^{-3} M, did not interfere with the conversion of [8-^{14}C]adenine to its nucleoside mono-, di-, and triphosphates.

One of the most toxic nucleosides encountered in nature is the antibiotic nucleocidin (Thomas *et al.*, 1956–1957; Hewitt *et al.*, 1956–1957). Its structure (Figure 1) has recently been redefined to be the 5′-*O*-sulfamoyl-4′-*C*-fluoroadenosine (Morton *et al.*, 1969). In analogy with nucleocidin, a number of sulfamoyl derivatives of the natural purine and pyrimidine nucleosides devoid of the fluorine atom have recently been prepared (Shuman *et al.*, 1969, 1970), and some of their biological and biochemical properties have been examined. The results of these studies are reported in this paper. A preliminary account of the findings has been presented (Bloch *et al.*, 1970).

Experimental Section

Materials. L-[U-^{14}C]Phenylalanine, L-[U-^{14}C]lysine, and L-[U-^{14}C]proline were purchased from New England Nuclear Corp. *Escherichia coli* B stripped sRNA was obtained from General Biochemicals.

5′-Adenylic acid deaminase from rabbit muscle (lot 66 B-7390), approximately 30 units/mg of protein, adenosine deaminase from intestinal mucosa (lot 30 C-2641), approximately 200 units/mg of protein, and snake venom (*Crotalus atrox*) were purchased from Sigma Chemical Co.

Antimicrobial Activity and Inhibition Analysis. S. faecium was grown in the medium of Flynn *et al.* (1951) from which uracil and the purines were omitted, and to which 1 mμg/ml of folic acid was added. *E. coli* was grown in the synthetic medium described by Gray and Tatum (1944). The procedures used for determining the potency of the compounds and for carrying out the inhibition analyses have been described previously (Bloch and Coutsogeorgopoulos, 1966).

The inhibition analyses were carried out by adding the following metabolites to the basal media containing the drug: adenine, guanine, xanthine, hypoxanthine, uracil, cytosine, thymine, and the corresponding ribonucleosides and deoxyribonucleosides, which were added individually or in combination, at concentrations ranging from 10^{-3} to 10^{-6} M. The amino acids DL-alanine, L-aspartic acid, L-arginine, L-cysteine, L-glutamic acid, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, DL-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, DL-tyrosine, and DL-valine were added to the medium either singly or in combination at concentrations of 600 μg/ml. The vitamin complement normally supplied and comprising thiamine, riboflavin, pantothenic acid, nicotinic acid, *p*-aminobenzoic acid, biotin, and pyridoxine was increased 10- to 20-fold.

Antileukemic Activity. The effect of the analogs on the *in vitro* growth of leukemia L-1210 cells was assayed by a micro method recently developed in this laboratory. The method, which constitutes a parallel to the microbial assays, involves the introduction of 0.5-ml aliquots of the medium (RPMI 1640 + 10% calf serum; Moore *et al.*, 1966) containing the drug, into 16 × 125 mm screw-cap culture tubes, followed by 0.5 ml of medium containing 3×10^5 L-1210 cells. The cultures are incubated for 40 hr at 37° with rotation in a roller drum. Under these conditions, an 8- to 10-fold increase in the number of L-1210 cells is achieved in the control. Growth is measured by counting the number of total and viable cells, the latter being determined by dye exclusion.

Assay of Growth and of RNA, DNA, and Protein Synthesis in E. coli B. All manipulations of the growing cultures were carried out in a constant-temperature room at 37°, and all glassware required for the growth experiments were kept at this temperature. Cell growth and the accompanying synthesis of the macromolecular cell components were determined as follows. A culture of *E. coli*, grown overnight, was centrifuged, and a sufficient number of the cells were added to 600 ml of

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fresh medium to give a reading of 10 in a Klett nephelometer. Incubation proceeded until a reading of 38–40 was obtained (approximately 2 hr), at which time the culture was divided in half. One portion was added to 1 ml of distilled water containing enough inhibitor to give a final concentration of 5×10^{-6} M, the other to the same amount of water free of the drug. The cultures were reincubated, and the extent of growth was followed. At stated intervals, 50-ml aliquots were withdrawn from the two flasks, chilled at once in an ice bath, and centrifuged in the cold. After washing with ice-cold saline, the pellets were frozen rapidly at −70°, and all samples collected in this manner were assayed simultaneously for their RNA and DNA content (Volkin and Cohn, 1954; Munro and Flack, 1966) and for protein (Lowry *et al.*, 1951). Yeast RNA, calf thymus DNA, and crystalline bovine albumin served as standards. Control experiments showed that the presence of the sulfamoyl analogs did not affect the color reactions.

Preparation of Ribosomes and Supernatant Fraction. All cell fractionations were carried out at 4°. Washed ribosomes were prepared from frozen *E. coli* B cells harvested in the middle of the logarithmic phase of growth. The nonpreincubated S-30 fraction (Nirenberg and Matthaei, 1961) was centrifuged at 100,000*g* for 4 hr. The upper two-thirds of the supernatant, constituting the S-100 fraction, was dialyzed overnight at 4° against the "standard buffer" of Nirenberg and Matthaei (1961). Any precipitate which developed during dialysis was discarded after centrifugation. The S-100 fraction was stored at −70° in small aliquots. The pellet fraction (ribosomes) was suspended in 0.5 M NH_4Cl–0.01 M $MgCl_2$–0.01 M Tris-HCl, pH 7.4 (NH_4Cl wash buffer), and was kept in ice overnight. After centrifugation at 100,000*g* for 6 hr at 4°, the pellets were resuspended in NH_4Cl wash buffer, and after remaining on ice overnight, they were centrifuged at 100,000*g* for 6 hr. Washing of the ribosomes by this procedure was repeated one more time. The washed ribosomes were finally suspended in "standard buffer," and were dialyzed for 4 hr at 4° against this buffer. They were then stored in small aliquots at −70°. The protein content of the S-100 fraction was determined according to the method of Lowry *et al.* (1951).

L-[^{14}C]Phe-RNA was prepared as previously described (Coutsogeorgopoulos, 1967), and was purified on a Sephadex G-25 column (2.5 × 20 cm for each 2500 A_{260} units) equilibrated with 0.01 M sodium acetate buffer (pH 5.5) at 4°.

Assay for Polyphenylalanine Synthesis and for Aminoacyl-RNA Formation. Polyphenylalanine synthesis was determined by the hot perchloric acid assay and aminoacyl-t-RNA formation by the cold perchloric acid assay, as described previously (Coutsogeorgopoulos, 1967). The incubation conditions used for polyphenylalanine formation are described in the legends to Tables II and III, those used for aminoacyl-tRNA formation appear in the legends to Tables IV–VI.

Susceptibility of 5′-Sulfamoyladenosine to the Action of Catabolic Enzymes. 5′-ADENYLIC ACID DEAMINASE. Dilutions of sulfamoyladenosine and of AMP in 0.01 M citrate buffer (pH 6.5) were prepared, at concentrations of 1×10^{-3} and 1×10^{-4} M. To 1-ml aliquots of these, 1 μM unit of the enzyme (each μM unit deaminates approximately 1 μmole of 5′-AMP to 5′-IMP per min at pH 6.5 at 25°), dissolved in 1.0 M KCl, was added. The reactions were monitored at 265 mμ for 1 hr at 25° with a Gilford Model 2000 spectrophotometer (Kalckar, 1947), and were terminated by immersion of the vessels into boiling water for 2 min. The reaction mixtures (after reduction in volume by evaporation *in vacuo*) were applied to Whatman No. 3MM paper, and were chromatographed in isobutyric acid–concentrated ammonia–water (66:1:33, v/v), in 5% sodium citrate–isoamyl alcohol (1:1, v/v), and in a solvent prepared as follows: 13.8 g of $NaH_2PO_4 \cdot H_2O$ was added to 900 ml of water, and the solution was adjusted to pH 6.8 with H_3PO_4. The volume was increased, with water, to 1 l., and 600 g of $(NH_4)_2SO_4$ and 20 ml of *n*-propyl alcohol were added.

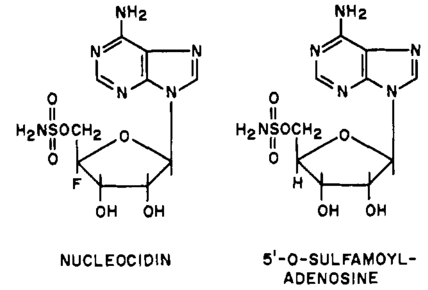

FIGURE 1

ADENOSINE DEAMINASE. Dilutions of sulfamoyladenosine and of AMP at concentrations of 1×10^{-3} and 1×10^{-4} M were prepared in 0.05 M phosphate buffer (pH 7.5). To 1.0-ml aliquots of these, 1 μM unit of the enzyme was added. The reactions were followed spectrophotometrically and chromatographically as described for adenylic acid deaminase. Parallel 4-hr incubation samples were treated in the same manner.

SNAKE VENOM (*Crotalus atrox*). To 1.0-ml aliquots of 1×10^{-3} or 1×10^{-4} M solutions of sulfamoyladenosine or of AMP in 0.1 M Tris buffer (pH 8.0) were added 0.05 ml of 0.1 M $MgCl_2$ and 0.55 mg of the venom. This amount converts approximately 1 μmole of AMP to adenosine within 1 min at 37°. Samples were chromatographed after 1- and 4-hr incubation in the same manner as described above for the deaminase determinations.

Conversion of [8-^{14}C]*Adenine to Nucleotides.* To 0.3 ml of 0.1 M Tris buffer (pH 6.8) containing adenine (1×10^{-5} M), [8-^{14}C]adenine (0.01 μCi, 0.5 mCi/1.1 mg), ATP (1×10^{-3} M), PRPP (1×10^{-3} M), $MgCl_2$ (1×10^{-5} M), and the analog at 1×10^{-3} or 1×10^{-4} M was added 0.1 ml of dialyzed *Streptococcus faecium* extract, prepared as follows. The *S. faecium* cells were harvested toward the end of their log phase of growth, and after washing with saline, they were disrupted with a Hughes press. The broken cells were suspended in 3 volumes of 0.05 M Tris buffer (pH 7.6) and were centrifuged at 29,500*g* for 1 hr. The supernatant solution was dialyzed overnight at 4° against 4 l. of the same buffer, and the recentrifuged supernatant constituting the crude cell extract was used for the assays. The assay mixtures were incubated for 5 min or for 30 min at 37°, and the reactions stopped by immersion of the vessels into boiling water for 2 min. The precipitates formed were removed by centrifugation, and 0.1-ml aliquots of the mixtures were applied in a band to Whatman No. 3MM paper, and were chromatographed together with authentic markers (adenine, adenosine, AMP, ADP, and ATP) in the following solvents: 95% ethanol–1 M ammonium acetate (pH 5.0) (7:3 v/v), isobutyric acid–concentrated ammonia– water (66:1:33, v/v), and 5% sodium citrate–isoamyl alcohol (1:1, v/v). Strips, 3 cm in width, extending from the origin to the solvent front were cut from the chromatograms and were divided into 1-cm segments. Each segment was placed into a vial containing 20 ml of phosphor containing 4 g of 2,5-diphenyloxazole and 100 mg of 1,4-bis[2-(5-phenyloxazolyl)]benzene per l. of toluene, and was counted in a Packard Tri-Carb scintillation counter. The total radioactivity per strip averaged 4900 cpm.

TABLE I: Biological Activity of 3′- and 5′-Sulfamoyl Nucleosides.

Compound	Concentration (M) for 50% Growth Inhibition of		
	S. faecium	*E. coli*	Leukemia L-1210
5′-*O*-Sulfamoyladenosine	4×10^{-6}	3×10^{-5}	2×10^{-7}
5′-*N*-Sulfamoylamino-5′-deoxyadenosine	9×10^{-5}	5×10^{-6}	1×10^{-7}
5′-*O*-Sulfamoyl-2′-deoxyadenosine	2×10^{-4}	4×10^{-5}	1×10^{-5}
3′-*O*-Sulfamoyl-2′-deoxyadenosine	9×10^{-5}	5×10^{-4}	$>10^{-3}$
5′-*O*-Sulfamoylinosine, or 5′-*O*-Sulfamoylcytidine, or 5′-*O*-sulfamoylthymidine, or 5′-*O*-sulfamoyl-3′-*O*-acetylthymidine	$>10^{-3}$	$>10^{3}$	$>10^{-3}$
5′-*O*-Sulfamoyluridine, or 3′-*O*-sulfamoyl-2′-deoxycytidine, or 3′-*O*-sulfamoyl-2′-*O*-methyladenosine	$>10^{-3}$	$>10^{-3}$	

TABLE II: Inhibition of Polyuridylate-Directed Polyphenylalanine Synthesis from L-[^{14}C]Phenylalanine by 5′-*O*-Sulfamoyladenosine.[a]

Length of Incubn (min)	Radioactivity (cpm)[b] Incorporated in		
	Absence[c]	Presence[c]	% Inhibn
4	2,178	284	87
8	6,884	577	92
16	18,160	732	96

[a] Added in the following order, the complete incubation mixture in a total volume of 0.25 ml contained: 25 μmoles of Tris-HCl buffer (pH 7.2), 25 μmoles of ammonium chloride adjusted with NH$_4$OH to pH 7.2, 2.5 μmoles of magnesium acetate, 1.5 μmoles of 2-mercaptoethanol, 0.2 μmole of GTP, 0.1 μmole of ATP, 10 μg of ammonium polyuridylate, 27.4 A_{260} units of sRNA, 8.0 A_{260} units of washed ribosomes, 0.22 mg of protein of the S-100 fraction, and 10 mμmoles of L-[U-^{14}C]phenylalanine with a specific activity of 50 μCi/μmole (60 cpm/μμmole). The inhibitor was added after the addition of the sRNA and before the addition of ribosomes. Incubation was carried out at 25° for the time specified. At the end of the incubation period, 0.1-ml aliquots of the reaction mixtures were applied to duplicate Whatman No. 3MM paper disks, and the hot acid-precipitable material was assayed as described under Methods. [b] Per 0.1 ml of incubation mixture. [c] Of 4×10^{-4} M 5′-*O*-sulfamoyladenosine.

Results

The effect of the analogs on *in vitro* cell growth was evaluated with *S. faecium*, *E. coli*, and leukemia L1210. The results of these tests are summarized in Table I. Whereas the 5′-sulfamate derivatives of adenosine or 2′-deoxyadenosine were inhibitory in all three test systems, the 3′-substituted analog was active only against the bacteria. The other purine or pyrimidine derivatives examined showed no inhibitory activity in the systems in which they were tested.

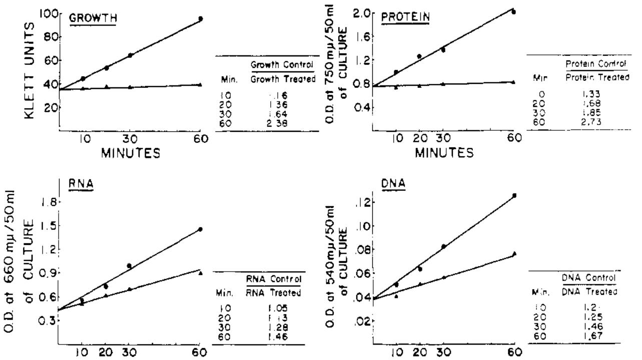

FIGURE 2: Effect of 5′-*O*-sulfamoyladenosine on growth and on protein, RNA, and DNA synthesis in *E. coli* B. A culture of *E. coli* was incubated at 37° until early log phase (Klett reading of 38). The culture was then divided into two, one-half serving as control (●), the other (▲) being immediately exposed to 5×10^{-6} M sulfamoyladenosine. The time of drug addition is taken as zero time At the specified intervals thereafter, 50-ml aliquots were removed from the two cultures, and were assayed for the macromolecular cell components as outlined in the Experimental Section. The values given in the graph are the averages of four separate experiments. An optical density of 0.1 at 750 mμ (1-cm light path) corresponds to approximately 40 μg/ml of crystalline bovine albumin, at 660 mμ to 15 μg/ml of yeast RNA, and at 540 mμ to 86 μg/ml of calf thymus DNA.

An inhibition analysis was carried out in the bacterial systems to gain information concerning the possible site of action of the growth-inhibiting sulfamates. The analysis showed that none of the natural purines or pyrimidines or their nucleosides, either alone or in combination, had any effect on the inhibition. Other metabolites, including the 22 amino acids and the cofactors listed in the Experimental Section, were also without effect. These results paralleled the response seen with certain purine and pyrimidine analogs which interfere with protein synthesis, such as puromycin and amicetin (Bloch and Coutsogeorgopoulos, 1966).

When 5′-*O*-sulfamoyladenosine at 5×10^{-6} M was added to a growing culture of *E. coli*, growth and protein synthesis were inhibited within 10 min (Figure 2). RNA and DNA synthesis continued for approximately 60 min, although at a reduced rate, as expressed quantitatively by the ratios provided in the figure. The RNA and DNA content of the treated cultures did not increase significantly after 60-min incubation. These data indicated that the primary effect of the analog is exerted on protein synthesis. To localize the site of such interference, a cell-free system from *E. coli* B was used. As shown in Table II, the 5′-*O*-sulfamoyl derivative, at 4×10^{-4} M, effectively inhibited the poly(U)-directed synthesis of polyphenylalanine from *free* phenylalanine. When precharged phenylalanyl-RNA was used in place of free phenylalanine (Table III), polyphenylalanine formation was inhibited only to a small extent (1–15%), suggesting that the compound interferes primarily with the synthesis of the aminoacyl-RNA. Indeed, as shown in Table IV, the sulfamate was found to interfere with phenylalanyl-RNA formation to about the same extent as it did with

TABLE III: Effect of 5′-*O*-Sulfamoyladenosine on Polyphenylalanine Synthesis from L-[^{14}C]Phenylalanyl-RNA.[a]

Length of Incubn (min)	Radioactivity (cpm)[b] Incorporated in		% Inhibn
	Absence[c]	Presence[c]	
1	3441	3322	1
2	5874	4969	15
4	5850	5059	12

[a] The complete system was identical with that of Table II except that ATP and sRNA were omitted, and instead of free L-[^{14}C]phenylalanine, 3.2 A_{260} units of L-[^{14}C]phenylalanyl-RNA charged with 50 $\mu\mu$moles of L-[^{14}C]phenylalanine (375 μCi/μmole; 450 cpm/$\mu\mu$mole) were added. The samples were incubated at 25° for the specified time and the reaction mixtures were assayed for hot perchloric acid precipitable material. [b] Per 0.1 ml of incubation mixture. [c] Of 4 × 10^{-4} M 5′-*O*-sulfamoyladenosine.

the synthesis of polyphenylalanine from free phenylalanine in the complete system (Table II). The inhibition was not affected by differences in the length of incubation (2, 4, and 10 min), or by differences in temperature (25 and 37°) (Table IV). The 5′-*N*-sulfamoylamino derivative, which was evaluated at 4 × 10^{-4} M for comparison, inhibited both the poly(U)-directed synthesis of polyphenylalanine from free phenylalanine and the formation of the aminoacyl-RNA to the same extent as did 5′-*O*-sulfamoyladenosine (Table IV). On the other hand, 5′-*O*-sulfamoylinosine, which is biologically inactive, did not interfere with these reactions, even when present at 10^{-3} M.

TABLE IV: Inhibition of L-[^{14}C]Phenylalanyl-RNA Formation by 5′-*O*- or 5′-*N*-Sulfamoyladenosine at Various Time and Temperature Intervals.[a]

Conditions	% Inhibition at		
	2 min/ 25°	4 min/ 25°	10 min/ 37°
Complete system + 5′-*O*-sulfamoyladenosine, 1 × 10^{-3} M	89	90	93
Complete system + 5′-*O*-sulfamoyladenosine, 4 × 10^{-4} M	81	81	79
Complete system + 5′-*O*-sulfamoyladenosine, 1 × 10^{-4} M	45	43	45
Complete system + 5′-*N*-sulfamoylamino-5′-deoxyadenosine, 4 × 10^{-4} M	80	77	78

[a] The complete system was the same as that described in Table II, with the following exceptions. The S-100 fraction was decreased to 0.055 mg of protein, polyuridylic acid and the ribosomes were omitted, and ATP was added at 1 × 10^{-4} M. At 2, 4, and 10 min the incorporation of label in the absence of inhibitor amounted to 3314, 4362, and 6283 cpm, respectively. With 0.2 mg of protein of S-100 fraction the control value for 4 min at 25° was 15,450 cpm, but the degree of inhibition remained the same.

TABLE V: Inhibition of L-[^{14}C]Lysyl-RNA Formation by 5′-*O*-Sulfamoyladenosine at Various Time and Temperature Intervals.[a]

Conditions	% Inhibition at		
	2 min/ 25°	4 min/ 25°	10 min/ 37°
Complete system + 5′-*O*-sulfamoyladenosine, 1 × 10^{-4} M	95	96	96
Complete system + 5′-*O*-sulfamoyladenosine, 1 × 10^{-5} M	98	89	87
Complete system + 5′-*O*-sulfamoyladenosine, 1 × 10^{-6} M	7	5	2

[a] The complete system was the same as that described in Table IV except that L-[^{14}C]lysine (10 μmoles, 50 μCi/μmole) was used in place of phenylalanine. At 2, 4, and 10 min, incorporation of label in the complete system (0.055 mg of S-100 fraction) free of inhibitor amounted to 25,861, 36,747, and 45,817 cpm, respectively. With 0.11 mg protein of the S-100 fraction the control value for 4 min at 25° was 37,907 cpm, but the degree of inhibition remained the same.

The inhibition of aminoacyl-RNA formation by 5′-sulfamoyladenosine also extends to amino acids other than phenylalanine. As shown in Table V, the formation of lysyl-RNA was affected by lower concentrations of the 5′-sulfamate than were required for the inhibition of phenylalanyl-RNA; 90% inhibition occurred at 10^{-5} M. The synthesis of prolyl-RNA was even more sensitive to the action of the analog (Table VI), 80% inhibition occurring at 1 × 10^{-6} M 5′-*O*-sulfamoyladenosine.

In view of the structural resemblance of 5′-*O*-sulfamoyladenosine to 5′-AMP, it was of interest to examine whether the analog can be acted upon by some enzymes which catabo-

TABLE VI: Inhibition of L-[^{14}C]Prolyl-RNA Formation by 5′-*O*-Sulfamoyladenosine at Various Time and Temperature Intervals.[a]

Conditions	% Inhibition of		
	2 min/ 25°	4 min/ 25°	10 min/ 37°
Complete system + 5′-*O*-sulfamoyladenosine, 1 × 10^{-6} M	80	83	83
Complete system + 5′-*O*-sulfamoyladenosine, 1 × 10^{-7} M	30	36	32

[a] The complete system was the same as that described in Table IV except that L-[^{14}C]proline (10 μmoles; 50 μCi/μmole) was used in place of phenylalanine and ATP was used at 4 × 10^{-4} M. At 2, 4, and 10 min, incorporation of label in the absence of inhibitor amounted to 4054, 5022, 8510 cpm, respectively. With 0.33 mg of protein of the S-100 fraction, the control value for 4 min at 25° was 24,000 cpm, but the degree of inhibition remained essentially the same.

lize the natural nucleotide. Under conditions where 1 μmole of 5′-AMP is converted, within 1 min, to IMP by AMP-deaminase or to adenosine by snake venom (1 μM unit), no effect on 5′-*O*-sulfamoyladenosine could be detected. Similarly, with a 1 μM unit of adenosine deaminase, no deamination of the sulfamate analog was demonstrable. When the incubation period was extended to 4 hr, approximately 7% of the analog was found deaminated in the presence of the AMP-deaminase. No deamination by adenosine deaminase or cleavage by the snake venom was chromatographically detectable after this extended period.

Sulfamoyladenosine did not interfere with the activity of the adenosine phosphokinases of *S. faecium*, as shown by the fact that the conversion of [8-^{14}C]adenine to its ribonucleoside mono-, di-, and triphosphates proceeded unhindered in the presence of 1×10^{-3} M of the analog. This was true during short-term 5-min incubation, and after 30 min, when the nucleotides formed amounted to approximately 15 and 80%, respectively, of the adenine supplied.

Like nucleocidin, the 5′-sulfamate of adenosine proved to be rather toxic. Its LD_{50} in DBA/2 mice was 0.48 mg/kg when given intraperitoneally once, and 0.03 mg/kg when administered on 5 consecutive days. The corresponding values in Sprague-Dawley rats were 0.44 and 0.1 mg per kg.

Discussion

5′-*O*-Sulfamoyladenosine lacks the fluorine atom which is present at the 4′-carbon of nucleocidin, but this lack does not abolish biological activity. The toxicity data for sulfamoyladenosine coincide rather closely with those reported for nucleocidin (Thomas *et al.*, 1956–1957; Hewitt *et al.*, 1956–1957). Whether the site of action of the two compounds is the same remains, however to be seen. As reported by Florini *et al.* (1966), nucleocidin, in a cell-free preparation obtained from rat liver, interfered not with aminoacyl-RNA formation as we have observed with the 5′-*O*- and 5′-*N*-sulfamoyladenosines in the *E. coli* system, but with the transfer of the amino acid from the charged RNA to the polymer. Whether this difference relates to the different systems used, or is conditioned by the presence or absence of the fluorine atom, remains to be determined. Since no nucleocidin was available to us, the needed comparison in *E. coli* could not be made.

In the absence of more detailed studies, one can only speculate on the mechanism responsible for the observed interference of sulfamoyladenosine with aminoacyl-RNA formation. The fact that only the sulfamate derivatives of adenosine or 2′-deoxyadenosine but not those of the other nucleosides are biologically active, and that the sulfamate derivative of inosine, for instance, showed no inhibitory activity in the cell-free polypeptide-synthesizing system, is an indication that the active compounds might act as analogs of adenosine or of one of its derivatives, such as for instance AMP. Such an AMP analog might, for example, inhibit protein synthesis by interfering with the formation of the aminoacyl-AMP–enzyme intermediate during amino acid–tRNA formation.

Acknowledgments

The excellent technical assistance of Miss R. Hromchak, and the Messrs. R. J. Maue, J. T. Miller, and R. L. Tisdale is gratefully acknowledged. The sulfamoyl derivatives were generously supplied by Dr. R. K. Robins and Dr. M. J. Robins of the University of Utah. The toxicity data were kindly provided by Dr. E. Mihich of this department.

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