BIOCHEMICAL STUDY OF MINOSAMINOMYCIN IN RELATION TO THE KASUGAMYCIN GROUP ANTIBIOTICS

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Minosaminomycin is structurally related to kasugamycin and inhibits protein synthesis in mycobacteria. It also inhibits phage f2 RNA-directed protein synthesis in a cell-free system of *Escherichia coli* by 50% at 2×10^{-7} M. It is 100-times more potent than kasugamycin in this system. At 10^{-7} M minosaminomycin inhibits EF-T dependent binding of aminoacyl-tRNA to ribosomes by 50%. This effect is markedly diminished if minosaminomycin is added to the assay system after a brief incubation of ribosomes with mRNA. Like kasugamycin, minosaminomycin preferentially inhibits the initiation of protein synthesis directed by phage f2 RNA *in vitro* and does not cause miscoding. Ribosomes from kasugamycin-resistant mutants Ksg A and Ksg C were as sensitive to minosaminomycin as those from each parent strain. In spite of the strong inhibitory activity of minosaminomycin manifested in cell-free systems of *E. coli*, this compound inhibits the growth of the organism itself only slightly. This discrepancy could be ascribed to impermeability, as *E. coli* cells with modified permeability show greater sensitivity to minosaminomycin. There is no indication that the antibiotic is inactivated in *E. coli* cells. On the basis of these results, the structural features of these antibiotics essential for interaction with ribosomes and for permeability into the cells are discussed.

Minosaminomycin (MSM) was isolated as an inhibitor of the growth of mycobacteria by HAMADA *et al.*[1] and its structure was determined by IINUMA *et al.*[2]. Based on its structural similarity to kasugamycin (KSM), we presumed that MSM was also an inhibitor of initiation of protein synthesis[3]. It was found that MSM inhibited the synthesis of protein in mycobacteria. Since the cell-free protein synthesizing system of *E. coli* is well established and was found to be strongly inhibited by MSM, this system was utilized for a detailed study of the mode of action of MSM. The reason for the lack of activity of MSM on *E. coli* was investigated. Structure-activity studies defining features essential for binding to ribosomes and those essential for cell permeation are considered in this paper.

Materials and Methods

In vitro protein synthesis and related reactions.

In vitro protein synthesis using S-30 extracts of *E. coli* Q13 and phage f2 RNA as mRNA was conducted as previously reported[4]. In brief, a reaction mixture contained in 100 μl, 50 mM Tris-HCl, pH 7.8, 3 mM ATP, 0.2 mM GTP, 60 mM NH_4Cl, 2 mM phosphoenol pyruvate, 5 μg of pyruvate kinase, 2 mM dithiothreitol, 18 μM[^{14}C]Val (233 mCi/m mole), 20 μM each of 19 amino acids, 10 mM $Mg(OAc)_2$, 0.6 A_{260} of uncharged tRNA, 0.2 mg protein equivalent of S-30, a desired amount of a test compound and 1.7 A_{260} of phage f2 RNA. After incubation at 37°C for 20 minutes, radioactivity in the protein fraction was determined[5].

Initiation-free polysomes were prepared as follows: Cells of *E. coli* B in early exponential phase

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(0.2 A_{600}, 80 ml culture) were harvested on crushed ice and submitted to gentle lysis as described by FLESSEL.[6] The lysate (1 ml) containing polysomes was spun at 130,000 *g* for 17 hours in a centrifuge tube containing 1 ml of 70% sucrose in TMN (10 mM Tris-HCl (pH 7.8)-10 mM $Mg(OAc)_2$-50 mM NH_4Cl) and 3 ml of 30% sucrose in TMN. The polysomes were suspended by gentle mixing in TMN containing 6 mM mercaptoethanol, divided into small portions, and stored at −90°C until use.

KSM-resistant strains; Ksg A (*E. coli* TPR201) and its parent strain (*E. coli* PR7)[7] were kindly supplied by Dr. J. E. DAVIES, University of Wisconsin, and Ksg C (*E. coli* AB312 Ksg 2) and its parent strain (*E. coli* AB312)[8] by Dr. M. YOSHIKAWA, University of Tokyo.

Plasmolysis of *E. coli* was performed according to the method of GROS *et al.*[9] with a minor modification; washed cells of *E. coli* Q13 were suspended in 2 M sucrose-0.01 M Tris-HCl, pH 8.0, at a density of about 2×10^{10} cells/ml and were kept at 30°C for 10 minutes (plasmolyzed cells). For comparison, a portion of washed cells was submitted to the same procedures except that sucrose was omitted from the buffer (untreated cells). After chilling, the cells were freed from the sucrose by centrifugation.

Results

Effect of MSM on Synthesis of Cellular Macromolecules in *Mycobacteria*

The effect of MSM on the synthesis of proteins, cell walls and nucleic acids by exponentially growing cells of mycobacteria is shown in Fig. 1. MSM strongly inhibited the incorporation of amino acids into acid-insoluble cell material, in other words, inhibited protein synthesis. Other macromolecular synthesis was relatively unaffected.

Permeability Barrier to MSM in *E. coli*

The antibiotic spectrum of MSM is relatively narrow. From the following observations, the resistance of *E. coli* to MSM seems to be due to a permeability barrier. Protein synthesis in *E. coli* cells subjected to plasmolysis, thus modifying their permeability, was inhibited more strongly than that in untreated cells (Table 1). However, complete inhibition could not be obtained. The plasmolysis was confirmed by the inhibitory effect of actinomycin D on the RNA synthesis in these cells. Attempts to alter cellular permeability to MSM by other means, such as osmotic shock[10] or treatment with phenethyl alcohol[11] was unsuccessful. Incubation of a MSM solution with a sonicate of cells of *E. coli* did not lower significantly its antibiotic titer (data not shown). Therefore, it is

Fig. 1. Effect of minosaminomycin on macromolecular synthesis in *Mycobacterium* 607

Mycobacterium 607 was grown in two culture tubes containing 10 ml each of casamino acids-glucose medium supplemented with 1% (w/v) glycerol and 1% (w/v) Tween 80 at 27°C with shaking. When the cultures reached a cell density of 0.1 A_{600}, they were combined and divided into 2 ml portions in short test tubes, which were grouped into 3 sets (A, B and C) of 2 tubes each. To each tube of (A), (B) and (C), 20 μl of [^{14}C] amino acid mixture (2 μCi), 20 μl of [^{14}C]N-acetylglucosamine (10 μCi/ml; 3 μCi/m mole) and 10 μl of [^{14}C]adenine (100 μCi/ml; 4.4 mCi/m mole) were added. One tube in each set received 30 μl of minosaminomycin solution to give a final concentration of 3×10^{-4}M while the other received 30 μl of water. Incubation was at 27°C and at a time indicated a 100 μl sample was transferred from a tube to a paper disc (Whatman 3 MM, 2.4 cm diameter), to determine the radioactivity incorporated into hot TCA insoluble materials (for [^{14}C]amino acids (A)) or cold TCA insoluble materials (for [^{14}C]N-acetylglucosamine (B) and [^{14}C]adenine (C))[14].

(○) without antibiotic.
(●) with 3×10^{-4}M minosaminomycin.

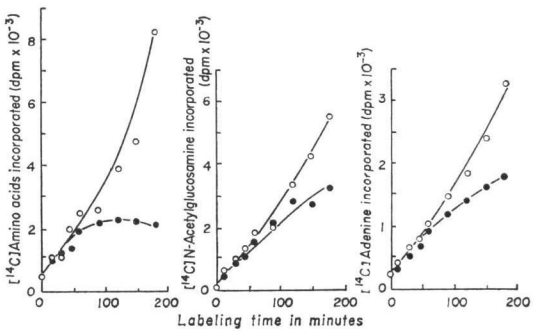


Table 1. Effect of minosaminomycin on protein synthesis in *E. coli* cells with and without plasmolysis

		% Inhibition				
		10 μg/ml	25 μg/ml	50 μg/ml	100 μg/ml	250 μg/ml
Untreated cells	Minosaminomycin	—	—	26	23	39
	Chloramphenicol	—	—	—	84	—
	Actinomycin D	—	—	4	—	—
Plasmolyzed cells	Minosaminomycin	33	39	50	65	—
	Chloramphenicol	—	—	—	93	—
	Actinomycin D	—	—	90	—	—

A reaction mixture for determination of protein synthesis contained in 100 μl, 5×10^7 cells, 8 mM Tris-HCl, pH 8.0, 100 mμCi of [^{14}C]Leu (348 mCi/m mole) and a desired amount of a test compound. A reaction mixture for determination of RNA synthesis contained in 100 μl, 5×10^7 plasmolyzed cells, 5 mM Tris-HCl, pH 8.0, 200 mμCi of [^{3}H]UTP (20 mCi/m mole), 4 mM $MnCl_2$, 50 mM KCl, 4 mM 2-mercaptoethanol, 0.5 mM ATP, 0.6 mM GTP, 0.6 mM CTP and a desired amount of a test compound. With the untreated cells, a reaction mixture for determination of RNA synthesis contained in 100 μl, 5×10^7 cells, 70 μl of Casein hydrolysate-glucose medium, 100 mμCi of [^{3}H]uridine (50 mCi/m mole), a desired amount of a test compound and water. After incubation at 37°C for 30 minutes, a 90-μl portion of a cell suspension was transferred to a paper disc for determination of radioactivity incorporated into acid-insoluble materials[14]. The effects of minosaminomycin and chloramphenicol were determined by [^{14}C]Leu incorporation while that of actinomycin D by [^{3}H]uridine or [^{3}H]UTP incorporation. Radioactivities of control runs receiving no antibiotics were 725 dpm and 432 dpm for [^{14}C]Leu-labeled untreated cells and plasmolyzed cells, respectively; and 7399 dpm for [^{3}H]uridine-labeled untreated cells and 3865 dpm for [^{3}H]UTP-labeled plasmolyzed cells.

Table 2. Effect of various antibiotics on poly (U)-directed [^{14}C]Phe or [^{14}C]Ile incorporation

	[^{14}C]Phe (% inhibition)			[^{14}C]Ile (Extent of stimulation)
	10^{-6} M	10^{-5} M	10^{-4} M	2×10^{-4} M
Minosaminomycin	35	81	91	0.72
Minobiosamine	0	0	0	1.03
Kasugamycin	0	0	0	1.27
Kasuganobiosamine	0	0	0	1.08
Kasugamycin KG-8	0	0	0	1.03
Kasugamycinic acid	—	—	—	0.96
Kanamycin	31	57	64	—
Negamycin	—	—	—	13.64

Protein synthesis directed by poly(U), was conducted as reported previously[12] with minor modifications as follows: The reaction mixture contained in 100 μl, 2 mM phosphoenol pyruvate, 4.7 μM [^{14}C]Phe (422 mCi/m mole) for poly (U), 10 μM each of 19 amino acids, 1.33 A_{260} of uncharged tRNA, 75 μg protein equivalent of S-30. The assay mixtures were incubated at 26°C for 30 minutes. The miscoding effect, *e.g.* stimulation of [^{14}C]Ile (270 mCi/m mole) incorporation into peptide chains directed by poly(U), was determined under similar conditions.

unlikely that the resistance of *E. coli* could be due to the enzymatic inactivation of MSM.

Effect of MSM on Poly(U)-Directed Protein Synthesis with an *E. coli* Cell-free System

The above experiments suggested that a cell-free protein synthesizing system of *E. coli* would be sensitive to MSM, as the permeability barrier is removed. This was tested by determining the effect on poly(U)-directed protein synthesis *in vitro* and the result is shown in Table 2. MSM, but

Table 3. Effect of minosaminomycin on protein synthesis with initiation-free polysomes

	Concentration (M)	% Inhibition
Minosaminomycin	10^{-3}	23
	2×10^{-4}	21
	2×10^{-5}	15
Kasugamycin	10^{-3}	0
Chloramphenicol	6×10^{-4}	91

Protein synthesis with initiation-free polysomes (see Methods) was performed as reported by TAI *et al.*[15]. Under these conditions, protein synthesis leveled off after 20 minutes of incubation at 37°C. The reaction speed, determined in the initial 5 minutes, paralleled the concentration of polysomes up to 1.0 A_{260} nm/0.1 ml of the assay mixture. Magnesium concentration (6~20 mM) did not influence the inhibitory effect of minosaminomycin. The assay was conducted with 100 μl reaction mixtures (10 mM Mg $(OAc)_2$ and 0.3 A_{260} polysomes) at 37°C for 20 minutes. [^{14}C]Val incorporation was determined.

Fig. 2. Effect of minosaminomycin on the amount of ribosome-bound peptides and released peptides (*in vitro* system directed by phage f2 RNA)

For analysis of the amounts of ribosome-bound peptides and released peptides, the volume of the reaction mixture (phage f2 RNA-directed protein synthesis; see Methods) was increased to 200 μl. After incubation, a 100 μl portion of each mixture was submitted to sucrose density gradient centrifugation analysis which was conducted as reported previously[4].

(○) no antibiotic. (●) with 2×10^{-4}M minosaminomycin.

The arrows indicate the time of minosaminomycin addition.

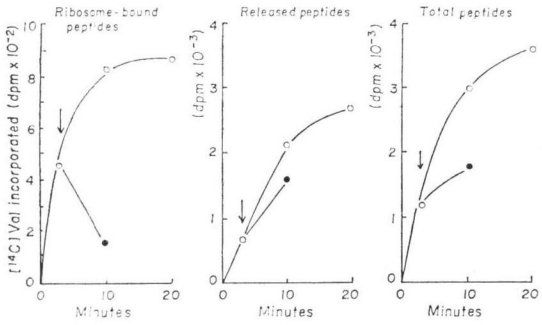
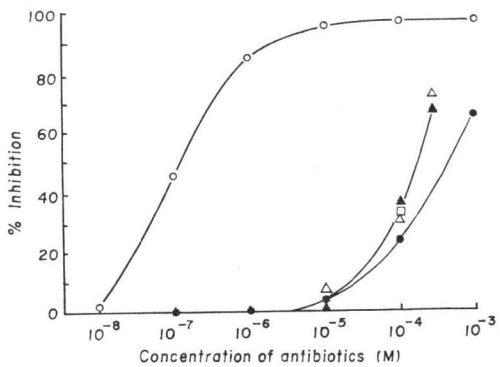


Fig. 3. Effect of minosaminomycin on EF-T dependent binding of aa-tRNA to ribosomes

The enzymatic binding of Phe-tRNA to ribosomes[18] was determined as follows. Reaction mixtures contained in 100 μl, 50 mM Tris-HCl, pH 7.5, 150 mM NH_4Cl, 10 mM $Mg(OAc)_2$, 10 mM 2-mercaptoethanol., 0.1 mM GTP, 0.2 A_{260} of uncharged tRNA, 31.5 μg of EF-T, 0.08 μM of [^{14}C]Phe-tRNA (200 mCi/m mole), 10 μg of poly (U), an indicated amount of an antibiotic, and water. The mixture was incubated at 37°C for 10 minutes and filtered on a nitrocellulose filter and radioactivity retained on the filter was determined[17].

(○), (□) and (△) represent minosaminomycin, kasugamycin and tetracycline, respectively. In a separate experiment, a reaction mixture containing all these components except EF-T, [^{14}C]Phe-tRNA and an antibiotic in 80 μl was incubated at 37°C for 5 minutes and chilled. Then the remaining components (in 20 μl) were added to the preincubated mixture in the cold and incubation was continued 37°C for 10 minutes. Bound radioactivity was determined as described above. Magnesium concentration was 10 mM throughout these 2 incubation processes. (●) and (▲) represent minosaminomycin and tetracycline, respectively.



not KSM or kasugamycin KG-8 (a chemical derivative of KSM, see Table 5 for the structure), was active. MSM inhibited this reaction as strongly as kanamycin, a member of a bactericidal group of aminosugar antibiotics. In view of the marked difference of the activity between MSM and the other two members of this family (KSM and kasugamycin KG-8), it seemed possible that MSM might have some other mode of action, for instance, miscoding. This was tested by measuring the poly(U)-directed incorporation of [^{14}C]isoleucine into

Fig. 4. Drug-sensitivities of ribosomes from kasugamycin-sensitive and kasugamycin-resistant *E.coli*

(a) Crude ribosomes, sedimented at 30,000~100,000 *g*, were obtained from *E. coli* strains TPR201 (Ksg A) and its parent PR7[7]. *In vitro* protein synthesis directed by phage f2 RNA as mRNA was performed as described in methods with the following modifications; S30 was replaced by crude ribosomes (1.8 A_{260} and 1.3 A_{260} for TPR201 and PR7, respectively) and 0.1 mg protein of S105 from *E. coli* Q13, and 17.5 μM each of 19 amino acids and 1.2 A_{260} of phage f2 RNA were used.

(b) S30 fractions were prepared from *E. coli* strain AB312 Ksg 2 (Ksg C) and its parent AB312[8], and *in vitro* protein synthesis was performed with phage f2 RNA as mRNA (see Methods).

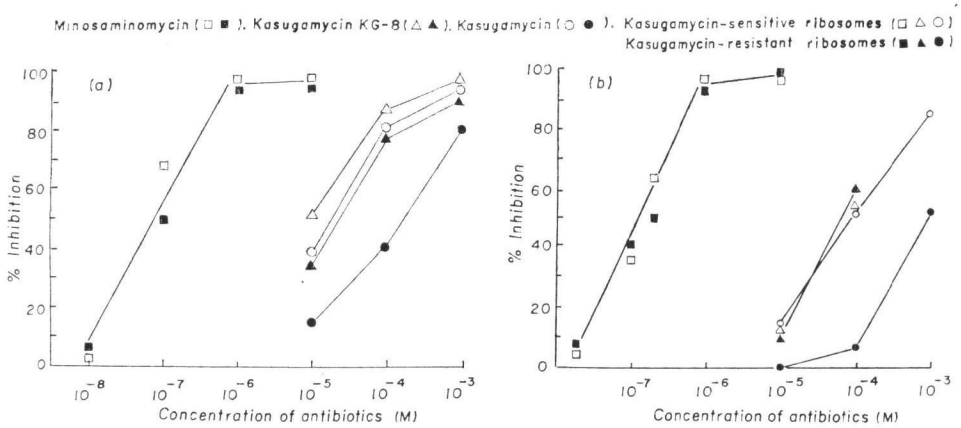


Table 4. Effect of various antibiotics on binding of f[^{14}C]Met-tRNA to ribosomes

Messenger		% Inhibition				
		10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M	10^{-3} M
AUG	Minosaminomycin	36	96	100	—	—
AUG	Kasugamycin	—	0	50	80	—
AUG	Kasugamycin KG-8	—	—	67	86	89
AUG	Streptomycin	—	—	89	89	—
f2RNA	Minosaminomycin	12	76	100	—	—
f2RNA	Kasugamycin	—	5	31	82	—
f2RNA	Kasugamycin KG-8	—	—	57	92	97
f2RNA	Streptomycin	—	—	—	80	—
Poly (G, U)	Minosaminomycin	—	—	96	—	—
Poly (G, U)	Kasugamycin	—	—	45	76	—
Poly (G, U)	Streptomycin	—	—	—	55	—
Poly (G, U)	Kanamycin	—	—	—	35	—

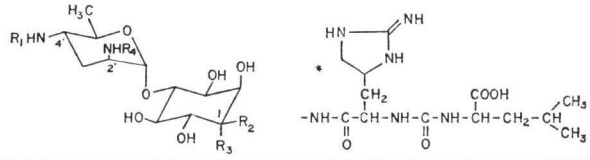
Binding of f[^{14}C]Met-tRNA to ribosomes in the presence of an appropriate template was determined according to the method of DUBNOFF and MAITRA[16]. A reaction mixture contained in 100 μl, 50 mM Tris-HCl, pH 7.5, 80 mM NH_4Cl, 6 mM $Mg(OAc)_2$, 4 mM dithiothreitol, 0.1 mg of crude initiation factors, a template (0.1 A_{260} of AUG, 0.2 A_{260} of poly(G, U) or 1.7 A_{260} of phage f2 RNA), 1.3 A_{260} of washed ribosomes, 0.15 μM f[^{14}C]Met-tRNA (255 mCi/m mole), and antibiotic and water to volume. Incubation conditions were varied depending on template; 25°C for 10 minutes for AUG, 37°C for 10 minutes for poly(G,U) and 37°C for 15 minutes for phage f2 RNA. Radioactivity retained on nitrocellulose filters was determined[17].

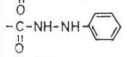
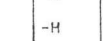
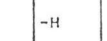
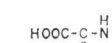
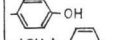
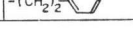
polypeptide chains (Table 2). MSM showed no miscoding effect. As reference, the miscoding activity of negamycin[12], determined under the same conditions, is shown. Similar results were obtained in poly(C)- and poly(A)-directed incorporation experiments.

Inhibition by MSM of Initiation of Protein Synthesis *In Vitro*

Protein synthesis can be divided into 3 distinct steps; initiation, elongation and termination. To determine on which step MSM acts, an *in vitro* protein synthesizing system directed by phage f2 RNA was utilized. Protein synthesis was initiated in the absence of MSM for 3 minutes. MSM was then added and incubation was continued for 10 or 20 minutes. Incorporation of [^{14}C] valine into ribosome-bound and released peptides were determined at the times indicated. As shown in Fig. 2, after addition of MSM, the amount of ribosome-bound peptides decreased rapidly, while that of released peptides continued to increase at a rate comparable to that of the control. These results indicated that MSM primarily inhibited initiation, but had no or a slight effect on the elongation and termination processes. This observation was confirmed by a separate experiment in which the effect of MSM on protein synthesis by initiation-free polysomes was determined. As shown in Table 3, MSM had a minor effect under conditions where ribosomes in the polysomal structure only run off from mRNA but do not initiate another round of protein synthesis. In addition, MSM did not inhibit the peptidyltransferase reaction; MSM at 10^{-4}M did not inhibit release of peptidyl puromycin in a reaction mixture containing [^{3}H]puromycin and derived monosomes carrying nascent peptides[13] under conditions where 6×10^{-4}M chloramphenicol showed 77% inhibition (data not shown).

Table 5. Structures of kasugamycin family antibiotics and their effects on f2 RNA-directed protein synthesis



	R_1	R_2	R_3	R_4	% Inhibition at 2×10^{-4}M or (1×10^{-6}M)
Minosaminomycin	-H	*	-H	-H	89 (90)
Minobiosamine	-H	$-NH_2$	-H	-H	6
Kasugamycin	$-C(=NH)-COOH$	-H	-OH	-H	81 (0)
Kasuganobiosamine	-H	-H	-OH	-H	0
Kasugamycinic acid	$-C(=O)-COOH$	-H	-OH	-H	31
Kasugamycin KG-8	$-C(=NH)-NH-CH_2-CH_2-NH_2$	-H	-OH	-H	65 (0)
TK-63	$-C(=O)-NH-NH_2$	-H	-OH	-H	58
TK-67	$-C(=O)-NH-NH-$ 	-H	-OH	-H	-237
TK-74	$-C(=O)-NH_2$	-H	-OH	-H	44
TK-27	$-C(=NH)-COOH$	-H	-OH	$-CH_2-$ 	50
TK-42	$-C(=NH)-COOH$	-H	-OH	$-CH_2-$  $N(CH_3)_2$	2
TK-9					2
TK-11					-17
TK-12					-13
TK-16	$-(CH_2)_2-$ 				-11

Protein synthesis was assayed as described in Methods. As reference, antimicrobial activities against *E. coli* K12 determined by the agar dilution method were 100 μg/ml for minosaminomycin, 50 μg/ml for minobiosamine, 200 μg/ml for kasugamycin and 25 μg/ml for kasugamycin KG-8.

Effect of MSM on Binding of f[^{14}C]Met-tRNA to Ribosomes

In the initiation process of protein synthesis, f[^{14}C]Met-tRNA binding to ribosomes is directed by an RNA template containing the AUG or GUG sequence. The effect of MSM on the binding of f[^{14}C]Met-tRNA to ribosomes in the presence of AUG, poly(G,U) or phage f2 RNA was determined. MSM at 10^{-6} M showed 70~90% of inhibition depending on templates, as shown in Table 4. On

a molar concentration basis, this inhibition was more than 100-times that of KSM and kasugamycin KG-8. These results indicated that MSM, like KSM, primarily inhibits the formation of the initiation complex[3].

Effect on Binding of [^{14}C]Phe-tRNA to Ribosomes

The data shown so far indicates that MSM primarily inhibits the initiation but has only a slight effect on the elongation and termination processes in protein synthesis (Fig. 2, Tables 3, 4). Therefore, one would presume that MSM has very little or no effect on binding of aminoacyl-tRNA to ribosomes. However this was not the case. As shown in Fig. 3, MSM strongly inhibited this reaction; 50% inhibition was observed at 10^{-7} M MSM. However, the inhibitory effect of MSM was markedly reduced if ribosomes and mRNA were incubated briefly before the assay was initiated (Fig. 3). It should be noted that the effect of tetracycline was unchanged under these conditions. These results indicate that ribosomes bound to mRNA are much less sensitive to MSM than free ribosomes. This mechanism must explain the specific inhibition by MSM of the initiation process, in which free ribosomes play a role, and the lack of inhibition of the elongation processes where ribosomes are in association with mRNA.

Sensitivity to MSM of Ribosomes from KSM Resistant Strains of *E. coli*

Considering the similarity in the structure and mode of action of MSM and KSM, we presumed that the ribosomes from a KSM resistant strain of *E. coli* would be insensitive to MSM as well as to KSM. This possibility was tested in an *in vitro* protein synthesizing system including ribosome preparations from either KSM resistant or sensitive strains. As Fig. 4 shows, ribosomes from the KSM-resistant strains required about 10 times the concentration of this antibiotic for equivalent inhibition than ribosomes from the sensitive strains. However, the KSM sensitive and resistant ribosome preparations showed the same sensitivity to MSM. An analogous result was obtained for kasugamycin KG-8. The minute structural differences of these compounds could be recognized by ribosomes, although these compounds appear to share a common site of action on the ribosome, leading to inhibition of the initiation step.

Relationship between Structure and *In Vitro* Activity

Various members and derivatives of the KSM family, including MSM, were tested for their effect on ribosomes with a cell-free protein synthesizing system directed by phage f2 RNA. The results are expressed in Table 5 as % inhibition of [^{14}C]Leu incorporation exerted by each compound at the indicated concentrations. Since MSM was a much stronger inhibitor than KSM *in vitro*, the equatorial N-blocked amino group on C-1, as in MSM, seems to be a preferred structure to the axial hydroxyl group on the same carbon, as in KSM. Since minobiosamine, in which the equatorial amino group is free, was only slightly active, the blocked amino group is the preferred structure. With regard to the substituent on C-4′, a free amino group, as in MSM, is sufficient for *in vitro* activity. However, a basic side chain in that position, as in kasugamycin KG-8, presumably increases the permeability of Gram-negative bacteria because kasugamycin KG-8 is about 10-times as active in inhibiting the growth of *E. coli* as KSM. The free amino group on C–2′ is not an essential structure for inhibition of ribosome function, since kasugamycin TK-27 showed some activity. All compounds lacking the inositol moiety were inactive. Based on these observations, we predict that a potent antimicrobial agent may be obtained by introduction of appropriate side chains on minobiosamine. The function of the presumptive side chains on the 4′ and 2′ amino groups may be involved in permeation while

those on the amino group at the C-1 position may be involved both in permeation and in association with ribosomes.

Discussion

The antibiotic MSM, structurally related to KSM, inhibited protein synthesis in *E. coli* cell-free systems more strongly than KSM. MSM inhibited preferentially the initiation step of protein synthesis with no or slight inhibition of elongation and termination (Fig. 2, Tables 3, 4). It neither inhibited the puromycin reaction and the translocation reaction[18] (data not shown) nor caused miscoding (Table 2). In these respects MSM resembled KSM. However, their modes of action were not identical. For instance, MSM inhibited the poly(U)-directed binding of Phe-tRNA to ribosomes ("Phe-tRNA binding") as strongly as it inhibited the binding of fMet-tRNA to ribosomes in the presence of poly (G,U), phage f2 RNA or AUG ("fMet-tRNA binding") (Fig. 3, Table 4). In contrast, KSM was a ten-fold more potent inhibitor of "fMet-tRNA binding" than "Phe-tRNA binding" (Fig. 3, Table 4). In view of the lack of inhibition by MSM of the elongation process, its inhibition of "Phe-tRNA binding" seemed contradictory. This question was answered by the finding that, if ribosomes and poly(U) were preincubated, MSM did not inhibit "Phe-tRNA binding" so strongly; MSM seems to act on free ribosomes but not on ribosomes which are in association with mRNA. Consistent with this notion was the observation that inhibition of polyphenylalanine synthesis by MSM (Table 2) was abolished if the assay was started with a preformed AcPhe-tRNA·ribosome·poly(U) complex (data not shown). The exact nature of the MSM-resistant poly(U)·ribosome complex, formed in 10mM magnesium is not known. In contrast to the results with the poly(U) systems, MSM inhibited "fMet-tRNA binding" even when the ribosomes were preincubated with poly(G,U) or not (data not shown). This supports the fact that MSM has a specific action on the initiation process.

Ribosomes from *E. coli* Ksg A and Ksg C mutants were still sensitive to MSM (Fig. 4). With kasugamycin KG-8, a resistant mutant of *E. coli* was isolated (*E. coli* Q13 KG-8 R). Ribosomes from this mutant compared with those from the parent strain were 10 times more resistant to KG-8 but not to KSM or MSM (data not shown). The lack of co-resistance between these structurally related compounds may reflect detailed differences in their modes of action on ribosomes.

The antimicrobial activity of a drug inhibiting protein synthesis is not solely dependent on its affinity for the ribosome but also on its permeability into and metabolism in target cells. The lack of antimicrobial activity of MSM to *E. coli* appears to be due to impermeability (Table I). Since MSM inhibited the growth of mycobacteria by inhibiting protein synthesis (Fig. 1), it indicated that MSM was permeable to this organism. It is presumed that hydrophilic drugs such as aminosugar antibiotics are taken up into cells through some specific transport systems of normal metabolites. Identification of the transport mechanism is important for future studies on chemical modification of this group of antibiotics.

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