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Mode of Action of Berninamycin. An Inhibitor of Protein Biosynthesis*

Fritz Reusser

ABSTRACT: Berninamycin acts as a specific inhibitor of protein synthesis in *Bacillus subtilis* cells. Experiments with bacterial cell-free macromolecular biosynthetic systems showed that berninamycin interferes with amino acid incorporation into peptides directed by natural messenger ribonucleic acid and synthetic polyribonucleotides. Deoxyribonucleic acid and ribonucleic acid synthesis remained unaffected in cell-free systems. Individual reaction sequences occurring during polypeptide synthesis including aminoacyl transfer ribonucleic acid formation, aggregation to the ternary aminoacyl transfer ribonucleic acid–messenger–ribosome complex, messenger attachment to ribosomes, and peptide-bond formation remained insensitive to the antibiotic. No misreading of the code was apparent in a test system sensitive to streptomycin. These data suggest that the most likely site of berninamycin interaction during polypeptide biosynthesis occurs after formation of the peptide bond and might involve interference with transfer ribonucleic acid release, movement of the nascent peptide chain on the ribosomes, or movement of the messenger.

Berninamycin, an antibacterial agent, was isolated from the culture broth of *Streptomyces bernensis* sp. nova. The compound crystallizes in the form of light beige needles which have very limited solubility in aqueous solutions (<100 μg/ml). Preparation, isolation, characterization, and biological properties will be described elsewhere (O. O. Bergy and F. Reusser, in preparation). Upon acid hydrolysis of the antibiotic, a new sulfur-containing acid, two unknown amino acids, L-threonine, glycine, and a trace of alanine were isolated from the hydrolysate. Structural studies are continuing. The agent is highly inhibitory to gram-positive bacteria *in vitro*, but has been ineffective in the systemic treatment of experimental infections caused by gram-positive organisms in mice.

Materials and Methods

Bacillus subtilis UC-564 cells were grown in a medium containing the following ingredients per liter of deionized water: KH_2PO_4, 6 g; K_2HPO_4, 14 g; $MgSO_4$, 0.1 g; $(NH_4)_2SO_4$, 2 g; sodium glutamate, 2 g; glucose monohydrate, 4 g; and tryptone (Difco), 0.2 g. Shaken flasks containing 100 ml of medium were inoculated with 5 ml of seed derived from an overnight culture. The flasks were incubated on a rotary shaker at 37°. Antibiotic was added at the early logarithmic phase of cell growth.

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The cellular protein and nucleic acid fractions of *B. subtilis* were isolated and assayed as described previously (Reusser, 1967).

DNA and RNA polymerases from *Escherichia coli* were prepared and assayed as described by Reusser (1967).

The cell-free polyribonucleotide directed amino acid incorporation systems were prepared as described by Nirenberg (1963). The 30S fraction was used as an enzyme source. Phage f_2 RNA was obtained from Dr. Nomura, University of Wisconsin, Madison, Wis.

Poly C attachment to ribosomes was assessed by the filtration technique of Moore (1966). Poly U attachment to ribosomes could not be measured by this technique due to a high degree of self-adsorption of poly U to the filters. Poly U binding to ribosomes was thus measured by separating the ribosome–poly U complex by sucrose density gradient centrifugation.

E. coli soluble RNA (tRNA), stripped, was purchased from General Biochemicals. The enzyme source used to catalyze acylation of tRNA with one amino-^{14}C acid and 19 unlabeled amino acids was a dialyzed ribosomal supernatant solution (100 S) prepared as described by Nirenberg (1963). The exact composition of the reaction mixtures is shown in Table V.

The samples were incubated at 37° for 15 min. Separate studies had indicated that the reaction was complete after this time. The reaction was stopped by the addition of 0.5 ml of cold 10% trichloroacetic acid containing 0.5 mg of Celite/ml. The acid-insoluble product was collected on Millipore filters

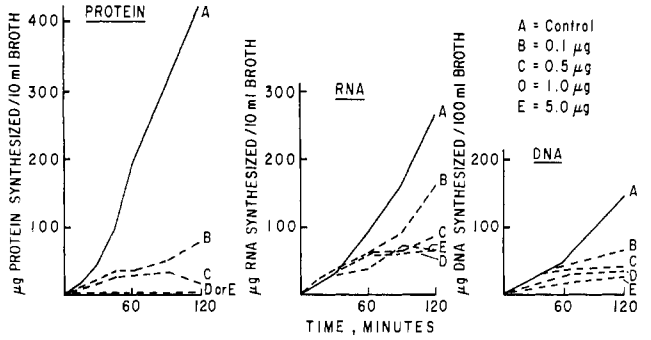

FIGURE 1: Effect of berninamycin on protein and nucleic acid synthesis in *B. subtilis* cells. Cellular protein and nucleic acid fractions were isolated and assayed as described previously (Reusser, 1967).

(0.45 μ). The tubes and filters were washed three times with 2 ml of 5% cold trichloroacetic acid, followed by three times with 2 ml of 70% cold ethanol. The filters were then dried and assayed for radioactivity.

The reaction mixtures used for the preparation of prolyl-^{14}C-tRNA and phenylalanyl-^{14}C-tRNA were those used for assessing transfer activity except: tRNA (2 mg/ml), proline-^{14}C or phenylalanine-^{14}C (8 μCi/ml), and 100S fraction (550 μg of protein/ml). The mixtures were incubated at 30° for 30 min and made acidic by adding 0.25 ml of 2 M sodium acetate buffer. The samples were extracted twice with an equal volume of water-saturated phenol, followed by several ether extractions. Residual ether was removed by gassing with N_2. The charged tRNA was precipitated with 2.2 volumes of cold absolute ethanol. The precipitate was redissolved in enough 0.01 M phosphate (K) buffer (pH 6.1) to yield a tRNA concentration of 2 mg/ml.

Formation of the ternary aminoacyl-^{14}C-tRNA–polyribonucleotide–ribosome complexes was determined by the technique of Nirenberg and Leder (1964).

The puromycin reaction was studied by the method described by Traut and Monro (1964). Ribosomes prelabeled with phenylalanine-^{14}C were prepared by incubating 2.5 ml of the poly U directed incorporation system described in Table III (supplemented with 800 μg/ml of tRNA) for 15 min at 30°. The ribosomes were then washed three times in 0.01 M Tris-HCl buffer (pH 7.3), containing 0.08 M magnesium acetate.

Incubation mixtures for the puromycin reaction were carried out in a total volume of 0.2 ml and contained per ml: Tris-HCl buffer, pH 7.4, 20 μmoles; magnesium acetate, 17.5 μmoles; NH_4Cl, 160 μmoles, mercaptoethanol, 6 μmoles; phosphoenolpyruvate, 6 μmoles; pyruvate kinase, 40 μg; GTP, 0.4 μmole; 100S fraction, 110 μg; and polyphenylalanine-^{14}C-labeled ribosomes, 500 μg. The mixtures were incubated for 5 min at 30°, chilled in ice, and made 0.015 M with EDTA and 0.5% with sodium dodecyl sulfate; 0.05 ml of reaction mixture was applied on 4.6-ml 5–12.5% linear sucrose density gradients containing 0.5% sodium dodecyl sulfate, 0.3 M LiCl, and 0.01 M Tris-HCl buffer (pH 7.4). The tubes were centrifuged in a SW39 rotor at 39,000 rpm for 12 hr at 10°. Four-drop fractions were collected and counted in 15 ml of diotol.

TABLE I: Effect of Berninamycin on Polyribonucleotide-Directed Amino Acid Incorporation.[a]

Sample	System	cpm/mg of 30S Protein	% of Control
Less poly C	Poly C–proline incorporation	70	7
Control, 0 min		50	5
Control		1000	100
Berninamycin, 10 μg/ml		240	24
Berninamycin, 5 μg/ml		270	27
Berninamycin, 1 μg/ml		670	67
Berninamycin, 0.5 μg/ml		770	77
Less poly U	Poly U–phenylalanine incorporation	100	3
Control, 0 min		70	2
Control		3440	100
Berninamycin, 20 μg/ml		2100	60
Berninamycin, 10 μg/ml		1960	56
Berninamycin, 5 μg/ml		2100	61
Berninamycin, 1 μg/ml		2700	78
Less poly A	Poly A–lysine incorporation	100	10
Control, 0 min		30	3
Control		940	100
Berninamycin, 20 μg/ml		1080	114
Berninamycin, 10 μg/ml		1070	113
Berninamycin, 5 μg/ml		900	95
Berninamycin, 1 μg/ml		870	92

[a] Assay mixtures (0.25 ml) were those described by Nirenberg with the following exceptions: poly C, poly U, or poly A, 15 μg; 30S protein, 1 mg. The mixtures were incubated at 37° for 15 min. The reaction was stopped by the addition of 3 ml of cold 10% trichloroacetic acid. The acid-insoluble product remaining after heating the samples for 20 min at 95° was collected on 0.45-μ Millipore filters and counted by liquid scintillation spectrometry. The acid digests of the samples containing polylysine were precipitated and washed with cold 5% trichloroacetic acid containing 0.5% sodium tungstate.

Results

Effect on Protein and Nucleic Acid Synthesis in B. subtilis. Synthesis of cellular protein in *B. subtilis* cells ceased immediately in the presence of 5 or 1 μg per ml of berninamycin; concentrations of 0.5 or 0.1 μg per ml caused only partial inhibition of this process (Figure 1). DNA and RNA syntheses on the other hand were partially inhibited at drug concentrations sufficient to cause effective cessation of protein synthesis (5 or 1 μg per ml). This suggested that berninamycin acts as a specific inhibitor of protein synthesis in *B. subtilis* cells. This conclusion was further substantiated in cell-free macromolecular biosynthetic systems.

Berninamycin had no significant effect on cell-free DNA and RNA polymerase systems when tested over a concentration range from 1 to 10 μg per ml.

Effect on Synthetic Polynucleotide and Natural mRNA-Di-

TABLE II: Effect of Berninamycin on f_2 Phase or Endogenous mRNA-Directed Amino Acid Incorporation.[a]

Sample	System	cpm/mg of 30S Protein	% of Control
Less f_2	f_2 phage–amino-	750	27
Control, 0 min	^{14}C acid	110	4
Control	incorporation	2740	100
Berninamycin, 20 μg/ml		2050	74
Berninamycin, 10 μg/ml		2310	84
Berninamycin, 5 μg/ml		2350	85
Berninamycin, 1 μg/ml		2640	96
Control, 0 min	Endogenous	120	15
Control	messenger–	800	100
Berninamycin, 100 μg/ml	amino-^{14}C	700	88
Berninamycin, 25 μg/ml	acid incor-	780	98
Berninamycin, 10 μg/ml	poration	710	89

[a] Reaction mixtures were those described in Table I except: f_2 phage RNA, 2 OD_{260} units where applicable; mixture of 15 L-amino-^{14}C acids (New England Nuclear), 0.5 μC; mixture of five amino-^{12}C acids, 0.05 μmole/sample.

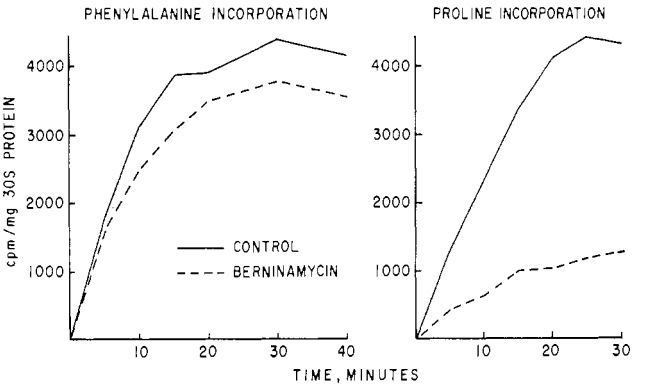

FIGURE 2: Effect of berninamycin on polyribonucleotide-directed phenylalanine and proline incorporation. Assay mixtures were those described in Table III. Berninamycin concentration was 20 μg/ml.

rected Amino Acid Incorporation Systems. Incorporation of phenylalanine-^{14}C or proline-^{14}C directed by synthetic polyribonucleotides was inhibited substantially (Table I). A reasonably strict dose–response relationship was obtained with the poly C directed proline incorporation system. An antibiotic concentration of approximately 2.3 μg/ml was necessary to cause 50% inhibition of the reaction. Poly U directed incorporation of phenylalanine was inhibited by approximately 40% in the presence of 20, 10, or 5 μg per ml of drug; 1 μg/ml caused somewhat less inhibition. The phenylalanine incorporation system was thus not as sensitive to berninamycin inhibition as the proline incorporation system and no strict dose–response relationship was apparent. Poly A directed incorporation of lysine-^{14}C was not inhibited by berninamycin.

A berninamycin concentration of 20 μg/ml caused approximately 25% inhibition of the f_2 phage RNA-directed amino-^{14}C acid incorporation system (Table II). Lower antibiotic concentrations induced correspondingly less inhibition. Concentrations in excess of 20 μg/ml (20–100 μg/ml) did not cause a further increase of inhibition. This indicates that a substantial portion of this reaction remained insensitive to berninamycin inhibition.

In a system where the endogenous messenger RNA contained in the 30S enzyme fraction served as the only primer, berninamycin inhibition of amino acid incorporation remained negligible (Table II).

Time studies with both the phenylalanine and proline incorporation systems showed that inhibition of phenylalanine incorporation by berninamycin was almost absent during the initial 5 min of the reaction. After this period, inhibition became more apparent. Proline incorporation on the other hand was significantly inhibited from the start of the reaction (Figure 2). These data suggested that the exertion of inhibitory activity by berninamycin in the phenylalanine incorporation system might be slower than in the proline incorporation system. Various preincubation times (0–40 min) of 30S enzyme or poly U with antibiotic prior to assay did not result in an increase of inhibitory activity in the phenylalanine incorporation system. The lower sensitivity of this system is thus not simply due to a slow expression of the antibiotic activity at its target site in the test system.

Increasing concentrations of 30S fraction (0.4–1.2 mg of 30S protein/sample) in the phenylalanine incorporation system in the presence of a constant amount of drug (3 μg/ml) did not reverse the activity of berninamycin. Inhibitions of 50–60% occurred in each case. Similarly, increasing concentrations of messenger (7.5–30 μg of poly C/sample) in the proline incorporation system did not result in reversal of the inhibitory activity of berninamycin. The system was inhibited by approximately 50% regardless of the poly C concentration. Variations of tRNA (50–200 μg/ml) in both the proline and phenylalanine incorporation systems had no effect on berninamycin inhibition. The phenylalanine incorporation system was inhibited by 10–12% in each case, the proline incorporation system by 64–69%.

The effect of the magnesium concentration in relation to berninamycin inhibition was studied in the poly C–proline-^{14}C incorporation system which proved to be the most sensitive to berninamycin inhibition. At or near optimal magnesium concentrations, berninamycin inhibition of proline incorporation remained constant and amounted to approximately 66% (Table III). At very low or excessive magnesium concentrations, inhibition was somewhat less and even absent at 10 μmoles of magnesium/sample. At extreme magnesium concentrations incorporation levels were also low in the control samples. The observed smaller extents of berninamycin-induced inhibition in these cases are thus probably technical and do not suggest a direct relationship between magnesium concentration and berninamycin inhibition.

Effect on Polyribonucleotide Attachment to Ribosomes. Attachment of poly-^{3}H C to ribosomes was assessed by the Millipore filtration technique and attachment of poly U by separation of the ribosome–poly U complex on sucrose density gradients. The results of these binding studies show that

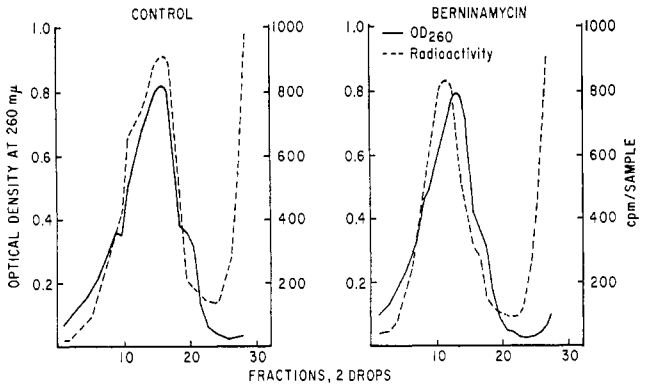

FIGURE 3: Effect of berninamycin on binding of poly U to ribosomes. The reaction mixtures contained in a total volume of 0.25 ml: 0.01 M Tris-HCl buffer (pH 7.8, 0.014 M magnesium acetate, 0.16 M NH_4Cl, 80 OD_{260} units of ribosomes, 12.5 μg of poly-^3H U (0.038 μCi), and 6 μg of berninamycin. All reactants except poly U were incubated for 10 min at 30°, poly U was then added and the mixtures were kept in ice for 20 min. The samples (0.25 ml) were then placed on 4.8-ml linear sucrose gradients (4–20%) prepared in 0.01 M Tris-HCl buffer (pH 7.8), 0.014 M magnesium acetate, and 0.16 M NH_4Cl. The tubes were centifuged at 38,000 rpm for 80 min in an SW39 Spinco rotor; two-drop fractions were collected and assayed for optical density and radioactivity.

berninamycin did not change the binding capacity of ribosomes toward either poly C or poly U (Table IV and Figure 3).

Effect on Charging Activity. Both the formation of prolyl-tRNA and phenylalanyl-tRNA remained unaffected in the presence of antibiotic concentrations ranging from 5 to 40 μg per ml (Table V).

Attachment of Aminoacyl-^{14}C-tRNA to Ribosomes. Formation of aminoacyl-tRNA–mRNA–ribosome complexes was tested in the two systems leading to either the formation of phenylalanyl-tRNA–poly U–ribosome complexes or prolyl-tRNA–poly C–ribosome complexes. As shown in Table VI, 10 or 5 μg per ml of berninamycin failed to significantly affect polyribonucleotide-directed binding of aminoacyl-tRNA to ribosomes. This observation pertains to both poly U and poly C mediated binding. As discussed above, polypeptide formation mediated by either of these two polyribonucleotides is inhibited significantly by 10 or 5 μg per ml of antibiotic.

Miscoding Effect. In an assay system where streptomycin increases poly U directed incorporation of isoleucine-^{14}C, berninamycin caused inhibition of the reaction (Table VII). Thus the antibiotic under study does not cause misreading of the code in a manner similar to streptomycin.

TABLE III: Effect of $MgCl_2$ Concentration on Berninamycin Inhibition of Poly C Directed Proline-^{14}C Incorporation.[a]

$MgCl_2$ (μmoles/ sample)	Incorporation (cpm/mg of 30S protein)		
	Control	Berninamycin (2.4 μg/ml)	% of Control
2	720	620	86
4	4880	3270	66
6	2930	1950	66
8	680	640	93
10	520	520	100

[a] Reaction mixtures were those described in Table I.

TABLE IV: Effect of Berninamycin on Poly C Attachment to Ribosomes.[a]

Sample	cpm/Sample	% of Control
Complete, control	3260	100
Berninamycin, 10 μg/ml	3160	97
Berninamycin, 5 μg/ml	2880	88
Less ribosomes	120	4

[a] The samples contained in a total volume of 0.15 ml: 0.005 M Tris-HCl buffer (pH 7.4), 0.01 M magnesium acetate, 2.4 OD_{260} units of ribosomes, and 10 μg of poly-^3H C containing 0.014 μCi. The mixtures were incubated at room temperature for 20 min, chilled in ice, diluted to 3 ml with Tris buffer, and filtered through 0.45-μ Millipore filters with slow suction. The filters were washed with three 3-ml portions of Tris-HCl buffer, dried, and counted by liquid scintillation spectrometry.

TABLE V: Effect of Berninamycin on Charging Activity of Phenylalanine and Proline to tRNA.[a]

System	Berninamycin (μg/ml)	cpm/ Sample	% of Control
Phenylalanyl-tRNA formation	None	2280	100
	40	1950	85
	20	2170	95
	10	2230	98
	5	2770	120
Prolyl-tRNA formation	None	5530	100
	40	5340	96
	20	5490	99
	10	5430	98
	5	5630	101

[a] Samples contained in a total volume of 0.5 ml: 10 μmoles of Tris-HCl buffer (pH 7.4), 5 μmoles of magnesium acetate, 5 μmoles of KCl, 0.25 μmole of mercaptoethanol, 5 μmoles of phosphoenolpyruvate (trisodium salt), 1.5 μmoles of ATP, 0.1 μmole each of 19 unlabeled amino acids minus either phenylalanine or proline, 0.1 μCi of either proline-^{14}C or phenylalanine-^{14}C, 10 μg of pyruvate kinase (Calbiochem), 100 μg of tRNA, and dialyzed 100S fraction containing 275 μg of protein. Samples were processed as described under Methods.

TABLE VI: Effect of Berninamycin on Aminoacyl-^{14}C-tRNA–mRNA–Ribosome Complex Formation.[a]

Sample	cpm/Sample	% of Control
Poly U, complete, control	8500	100
Berninamycin, 10 μg/ml	9710	114
Less poly U	310	3
Poly C, complete, control	940	100
Berninamycin, 10 μg/ml	890	94
Berninamycin, 5 μg/ml	890	95
Less poly C	190	20

[a] Samples (0.1 ml) contained 0.1 M Tris-HCl buffer (pH 7.2), 0.02 M magnesium acetate, 0.05 M KCl, 2.6 OD_{260} units of washed ribosomes, 10 μg of poly U or poly C, and 100 μg of either phenylalanyl-^{14}C-tRNA or prolyl-^{14}C-tRNA. Incubation was at room temperature for 20 min. The tubes were then chilled in ice, diluted with 3 ml of cold Tris–magnesium acetate–KCl buffer, filtered through 0.45-μ Millipore filters, and assayed for radioactivity (Nirenberg and Leder, 1964).

Effect on Puromycin Reaction. Puromycin inhibits polypeptide biosynthesis by acting as an analog of aminoacyl-tRNA. During the reaction puromycin binds to the nascent peptide chains and causes simultaneous release of the newly formed peptidylpuromycin chains from tRNA bound to ribosomes. To study the effect of berninamycin on release of peptide chains from ribosomes and on peptide-bond formation, ribosomes prelabeled with polyphenylalanine-^{14}C were incubated with berninamycin alone or in combination with puromycin. Addition of puromycin to the reaction mixture leads to the release of polyphenylalanylpuromycin from polyphenylalanyl-tRNA attached to ribosomes. The release can be assessed by sucrose density centrifugation. Polyphenylalanine attached to tRNA sediments more rapidly than released polyphenylalanylpuromycin. The results assembled in Figure 4 show that berninamycin did not cause release of label from ribosomes in the absence of puromycin. In the presence of puromycin, berninamycin did not prevent release of polyphenylalanine from ribosomal-bound tRNA. These results indicate that berninamycin does not induce premature release of peptide chains from ribosomes. In addition, no interference with the peptide-bond formation step appears likely since attachment of puromycin to polyphenylalanine remained unaffected by berninamycin.

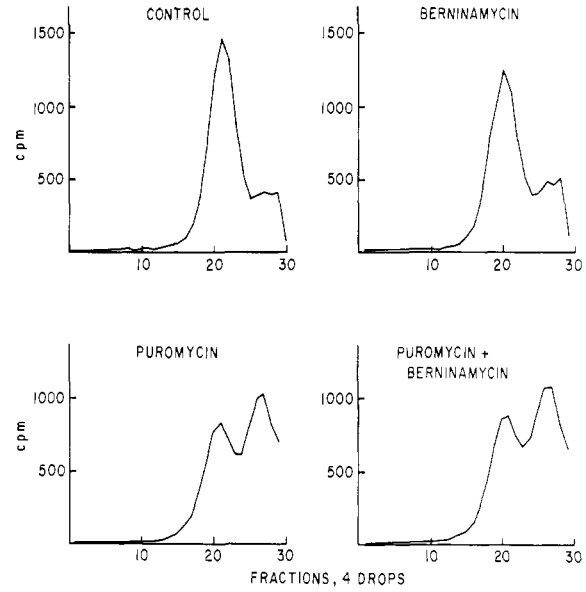

FIGURE 4: Effect of berninamycin on puromycin-induced release of polyphenylalanine from ribosomes. Ribosomes were prelabeled with phenylalanine-^{14}C as described under Methods. Reaction mixtures (0.2 ml) contained per ml: Tris-HCl buffer (pH 7.4), 20 μmoles; magnesium acetate, 17.5 μmoles; NH_4Cl, 160 μmoles; mercaptoethanol, 6 μmoles; phosphoenolpyruvate, 6 μmoles; pyruvate kinase, 40 μg; GTP, 0.4 μmole; 100S fraction, 110 μg; phenylalanine-^{14}C-labeled ribosomes, 4.4 OD_{260} units, 35,500 cpm; puromycin (10^{-3} M), and berninamycin, 40 μg/ml, as indicated. After incubation for 5 min at 30° the reaction was stopped by the addition of EDTA (0.015 M) and sodium dodecyl sulfate (0.5%). tRNA-bound polyphenylalanine and released polyphenylalanine were separated on sucrose density gradients as described under Methods. Four-drop fractions were collected, mixed with 15 ml of diotol, and assayed for radioactivity.

Discussion

Berninamycin acts as a potent and specific inhibitor of protein synthesis in *B. subtilis* cells. A drug concentration of 1 μg/ml in the culture broth was sufficient to cause complete cessation of protein synthesis while some nucleic acid synthesis persisted at a reduced rate.

Experiments in cell-free systems established that the antibiotic inhibits synthetic and natural mRNA directed amino acid incorporation into polypeptides. The extent of inhibition caused by berninamycin is messenger dependent. Poly C directed incorporation of proline was the most sensitive system studied. Although the exact degree of inhibition varied somewhat with different 30S enzyme preparations, an antibiotic concentration of 2–3 μg/ml caused approximately 50% inhibition of proline incorporation. Maximum inhibition attained in the phenylalanine incorporation system was only 40% despite the presence of relatively large antibiotic concentrations. Lysine incorporation was almost insensitive to berninamycin

TABLE VII: Effect of Berninamycin on Poly U Directed Incorporation of Isoleucine.[a]

Sample	cmp/ Sample	% of Control
Control	130	100
Streptomycin, 20 μg/ml	600	460
Streptomycin, 10 μg/ml	620	476
Berninamycin, 10 μg/ml	80	61
Berninamycin, 5 μg/ml	80	61

[a] The assay mixtures were those described under Table III except that L-isoleucine-^{14}C was substituted for phenylalanine-^{14}C.

inhibition. Berninamycin inhibition of the phage RNA mediated amino acid incorporation system reached a plateau at 25% inhibition. Increasing amounts of antibiotic did not cause a further increase of inhibition. Amino acid incorporation with endogenous messenger remained practically insensitive to berninamycin inhibition.

Kinetic studies indicated that berninamycin does not significantly inhibit phenylalanine incorporation during the first 5 min of the reaction. Pretreatment of 30S enzyme or messenger with berninamycin for 30–40 min prior to assay did not increase inhibition. This indicates that the slow manifestation of inhibition by the antibiotic does not merely reflect a slow reaction time at the target site.

Individual reaction sequences occurring during polypeptide synthesis (aminoacyl-tRNA formation, aggregation to the ternary aminoacyl-tRNA–messenger–ribosome complex, messenger attachment to ribosomes) were not impaired by the antibiotic. No misreading of the code was apparent in a system sensitive to streptomycin. Chain initiation is an unlikely target for berninamycin activity since the synthetic messengers used (poly U and poly C) do not contain any known initiation codons. This conclusion is further substantiated by the observation that phage-directed peptide biosynthesis was not very sensitive to berninamycin inhibition. The antibiotic does not cause premature release of newly formed polypeptide chains from the ribosomes and failed to interfere with the peptide-bond-forming step studied with the puromycin reaction. Conceivably, the puromycin system might be deficient in one or several specific steps susceptible to the antibiotic. However, the relative small extent and slow exertion of inhibition observed during poly U directed phenylalanine incorporation support the conclusion that peptide-bond formation is probably not the target of berninamycin activity. Since berninamycin does not interfere with any of the reaction steps discussed above, we assume that the most likely function of the peptide synthesis process impaired by the antibiotic occurs after formation of the peptide bond. The most likely site of berninamycin interaction seems to occur during the interrelated processes of tRNA release, movement of peptidyl-tRNA, and movement of messenger on the ribosomes.

The structure of berninamycin has not been established yet. Preliminary results indicate that the molecule consists of a small peptide which is linked with both its N and C terminals to an unknown sulfur- and nitrogen-containing acid. It is of interest that berninamycin interacts with the peptide synthesis process at or close to the region where the aminoglycoside-type antibiotics such as spectinomycin are presumed to interact (Anderson *et al.*, 1967). The chemical structure of berninamycin bears no obvious relation to the structure of the aminoglycoside antibiotics.

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