MICROBIAL TRANSFORMATION OF PEPTIDE ANTIBIOTICS. III

RELATIONSHIP OF INDUCER STRUCTURE TO INDUCTION OF ACTINOMYCIN DEGRADING ENZYMES IN *ACTINOPLANES MISSOURIENSIS*

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Induced enzymes in *Actinoplanes missouriensis* convert actinomycins to the monolactone and to actinomycinic acid. These enzymes are induced by adding actinomycin, actinomycin monolactone, and peptide derivatives of 4-methyl-3-hydroxyanthranilic acid to washed cell systems. Among the compounds which do not induce these enzymes are the peptide portion of actinomycin, actinomycinic acid, and the heterodetic peptide antibiotics etamycin, vernamycin B, and albomycin. The biosynthesis of the actinomycin degrading enzymes induced by addition of actinomycin was inhibited by simultaneous addition to the washed cells of chloramphenicol, tetracycline, neomycin, streptomycin, KCN, or Ag_2SO_4.

The induction of enzymes in *Actinoplanes missouriensis* degrading actinomycin to actinomycin monolactone and to actinomycinic acid was described in an earlier publication[1]. In this communication we will summarize our observations on the relationship of the chemical structure of the inducer to the induction of this enzyme system.

Materials and Methods

1. Growth of *A. missouriensis.*

Stock cultures of *A. missouriensis* (IMRU no. 824) were maintained on slants of BERGER's tomato paste-oatmeal agar and by storage of vegetative cells in a liquid nitrogen refrigerator. Cells for experimental purposes were obtained by transferring stock culture cells into a cotton plugged 250 ml Erlenmeyer flask containing 100 ml of sterile 3 % soybean meal-3 % glycerol medium. After 2 to 4 days' incubation on a rotary shaker (280 rpm, 1″ throw) at 30°C, some of this vegetative growth was used to inoculate experimental flasks (10 ml per flask in most studies).

2. Preparation of washed cells and cell fractions.

Samples of the *A. missouriensis* cell suspensions were removed from the incubation flasks and centrifuged at $1,000 \times g$ for 10 minutes. The collected cells were suspended in 0.05 M phosphate buffer (pH 7.0), recentrifuged, and resuspended in the buffer, and placed back on the shaker. Inducers were added as described below and the suspension incubated for 18 hours (unless otherwise indicated). At the end of the induction period the cells were collected by centrifugation and resuspended in 0.1 volume of buffer, *e. g.* 10 fold concentration of cells, chilled, and crushed in a French press. The chilled exudates from the press were centrifuged for 30 minutes at $8,000 \times g$ and the supernatant solution retained for study.

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3. Chemicals.

The actinomycin-^{3}H used in these experiments was obtained from Schwarz Bioresearch and the non-radioactive actinomycin was a gift from the Squibb Institute for Medical Research through Dr. A. Aszalos. The 4-methyl-3-hydroxyanthranilic acid peptides were gifts from Dr. H. Weissbach and the peptide portions of the actinomycin peptides were obtained from Dr. J. Meienhofer. Etamycin was a gift from Dr. A. B. Mauger, vernamycin B from Dr. M. Ondetti, and the actinomycin monolactone was prepared chemically as described earlier[1]. The neomycin sulfate, tetracycline hydrochloride, streptomycin sulfate, and chloramphenicol were commercial grade materials and were not purified further prior to use in our experiments.

4. Analytical methods.

The enzymatic activity of the *Actinoplanes* preparations was determined by the procedure mentioned earlier[1]. One enzyme unit converts 1 mcg of actinomycin to actinomycin monolactone in 60 minutes at 40°C. Soluble protein was determined by a modification of the Lowry *et al.* method[2] with bovine serum albumin as standard.

Radioactivity of various samples was determined with a Packard Tricarb Scintillation Spectrometer (model 3002) using Bary's solution as scintillation fluid[3].

Actinomycin, actinomycin monolactone, and actinomycinic acid were identified by filter paper chromatography using Whatman no. 3 MM filterpaper and *n*-butanol - pyridine - water (4 : 1 : 5, upper phase) as developing solvent.

Results and Discussion

Since preliminary experiments showed the somewhat variable rate of induction of actinomycin degrading enzymes in growing cells of *A. missouriensis* (perhaps due to the unusual growth cycle of this organism), we decided to use resting cells for the study of relationship of inducer structure to enzyme induction. Enzyme levels were detected in the French press exudates of the washed cells within 8 hours after addition of the actinomycin to the cell suspensions (see Table 1), and the optimum induction period was about 18 hours. The optimum pH for induction was determined to be pH 7.0 with 0.05 M phosphate buffer, and 30°C as incubation temperature. The French press exudates contained only the lactonase enzyme hydrolyzing actinomycin to the monolactone, and very little activity converting actinomycin to actinomycinic acid was detected.

The optimum level of actinomycin for induction of lactonase was determined to be 10 mcg/ml washed cell suspension (see Table 2), though enzyme activity was detected

Table 1. Actinomycin induction of lactonase enzyme in washed cells of *Actinoplanes missouriensis*

Incubation time after inducer addition (hrs)	Enzyme units/mg protein	
	24 hour old cells	48 hour old cells
0	0	0
1	0	0
2	0	0
4	0	0
8	0.14	0.06
12	1.45	1.53
16	3.36	2.76
24	4.35	3.70
48	3.38	3.55

Table 2. Effect of actinomycin inducer concentration on lactonase enzyme level in washed cells

Actinomycin concentration (mcg/ml)	Enzyme units/mg pratein
0	0
0.0002	0
0.002	0.02
0.02	0.09
0.2	0.86
2	2.73
5	6.06
10	5.31
20	0.15
30	0.06

Table 3. Inhibition of actinomycin induction of lactonase enzyme

Inhibitor	mcg/ml	Enzyme units/mg protein	Inhibitor	mcg/ml	Enzyme units/mg protein
None		6.05	Neomycin sulfate	0.2	5.52
Chloramphenicol	0.1	4.47		0.5	5.15
	0.2	4.01		1	3.95
	1	3.50		2	3.10
	2	2.88		4	2.53
	4	2.70	Streptomycin sulfate	0.25	5.44
Tetracycline hydrochloride	0.02	5.90		0.5	5.23
	0.1	3.39		1	4.02
	0.2	2.46		2	3.05
	0.5	1.98		4	2.45
Ag_2SO_4	50	0	KCN	50	0

at much lower levels of the inducer. Induction of enzyme was inhibited by a variety of antibioitcs and chemicals and some of the data collected are summarized in Table 3. Tetracycline was more effective on a molar basis than the other compounds tested.

Table 4. Relationship of structure to inducer activity

Compound tested	Enzyme units/mg protein
Actinomycin	6.40
Actinomycin monolactone	3.82
Actinomycinic acid	0.24
4-Methyl-3-hydroxyanthranilic acid-Thr-Val	0.90
4-Methyl-3-hydroxyanthranilic acid-Thr-Val-Pro-Sar- N-MeVal	0.73
4-Methyl-3-hydroxyanthranilic acid-Thr-Val-Pro-Sar	0.60
Thr-Val-Pro-Sar- N-MeVal	0.26
N-MeVal- O-Thr	0.22
Sar- N-MeVal- O-Thr	0.14
Etamycin	0.33
Vernamycin B	0.16
Albomycin	0.09

The study of the relationship of structure to inducer activity in this washed cell system is summarized in Table 4. Of the various compounds examined, actinomycin monolactone, and the peptides of 4-methyl-3-hydroxyanthranilic acid show definite activity while the peptide portions of the actinomycin (as represented by Thr - Val - Pro - Sar - N-MeVal, *etc.*) showed no appreciable inducing activity. The N-MeVal - O-Thr and the etamycin and vernamycin B representing the compounds with the lactone bond also were inactive. These findings suggest that the chromophore (or parts of it) have a definite role in the induction of the lactonase splitting actinomycin to the monolactone.

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