ENZYMATIC MODIFICATION OF HYGROMYCIN B IN *STREPTOMYCES HYGROSCOPICUS*

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Antibiotic producing-organisms generally exhibit resistance to the antibiotics that they produce[1]. Such resistance mechanisms may take the form of target site modification, as is the case with erythromycin[2] and thiostrepton[3], or enzymatic modification of the antibiotic itself, as has been shown for a number of aminocyclitols[4].

The existence of these resistance mechanisms in producing organisms is thought to be the means by which the organism avoids growth inhibition by its own antibiotic product although other possibilities cannot be ruled out. For example, in the case of the aminocyclitols the enzymatic modifications may be concerned with specific steps in antibiotic biosynthesis or transport and the fact that the antibiotics are inactivated may be coincidental. It has been suggested that these genes may be evolutionary precursors of the resistance determinants of R-plasmids in clinical isolates of bacteria[5]. Regardless of the role or evolution of antibiotic modifications in producing organisms, the fact that they are biochemically homologous to the determinants of R-plasmids provides a useful means of predicting possible resistance mechanisms that may be encountered in the therapeutic use of antibiotics. Chemical modifications of antibiotics to block these enzymatic modifications could be undertaken in anticipation of the appearance of resistant bacterial strains.

The hygromycin group of antibiotics have not been used extensively, apart from veterinary applications. Hygromycin B, produced by *Streptomyces hygroscopicus*, has a good range of antibacterial activity; a limited number of R-plasmids have been screened for resistant determinants to this drug but the results were negative (J. D. unpublished observations). Since no resistance mechanism had been detected to date, we have examined extracts of *S. hygroscopicus* to see if an enzymatic modification exists for hygromycin in the producing organism.

Streptomyces hygroscopicus (NRRL 2387) was grown in trypticase soy broth and the cells were harvested, washed and resuspended in buffer. The cells were broken by passing the suspension through a French pressure cell (Aminco) at 1,400 kg/cm^2 and the resulting extract centrifuged at 30,000$\times g$ for 30 minutes to remove the cell debris. Samples of the extract were tested for acetyltransferase, adenylyltransferase and phosphotransferase activity, using a variety of substrates. A phosphotransferase activity was detected with hygromycin B and structurally related antibiotics as substrates (Table 1). Note that weak acetyltransferase activity against sisomicin was detected; the enzyme aminoglycoside 3-acetyltransferase appears to be widely distributed in Streptomycetes[6]. Since previous experience with phosphotransferases had shown that detection was often made difficult because of the presence of potent endogenous ATPase activity, the *S. hygroscopicus* extract was passed through a column of Bio-Gel P-100; the protein eluting in the void volume was discarded and assay of material retarded by gel filtration chromatography demonstrated potent hygromycin phosphotrans-

Table 1. Modification of aminocyclitol antibiotics catalyzed by extracts* of *Streptomyces hygroscopicus*.

	Substrate	cpm transferred**
Acetylation	Hygromycin B	208
	Sisomicin	1,048
Adenylylation	Tobramycin	0
	Spectinomycin	0
	Hygromycin B	178
Phosphorylation	Neomycin	1,708
	Sisomicin	2,018
	Streptomycin	1,630
	Hygromycin B	68,797

* In the case of phosphorylation, a crude extract was partially purified by gel-filtration on Bio-Gel P-100.

** Activity transferred in phosphocellulose paper binding assay[4].

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ferase activity. A number of aminocyclitol antibiotics were tested as substrates for the phosphotransferase but none were active; the enzyme demonstrates high specificity for the destomycin-hygromycin antibiotics.

This finding extends the notion that enzymatic inactivation of antibiotics is common among producing organisms and we suggest that should R-plasmid determined resistance to the hygromycin antibiotics appear, phosphorylation is likely to be an important biochemical mechanism. It has been demonstrated, recently, that phosphorylation is the major mechanism of self-protection for a number of aminoglycoside-producing organisms[7]. It would be profitable to study the nature of phosphorylated hygromycin B in the event that extensive clinical use of this antibiotic (or related compounds) is anticipated.

In addition, since hygromycin B is a potent inhibitor of protein synthesis in a wide range of pro- and eukaryotes[8] this drug, in combination with the resistance gene, might be a useful selection system in gene transfer experiments, as has been shown previously for the aminoglycoside antibiotic G418[9].

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