MICROBIAL TRANSFORMATIONS OF ANTIBIOTICS

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ABSTRACT

In spite of their unusual molecular structures and seemingly high degree of biological recalcitrance, many antibiotics have been shown to be susceptible to microbial attack. Among the types of changes brought about by microbial systems are: (i) acylation; (ii) phosphorylation; (iii) adenylylation and ribonucleotide formation; (iv) hydrolysis; (v) oxidation; (vi) sulphoxidation; (vii) reduction; (viii) demethylation; and (ix) deamination.

The choice of organisms to carry out specific types of transformations of an antibiotic has rarely been done on a logical basis. Random screening of cultures known to transform steroids, of cultures which are known to be resistant to the antibiotic under study, of the antibiotic-producing organism, and of organisms selected from soil (or other sources) by enrichment techniques, have all been successfully used. With a few exceptions the transformations so far reported have resulted in biological inactivation of the antibiotic. However, some of the products of the transformation are useful as intermediates in chemical synthesis of new antibiotics, e.g. 6-aminopenicillanic acid for the preparation of new penicillins. Others including streptomycin (formed from mannosidostreptomycin) are more active than the starting material.

I. INTRODUCTION

Although more than thirty years have passed since the first reports of the therapeutic promise of penicillin, and the description of a microbiological transformation of this antibiotic[1], only a limited effort has been invested in using enzymes to produce new and potentially useful antibiotic derivatives. The success of the chemical modification programmes with a number of clinically important antibiotics including penicillin[2], cephalosporin[3], tetracycline[4], lincomycin[5], and rifamycin[6], encouraged large-scale efforts to modify coumermycin A_1^7, kasugamycin[8] and mitomycin[9]. It is one of the purposes of this review to summarize the information on microbiological transformation of antibiotics and to suggest some possible applications of this knowledge.

A brief survey of the literature shows that there are numerous reports on complete loss of antimicrobial activity when actinomycin, chloramphenicol, chlortetracycline, cycloheximide, griseofulvin, mycophenolic acid, oxytetracycline, patulin, penicillin and streptomycin were added to different types of

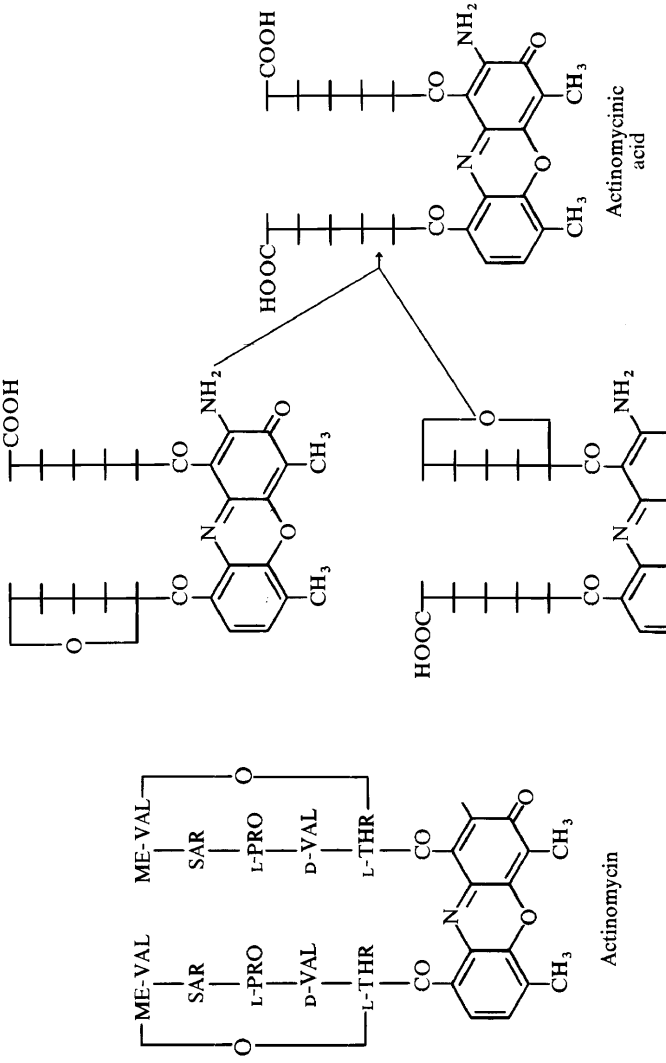

Figure 1. Enzymic transformation of actinomycin.

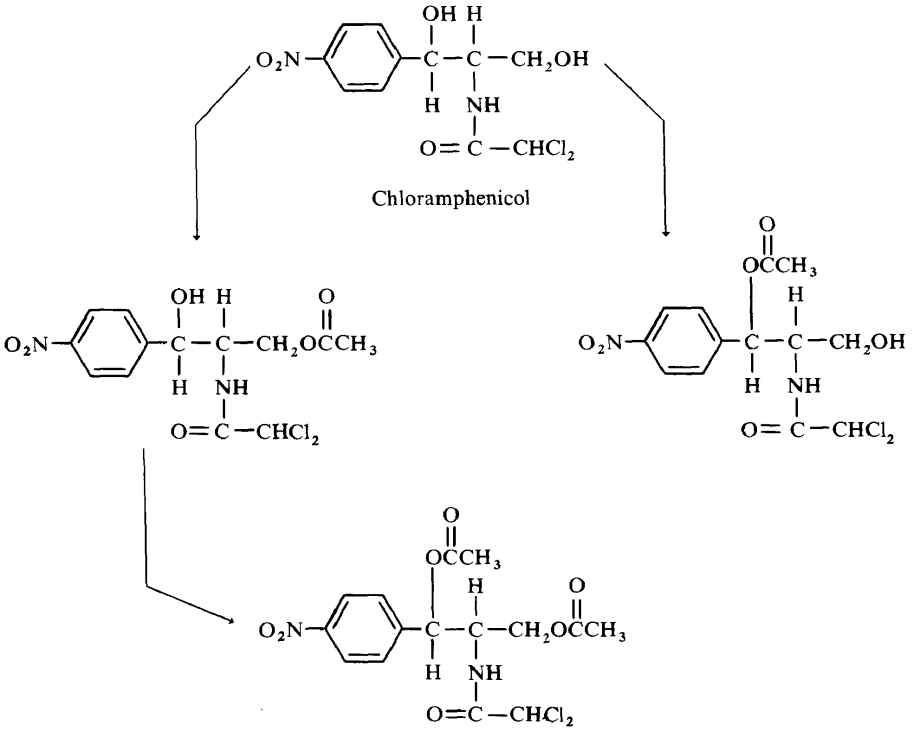

Figure 2. Acetylation of chloramphenicol.

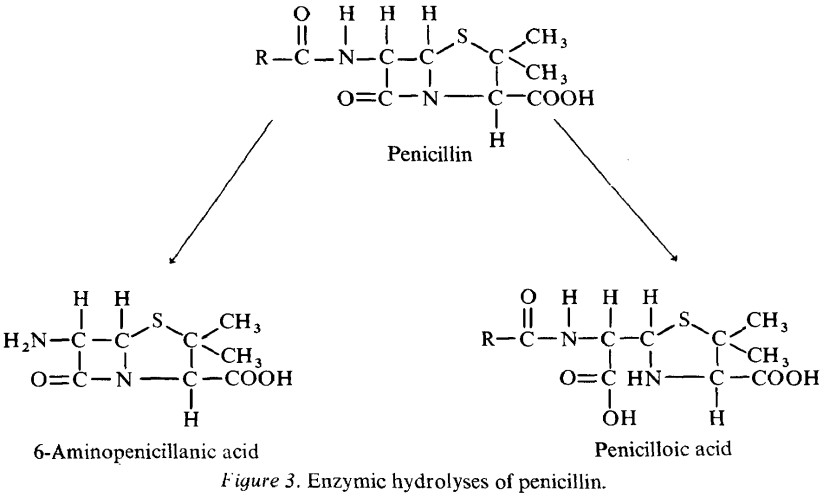

Figure 3. Enzymic hydrolyses of penicillin.

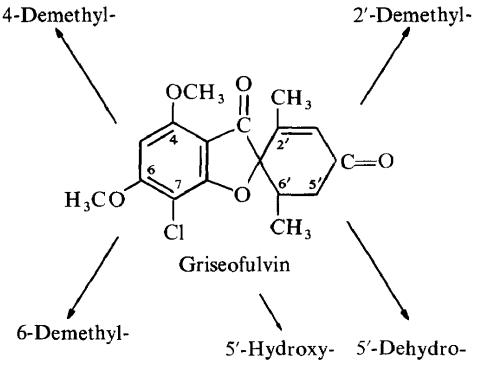

Figure 4. Microbial degradation of griseofulvin.

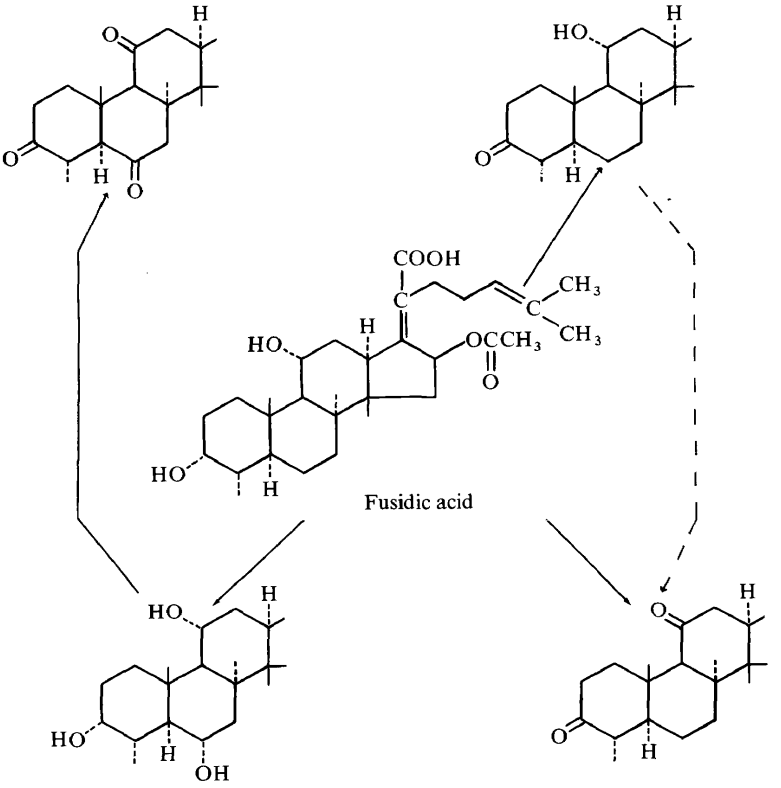

Figure 5. Microbial transformation of fusidic acid and related compounds.

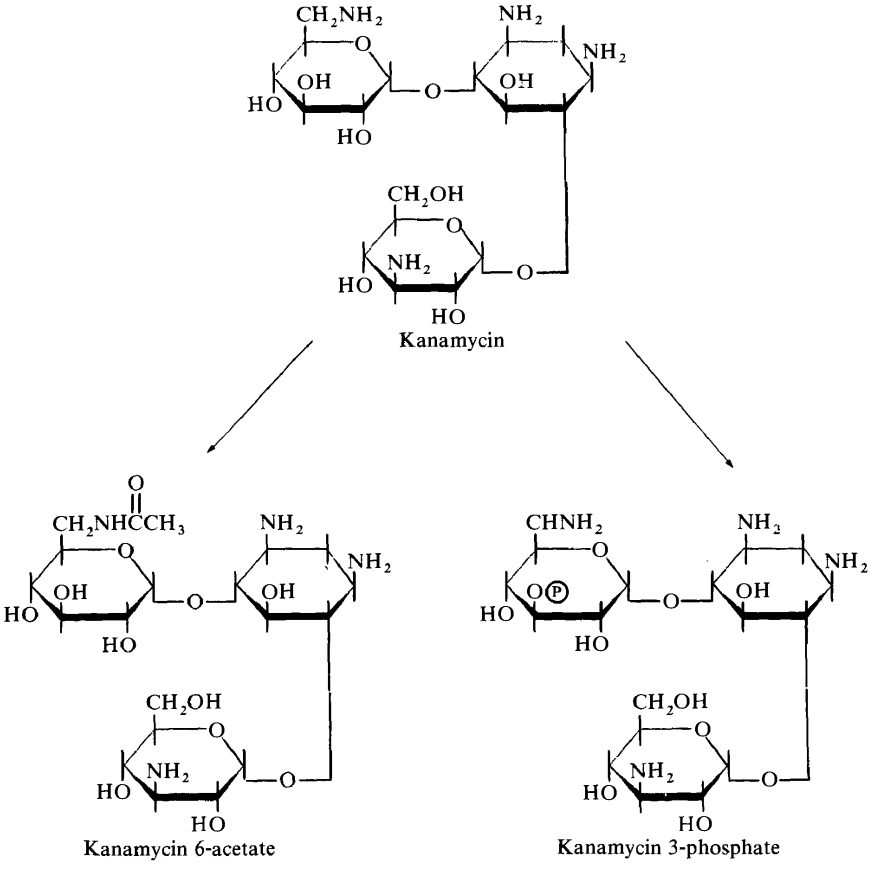

Figure 6. Enzymic transformation of kanamycin A.

soil[10, 11], and both biological and chemical inactivation have been implicated.

The successes of programmes studying microbial transformations of the cyclopentanophenanthrene nucleus and functional groups of steroids[12, 13, 14] have shown that microbial systems can carry out the following types of transformations:

1. Oxidations (including dehydrogenations),
2. Reductions,
3. Esterification, and
4. Hydrolysis.

In addition to these changes, the following are found in the literature on microbial modification of antibiotics:

(i) Adenylylation and ribonucleotide formation,
(ii) Phosphorylation,
(iii) Demethylation, and
(iv) Deamination.

Table 1. Microbial transformations of antibiotics

Antibiotic	*Change noted*	*Reference*
Actinomycin	Hydrolysis of lactones	15, 16, 17
Cephalosporin	Hydrolysis of lactam	18, 19
	Hydrolysis of peptide bond	20
	Hydrolysis of ester	21
Chloramphenicol	Hydrolysis of amide	22, 23
	Reduction of nitro group	22, 24
	Acylation of hydroxyl	25, 26, 27
Circulin	Hydrolysis of peptide ring	28
Clindamycin	Sulphoxide formation	29
	Phosphorylation	30
	Demethylation	29
	Ribonucleotidation	31
Cycloheximide	Acylation	32
Colistin	Hydrolysis of peptide chain	33
Cordycepin	Deamination	34, 35
Echinomycin	Hydrolysis of lactone	17
Etamycin	Hydrolysis of lactone	17
Formycin B	Deamination	36
	Oxidation	37
Fusidic acid	Oxidation (hydroxyl to ketone)	38
	Hydroxylation	39
Gentamicin A	Phosphorylation	40
Gramicidin S	Hydrolysis of peptide ring	41
Griseofulvin	Demethylation	42
	Hydroxylation	43
	Reduction (of dehydrogriseofulvin)	43
Kanamycin	Phosphorylation	44, 45, 46, 47, 48
	Acylation	49
Lincomycin	Sulphoxidation	50
	Demethylation	50
	Phosphorylation	51
Mannosidostreptomycin	Hydrolysis	52, 53
	Adenylylation	54
Mycophenolic acid	Oxidation	55
Nisin	Hydrolysis	56, 57
Paromamine	Phosphorylation	58
Penicillin	Hydrolysis of lactam	59, 60
	Hydrolysis of peptide	61, 62, 63
Polymyxin	Hydrolysis of peptide	28
Rifamycin S	Acylation	64
	Esterification	64
Rifamycin B	Aeacetylation	65
Staphylomycin S	Hydrolysis of lactone	15, 17
Stendomycin	Hydrolysis of lactone	17
Spectinomycin	Adenylylation	66
Spiramycin	Acylation	67
Streptomycin	Adenylylation	47, 68, 69, 70
	Phosphorylation	70, 71, 72
5a,6-Anhydrotetracylcine	Rehydration	73
12a-Deoxytetracycline	Hydroxylation	74, 75
Toyocamycin	Hydrolysis	76
Tylosin	Reduction	77
T-2636 antibiotics	Deacetylation	78, 79
	Dehydrogenation	78, 79
	Acylation	78, 79

Some of the antibiotics reported to undergo these transformations are listed in *Table 1*. The structures of some of these antibiotics, the types of transformations, and the structures of some of the products are shown in *Figures 1* to 6. In addition, there are reports of non-specific degradations of antibiotics: Several antibiotics have been shown to serve as the only source of C, N and energy for bacterial growth, or to serve as growth factors for antibiotic-dependent bacteria. Streptomycin, a growth substance for certain pseudomonads[80], is decomposed by several pathways depending on the culture used[81,82], with urea and streptamine as intermediates.

In a study with a neamine-dependent *Staphylococcus aureus* it was found that those antibiotics containing amino-hexoses and NH_2-groups at C-2 of the hexose, e.g. streptomycin, paromomycin, zygomycin and neomycin C, could substitute for neamine, while those with an NH_2-group at C-3, e.g. kanamycin, erythromycin, or no amino-hexoses, e.g. spectinomycin, vancomycin, did not support growth of the coccus[83]. Similar results were obtained with streptomycin-dependent *Staphylococcus aureus* and *Salmonella paratyphi* B but not with streptomycin-dependent strains of *Escherichia coli* and *Mycobacteria*[83]. The components of the streptomycin molecule as well as streptomycin derivatives were also able to substitute for growth of streptomycin- and neomycin-dependent mutants of *Escherichia coli*[84]. These and other reports[85] are suggestive of antibiotic modification but do not identify the changes in chemical or enzymatic terms.

Similar studies with tetracyclines showed *Xylaria* species capable of inactivating the antibiotics[86], but the chemistry of the degradation products was not reported.

II. TECHNIQUES USEFUL IN STUDYING MICROBIAL TRANSFORMATIONS OF ANTIBIOTICS

Review of the reports on microbial transformations of a number of antibiotics shows that selection of the microorganism depends in part on the background of the investigator. The biochemist and chemist have usually been confident of the value of testing pure cultures obtained from recognized culture collections, while the microbiologist has often utilized the classical enrichment techniques[87,88] so helpful in isolating microorganisms with unusual enzymatic abilities.

Other factors, however, must be considered before more definitive conclusions are drawn. Some antibiotics, especially albidin, frequentin, gliotoxin, penicillin and viridin, have been inactivated due to instability of the natural pH of the soil. Others, including streptomycin and tetracyclines, were inactivated by absorption on clay minerals or organic matter of the soil[11]. Only griseofulvin, mycophenolic acid and patulin appear to be biologically degraded in soil[10,89].

Since antibiotics may serve as both providing C, N and energy for growth, and also interfering with the life processes of the cells themselves, enrichment of the 'open' type is sometimes preferred. Under these conditions non-inhibitory constant antibiotic concentrations are maintained while the metabolic products are continuously removed.

In many studies more or less randomly chosen microorganisms have been

grown in complex media to promote rapid and heavy cell growth. The antibiotic is then added, incubation is continued for various lengths of time, and the mixture studied for any change in characteristics, e.g. antibacterial activity, that may have occurred. (This methodology is patterned after that successfully used in the study of steroid transformations[12,13].) If changes have taken place, they are often minor in the chemical sense and as noted above, may include acylation. oxidation, reduction, phosphorylation and/or adenylylation.

Since nearly all antibiotic-producing microorganisms have the ability to produce families of closely related antibiotics, it has usually been profitable to search among these organisms for enzymatic ability to transform given antibiotics. Phosphorylation of streptomycin[90,91], phosphorylation of neomycin[92], acylation of spiramycin[67] and hydrolysis of mannosidostreptomycin[52,53] are among the transformations carried out by the antibiotic-producing cultures.

It is common experience that even a very minor chemical change in an antibiotic molecule results in a drastic decrease or complete loss of antimicrobial activity. Only rarely there is an increase in biopotency. Hence a convenient method to detect changes due to microbial action is to monitor the bioactivity by a microorganism sensitive to the substrate antibiotic. However, since there is a possibility that bioactive products may also be formed, the traditional agar diffusion disc-plate assay method should be supplemented with chromatography on paper or thin-layer. Use of the latter results in separation and purification of the metabolic products for further testing and study. If it is possible to obtain the substrate antibiotic in radioactively labelled form (both ^{14}C and ^{3}H have been used), much time and effort can be saved by locating radioactive areas on the chromatogram. Once isolated and purified, the radioactive compound may be identified and characterized by conventional chemical and physical methods.

Inactivation of the aminoglycoside antibiotics, e.g. streptomycin, kanamycin, etc., by cell-free preparations of the R factor-carrying bacteria, has led to the expected conclusion that several closely related enzymes obtained from different organisms carry out the same type of transformation. Each has some substrate specificity and it is possible by genetic techniques to obtain mutants more suitable for the intended study (higher levels of the desired enzyme or its absence). Although the possibility of finding enzymes transforming various antibiotics among the microorganisms resistant to the antibiotic is attractive, studies have shown that the *in vivo* resistance to an antibiotic is due to the lack of its absorption or serum binding in the body rather than to the enzymatically catalysed alterations of its molecules.

III. TRANSFORMATIONS OF ANTIBIOTICS BY PURIFIED ENZYMES

Practically all of the transformations listed in *Table 1* have been carried out with growing cultures or resting cells of microorganisms selected in different ways. In the case of mycophenolic acid transformations[55], hundreds of organisms have been examined for the ability to carry out changes of the

molecule, and approximately ten per cent of them have had this ability. In many instances the enzymes carrying out the transformations have been shown to be inducible (rather than constitutive) and at least a portion of the antibiotic molecule was required to be present in the growth medium before the degrading enzymes were detected in the cells.

Isolation and purification of the enzymes involved in the transformations has been infrequently attempted. Some of the data reported are summarized in *Table 2*. The β-lactamases have been crystallized[93] and a few of the other

Table 2. Purification of some antibiotic transforming enzymes

Antibiotic	Transformation noted	Microbial source	Type of enzyme	Purification	Reference
Actinomycin	Hydrolysis of lactone	*Actinoplanes missouriensis*	I†	90-fold	16
Cephalosporin	Hydrolysis of lactam	*Bacillus cereus*	C	crystals	93
Chloramphenicol	Acetylation	*Streptococcus epidermidis*	C	171-fold	94
Echinomycin	Hydrolysis of lactone	*A. missouriensis*	C	200-fold	15
Etamycin	Hydrolysis of lactone	*A. missouriensis*	C	200-fold	15
Gramicidin S	Hydrolysis of peptide	*Bacillus subtilis* (Nagarse)	C	crystals	41
Kanamycin	Phosphorylation	*Pseudomonas aeruginosa*	C	350-fold	45
Penicillin	Hydrolysis of lactam	*Bacillus cereus*	C	crystals	60
Penicillin	Hydrolysis of peptide	*Bacillus megaterium*	I	91-fold	95
Streptomycin	Adenylylation	*Escherichia coli*	C	100-fold	66
Streptomycin-PO_4	Dephosphorylation	*Streptomyces griseus*	C	50-fold	96

† I = inducible, C = constitutive.

enzymes have been purified more than 100-fold. One might expect that attempts would be made to purify those enzymes involved in a transformation where the product of the enzyme catalysed reaction had economic value. However, this does not seem to be the case, as far as it can be determined from the literature on penicillin acylase, mannosidostreptomycinase and the acylases attacking the macrolide antibiotics. With the advent of the fixed-bed enzyme technology, it should be feasible to attach the enzyme to an inert support and to use it for large-scale transformation of the antibiotic in question. Under these circumstances the increased use of the enzyme would probably be more than equal to the effort needed to release it from the microbial cells and to purify.

IV. SUMMARY AND PROSPECT FOR THE FUTURE

The studies published to date indicate that all antibiotics can be expected to undergo microbial attack. In some instances, the attack has resulted in rather complete degradation of the molecule; and in other instances the changes have been limited to hydrolysis oxidation, reduction, deamination,

demethylation, acylation, phosphorylation or adenylylation. Some of these latter transformations are difficult to carry out by chemical means, and their utility should lead to further studies.

The diversity of enzyme attack on antibiotic structures should encourage further exploration. It took some ten years of study of microbial transformation of steroids (and survey of over 10000 organisms) to realize the limitations and advantages of this method for the production of new compounds. It should not be expected that antibiotic transformation will require less effort for similar returns.

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