## MICROBIOLOGY

# Activation of Amino-Acids in Micro-Organisms which produce Antibiotics

It seems well established that the first steps in the biosynthesis of proteins are the activation of amino-acids in the presence of adenosine triphosphate (ATP) and the transfer of the activated amino-acids to a soluble fraction of ribonucleic acid (RNA)[1]. Such reactions are also involved in the biosynthesis of some peptides, for example, the condensation of glutamyl-cysteine with glycine to give glutathione[2]. Enzymes activating almost all the naturally occurring L-amino-acids have been isolated from animal, plant, and microbial sources. Only one enzyme active on a D-amino acid, D-alanine, has been demonstrated in cells of *Lactobacillus arabinosus* and other Lactobacillaceae[3].

If the mechanisms for protein synthesis are involved also in the synthesis of peptides, it should be possible to demonstrate activation and transfer of amino-acids present in peptides but absent from proteins, such as many of the D-amino-acids. To test this hypothesis the following micro-organisms were selected, since they produce antibiotics containing one or more of the amino-acids that do not occur in proteins: *Penicillium chrysogenum P*–4 (Lepetit S.p.A.), producing penicillin that contains D-valine; *Streptomyces antibioticus* 23 (Lepetit S.p.A.) and *Streptomyces parvus N.R.R.L. B*-1455 (both producing antibiotics of the actinomycin group in which D-valine, sarcosine, D-allo*iso*leucine and N-methyl-L-valine are present); *Bacillus polymyxa A.T.C.C.* 10401 and *B. polymyxa* 2459 (Ch. Pfizer and Co.) producing, respectively, polymyxin-D and polymyxin-B, containing D-phenylalanine, D-leucine, D-serine, D- and L-α,γ-diaminobutyric acid; *Bacillus circulans A.T.C.C.* 14040, producing circulin containing D-leucine and L-α,γ-diaminobutyric acid. The micro-organisms were grown on complex media capable of supporting antibiotic synthesis and were collected when the production of the antibiotic reached its maximum (2 days for the bacteria, 3 for *P. chrysogenum*, and 6 to 8 for the Streptomycetes). Cell-free extracts were prepared by suspending the centrifuged cells in one volume of 0·066 *M* phosphate buffer and 0·002 *M* reduced glutathione (*p*H 6·9) and disrupting them with glass beads in a refrigerated mechanical disintegrator. Following centrifugation at 20,000*g* for 20 min., the presence of amino-acid-activating enzymes was assayed on the supernatants by measuring the exchange between ATP and radioactive pyrophosphate[4].

The results (Table 1) indicate that, while a fair number of the L-amino-acids is activated, no activation of the D-amino-acids is detectable even if the micro-organism is capable of synthesizing compounds containing amino-acids with such an optical configuration. In addition, sarcosine, which has no optical activity, is not activated by the tested Streptomycetes. L-α,γ-diaminobutyric acid, which has not been found in proteins, is activated by strain 2459 of *B. polymyxa*; this is the first time that this amino-acid is reported to be activated.

The possibility that the enzymes activating the D-amino-acids and sarcosine through an ATP-pyrophosphate exchange are destroyed during the process of extraction or are present in too small amounts for detection cannot be ruled out, but seems unlikely. It appears more probable that the pathway of biosynthesis of these peptides does not involve the activation of the D-amino-acids. Such a hypothesis is in agreement with the recent findings that exclude a process of amino-acid activation in the biosynthesis of some petides containing D-alanine and other L-amino-acids by *Staphylococcus aureus*[5]. Work under way in this laboratory[6] has shown that while D-leucine-$^{14}$C is incorporated in the polymyxin-D synthesized by *B. polymyxa*, the amino-acid is not activated by an ATP-pyrophosphate exchange nor transferred to soluble RNA.

Table 1. Activation of Amino-Acids
Micromoles of pyrophosphate-phosphorus-32 exchanged per 15 min. per mgm. of protein

| | *B. polymyxa* 10401 | *B. polymyxa* 2459 | *B. circulans* | *Str. antibioticus* | *Str. parvus* | *P. chrysogenum* |
|---|---|---|---|---|---|---|
| L-Alanine | 0 | 0 | 0·077 | 0 | 0 | 0·012 |
| L-Arginine | 0·013 | 0 | 0 | 0·115 | 0·044 | 0 |
| L-Aspartic acid | 0·080 | 0 | 0 | 0 | 0 | 0 |
| L-Cysteine | 0·243 | — | — | 0·107 | 0·059 | 0 |
| L-α,γ-Diaminobutyric acid | 0 | 0·641 | 0 | — | — | — |
| L-Glutamic acid | 0 | 0 | 0 | 0·062 | 0 | 0 |
| Glycine | 0 | 0 | 0 | 0·133 | 0·075 | 0 |
| L-Histidine | 0 | 0·182 | 0 | 0·986 | 0 | 0 |
| L-*iso*Leucine | 2·001 | 0·695 | 0·490 | 1·129 | 0·162 | 0·116 |
| L-Leucine | 2·073 | 1·690 | 3·623 | 0·498 | 0·044 | 0·124 |
| L-Lysine | 0·094 | 0·140 | 0 | 0·098 | 0 | 0·020 |
| L-Methionine | 0·278 | 0·416 | 0·487 | 0·444 | 0 | 0 |
| L-Phenylalanine | 0·064 | 0 | 0 | 0 | 0 | 0·016 |
| L-Proline | 0·144 | 0 | 0 | 0·204 | 0 | 0 |
| L-Serine | 0 | 0·055 | 0 | 0·302 | 0 | 0·039 |
| L-Threonine | 0·210 | 0·050 | 0·035 | 0·088 | 0·044 | 0 |
| L-Tryptophan | 0·083 | 0 | — | 0·133 | 0 | 0 |
| L-Valine | 0·106 | 1·790 | 0·986 | 0·489 | 0·235 | 0·076 |
| Sarcosine | — | — | — | 0 | 0 | — |
| D-Alanine | 0 | 0·162 | 0 | 0 | 0 | 0 |
| D-*iso*Leucine | 0 | 0 | — | — | — | — |
| D-Leucine | 0 | 0 | 0 | 0 | 0 | 0 |
| D-Phenylalanine | 0 | 0 | 0 | — | — | 0 |
| D-Serine | 0 | 0 | 0 | — | — | — |
| D-Threonine | 0 | 0 | 0 | — | — | — |
| D-Valine | — | — | — | 0 | 0 | 0 |
| None (control) | 0·133 | 0·280 | 0·355 | 0·507 | 0·073 | 0·100 |

The assay system contained 20 μ*M* of *tris* buffer (*p*H 7·9); 5 μ*M* of ATP (*p*H 6·5); 2·5 μ*M* of $MgCl_2$; 3·2 μ*M* of sodium pyrophosphate-phosphorus-32 (corresponding to approximately 340,000 counts per min. on a 'Tracerlab' mica window counter); 10 μ*M* of amino-acid; cell-free extracts ranging from 0·068 to 0·49 mgm. of protein; glass distilled water, 0·5 ml.

The values obtained in the controls containing no amino-acids were subtracted from all the experiments. The activation was considered present when the experimental data were at least 10 per cent higher than the controls.

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