EFFECT OF CHLOROMYCETIN AND PENICILLIN ON THE INCORPORATION OF AMINO ACIDS INTO ACTINOMYCIN AND PROTEIN BY STREPTOMYCES ANTIBIOTICUS

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During an investigation concerned with the precursors of the actinomycin molecule (Figure 1) a simple and sensitive method of assay was developed for the antibiotic based on its solubility in ethyl acetate. By means of this assay it has been possible to study the rates of incorporation of amino acids into actinomycin during short-term experiments and to compare these with the rates of incorporation of amino acids into protein by Streptomyces antibioticus.

S. antibioticus was cultured as described previously (Katz and Goss, 1959). After 24 hours of growth antibiotic production began; at 48 hours the experiments were started by the addition of 0.1 to 1.0 μmoles of C^{14}-labeled amino acid (1 μcurie) per 100 ml of culture medium. Four ml of the medium were then removed at different intervals and filtered free of mycelium through glass wool. Actinomycin from 3 ml of culture filtrate was extracted into 5 ml of ethyl acetate; after centrifugation, 4.5 ml of the ethyl acetate layer was washed with 5 ml distilled water to remove any residual water soluble radioactive compounds. Following centrifugation, 1 ml of the ethyl acetate layer was mixed with 10 ml of a naphthalene-dioxane solution (Bray, 1960) and counted in a liquid scintillation counter.

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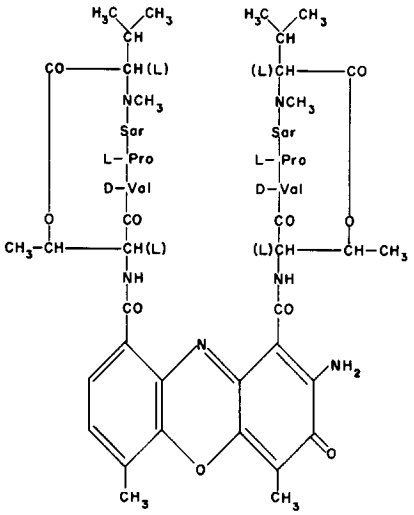

Fig. 1. Structure of actinomycin IV. The sequence of amino acids: L-threonine, D-valine (D-val), L-proline (L-Pro), sarcosine (Sar), N-methyl-L-valine.

The radioactive product in the ethyl acetate phase was shown to be actinomycin by several procedures. The radioactivity could not be separated from actinomycin using paper chromatographic techniques (butanol:acetic acid:water 4:1:5; methanol:benzene:butanol:water, 2:1:1:1; 10% aq. sodium o-cresotinate:di-butyl ether:sym. tetrachloroethane, 3:2:1). In two separate experiments, the antibiotic formed in the radioactive experiments was isolated by solvent extraction and silicic acid chromatography (Roussos and Vining, 1956; Goss and Katz, 1960) and shown to have the same specific radioactivity as the actinomycin in the original ethyl acetate extract.

With this assay procedure it was possible to demonstrate that within 3 minutes after the addition of L-valine-1-C^{14}, radioactive antibiotic could be isolated from the medium (Figure 2). Furthermore, during the first 30 minutes, synthesis of labeled antibiotic was linear; it was also observed that virtually all the radioactivity that was incorporated into the antibiotic occurred during the first 60 minutes. As shown in Figure 2, D-valine-1-C^{14} was not

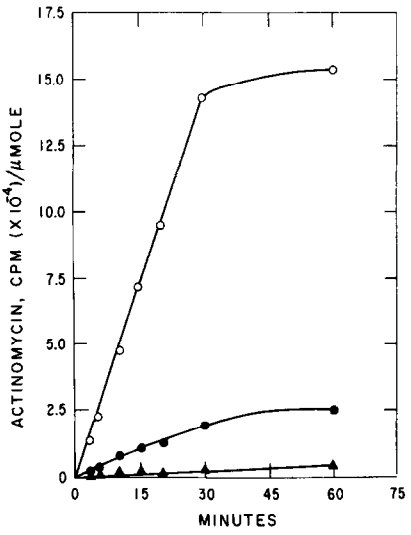

Fig. 2. Rate of incorporation of D- and L-valine-1-C^{14} into actinomycin.

○——○ L-valine-1-C^{14}.

●——● L-valine-1-C^{14} plus D-valine, 2 x 10^{-3}M.

▲——▲ D-valine-1-C^{14}.

Incubation conditions were as described in the text.

incorporated into the antibiotic to any significant extent. However, D-valine-1-C^{14}, when added to the culture medium, was rapidly taken up by the organism; within 15 minutes, over 97% of the radioactivity was removed from the medium. Thus, penetration of the D-isomer into the organism is not a factor in its failure to serve as a precursor of the D-valine in the actinomycin peptide. These findings are in agreement with our recent radioisotope studies which revealed that L-valine-1-C^{14} is the precursor of both the N-methyl-L-valine and D-valine of the actinomycin peptide.

In long-term experiments D-valine has been shown to inhibit total antibiotic synthesis without affecting the growth of S. antibioticus (Katz, 1960). In fact, a slight increase in mycelium

weight was observed when D-valine was supplied to the organism. In the present studies the D-isomer produced an 80 to 90% inhibition of the incorporation of L-valine-C^{14} into the antibiotic (Figure 2).

Kinetic data similar to that reported for L-valine-1-C^{14} have also been obtained with other precursors of the actinomycin molecule, e.g., L-proline-U-C^{14}, L-threonine-U-C^{14}, glycine-1-C^{14} (precursor of sarcosine) and L-methionine-$C^{14}H_3$ (methyl donor).

Recent studies have shown that penicillin inhibits cell-wall synthesis in certain microorganisms but does not affect protein synthesis and that chloromycetin blocks protein synthesis without inhibiting cell-wall formation (Gale, 1959). These inhibitors were used to determine whether actinomycin formation was similar to or differed from cell-wall or protein synthesis. It was found (Table 1) that penicillin had little effect on the incorporation of L-valine-C^{14} into either actinomycin or protein, suggesting that actinomycin synthesis differs from bacterial cell-wall formation. In contrast, chloromycetin markedly inhibited incorporation of the C^{14}-label into protein (about 90%) whereas it produced a 2- to 3-fold stimulation of the rate of L-valine-C^{14} incorporation into actinomycin. A similar stimulation of amino acid incorporation into antibiotic occurred when either L-proline-U-C^{14} or L-threonine-U-C^{14} was substituted for L-valine-1-C^{14} in the presence of chloromycetin. Conversely, when D-valine (2×10^{-3} M) was employed to inhibit actinomycin synthesis, the incorporation of L-valine-1-C^{14} into protein was found to increase by 25%.

These findings point to a far closer relationship between protein synthesis and the formation of peptide antibiotics than has been previously suggested. While the chloromycetin experiment shows that the mechanism of antibiotic formation is different from protein synthesis, the two processes appear to compete for certain

TABLE I

Effect of Chloromycetin and Penicillin on the Incorporation of L-valine-1-C^{14} into Actinomycin and Protein by Streptomyces antibioticus

Medium Supplement	30 min		60 min	
	Actinomycin, cpm/μmole x 10^{-5}	Protein, cpm/mg	Actinomycin, cpm/μmole x 10^{-5}	Protein, cpm/mg
L-valine-1-C^{14}	1.3	9400	1.5	12450
L-valine-1-C^{14} + penicillin, 30 units/ml	1.4	12700	1.4	11600
L-valine-1-C^{14} + chloromycetin, 30 μg/ml	2.9	400	4.0	1270

Conditions of incubation are described in the text. Actinomycin titer of culture filtrates was 15 μg per ml. Protein was determined as follows: Six ml of culture medium were added to 1 ml of 50% cold TCA to precipitate the protein. The TCA precipitate was washed twice with 5% cold TCA, once with 5% hot TCA, twice with ethanol-ether and finally with ether. The precipitate was suspended in 1 ml of 0.5 N NaOH and the protein was dissolved by heating for 30 min. at 100°. Soluble protein was determined by the method of Sutherland et al (1949).

of the available amino acids in the cell. When the demand for amino acids for protein synthesis is high, such as in the early stages of growth little if any antibiotic is synthesized; in contrast, when the requirements for protein synthesis are low such as at the end of the log phase, or when protein synthesis is inhibited, antibiotic formation is markedly enhanced.

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