Synthesis and Degradation of Proteins and DNA in Streptomyces aureofaciens

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ABSTRACT. The rate of protein synthesis in Streptomyces aureofaciens, measured by incorporation of U-14C-L-leucine into cells, fluctuated during the production phase in the range of 10–15 % of the values determined in the phase of intensive growth. Tetracycline partially inhibited the protein synthesis during the growth phase only. The proteins synthesized between the 6th and 18th hour of growth, were 75 % degraded by the 48th hour. The DNA synthesis, measured by means of incorporation of 2-14C-thymine into the mycelium, occurred predominantly during the first 24 h of cultivation. Similarly, DNA synthesized between the 6th and 12th hour of cultivation was degraded by 75 % after 48 h. The turnover of culture proteins is thus caused largely by degradation of old cells and growth of new ones which are more resistant to tetracycline. The activity of alanine aminotransferase and aspartate aminotransferase increase substantially towards the end of fermentation.

Considering the classical division of the growth curve into four phases (lag, growth, stationary and degradation of cells), the excessive production of a secondary metabolite occurs mostly at the end of the growth phase and during the stationary phase. In the case that the synthesized metabolite inhibits the protein synthesis the question arises of the resistance of the protein synthesizing apparatus of the cell to its own product.

Tetracycline, whose antibiotic activity is mainly based on the inhibition of protein synthesis, produces in vitro inactive aggregates with the ribosomes of its producer (the actinomycete Streptomyces aureofaciens) (Ludvík et al. 1971). The content of ribosomes and ribonucleic acids in S. aureofaciens mycelium decreases from the 12th to the 24th hour of fermentation and is practically constant after 36 h (Timko et al. 1976). Tetracycline added to a S. aureofaciens culture during the lag phase practically inhibits further protein synthesis (Mikulík et al. 1971). For this reason it is not clear whether the synthesis of proteins (predominantly the enzymic systems synthesizing excessive metabolites) in S. aureofaciens takes place even in the period when the level of produced tetracyclines rises sharply.

We attempted to resolve this question by monitoring the synthesis of proteins and DNA, and the rate of their degradation. By studying the aminotransferases we monitored the activity of reactions linking up the metabolic pathways of amino acids and the biosynthesis of intermediates of the tetracycline skeleton. The coupling of these metabolic pathways in streptomycetes was described by Gräfe et al. (1974). The utilization of carbon residues of amino acids for the biosynthesis of the tetracycline nucleus was described previously (Běhal et al. 1977).
MATERIALS AND METHODS

Microorganisms and cultivation. The strains used were the production variant Streptomyces aureofaciens 8425 (Research Institute for Antibiotics and Biotransformations, Roztoky u Prahy) and the low-production strain S. aureofaciens RIA 57 (Research Institute of Antibiotics, Moscow); for cultivation media and procedure see Hoštálek (1964).

Cell-free extract. Mycelium washed with water was disintegrated in a bacterial press (Biox, X-press) and suspended in a homogenizing buffer (0.1 M Tris. HCl, pH 7.4, 2 mM EDTA; 15% glycerol; 1 mM 2-mercaptoethanol). The homogenate was centrifuged at 20,000 g for 30 min, lowmolecular weight components were removed on a Sephadex G-25 column (10×100 mm) and the homogenate was used for measuring the enzyme activity.

Incorporation of U-14C-L-leucine and 2-14C-thymine. Radioactive leucine (222 kBq in 35 μmol per 60 ml of medium) or thymine (185 kBq in 50 μmol per 60 ml of medium) was added into fermentation flasks at predetermined intervals during the cultivation and incorporated into the cells for 1 h. The contents of parallel flasks were then pooled, the mycelium was centrifuged at 300 g, acidified with 5 M HCl, and washed three times with water, dehydrated by washing twice with acetone and dried in a desiccator. The radioactivity of 10 mg of dry mycelium was measured in 5 ml of scintillation solution in a Mark I Liquid Scintillation System (Nuclear Chicago). In experiments where the effect of tetracycline was monitored, the antibiotic was added to a concentration of 1 mg per ml of fermentation medium together with radioactive substrate.

Degradation of proteins and DNA. U-14C-L-Leucine (222 kBq in 35 μmol per 60 ml of medium) was added after six hours of fermentation and incorporated for another 12 h. 2-14C-Thymine (185 kBq in 50 μmol per 60 ml of medium) was added after 6 h of fermentation and incorporated for another 6 h.

After the termination of incorporation the contents of all the flasks were pooled; the mycelium was centrifuged, washed with physiological saline and aliquots were distributed into flasks with a filtrate of culture of the same age but containing no radioactivity, and further cultivated. The contents of parallel flasks were pooled at predetermined intervals, further procedure being the same as described in the measuring the incorporation level. Tetracycline (1 mg per ml of medium) was added simultaneously with the radioactive substrate. No L-leucine or thymine were added into the medium as carriers to prevent reutilization of the degraded radioactive components. (This precaution was unnecessary in the case of L-leucine because the mycelium was cultivated on a complete medium containing sufficient amounts and variety of amino acids.) When monitoring the course of DNA degradation, the degradation ending with nonutilizable fragments was thus studied. As the rate of DNA synthesis is at its minimum after 24 h of cultivation, the reutilization of thymine is also imperceptible. It was thus possible to measure the degradation of proteins and DNA under conditions equal to the rest of experiments.

Aminotransferases. Aspartate aminotransferase (EC 2.6.1.1) was measured according to Sitzer and Jenkins (1961); alanine aminotransferase (EC 2.6.1.2) by the same method, L-aspartic acid being replaced with L-alanine and malate dehydrogenase with lactate dehydrogenase.