Nucleoside Triphosphate Levels in *Streptomyces hydrogenans* during Growth and Induction of 20β-Hydroxysteroid Dehydrogenase

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Levels of the purine nucleoside triphosphates are decreasing towards the end of log phase growth of *Streptomyces hydrogenans.* Induction of 20β-hydroxysteroid dehydrogenase by addition of 11β,21-dihydroxy-4,17(20)-pregnadien-3-one to the growth medium leads to a pronounced drop in purine nucleoside triphosphate levels with is irreversible in contrast to the initial loss and later accumulation of RNA.

In the presence of various steroids *Streptomyces hydrogenans* enhances the synthesis of 20β-hydroxysteroid dehydrogenase (EC 1.1.1.53) [1, 2]. The induction of the enzyme, the initial appearance of which can be determined within 15 min after application of the inducer to the culture medium by measuring enzyme activity and by immunological methods [3], is accompanied by a rapid and pronounced drop in RNA content and decreased RNA synthesis. Despite the initial decline of total RNA synthesis the production of specific mRNA for 20β-hydroxysteroid dehydrogenase seems to be enhanced as shown by double isotope labelling experiments [4].

Previous findings suggest that nucleotide pools are affected, too [5]. The reduction of total RNA, increased stability of mRNA, as suggested from longer mRNA half-lives in induced cells, and the diminished rate of RNA synthesis show interesting parallels to the biochemical effects accompanied with stringency in *Escherichia coli* [6]. Our data allow the assumption that inducing steroids may have similar effects on *Strectomyces hydrogenans* as starvation has on *E. coli* [7]. Therefore, the determination of nucleoside triphosphate pool sizes following induction would be helpful for a better understanding of the mechanism of enzyme induction in *Streptomyces hydrogenans.*

Methods

Cultivation and homogenization of *Streptomyces hydrogenans* are described elsewhere [1, 8]. For the estimation of the relative amount of nucleoside triphosphates, the cells were cultivated in the presence of 0.33 mCi $^{32}PO_4^{3-}$ per ml and diluted twofold at the beginning of the experiment. Ten ml-samples of the suspension were taken from the culture and the cells were harvested on Whatman glass fibre filters. The filter discs were transferred into 2 ml 1 M formic acid to extract soluble nucleotides. Filtrates were prepared after incubation for 15 min at 4 °C and 10 µl samples were spotted on PEI impregnated cellulose plastic sheets. Sheets were washed twice in dest. water, dried and then developed in 4 M $(NH_4)_2SO_4$ up to 4 cm above the origin, followed by developing in 7 M $(NH_4)_2SO_4$ [9, 10]. Nucleoside triphosphates were identified by their UV absorption. The spots were wetted with nitrocellulose solution and transferred as a hard pellet to scintillation vials [11].

Results and Discussion

During quasi-logarithmic growth of *Streptomyces hydrogenans* the relation of the content of nucleoside triphosphates to total DNA remains constant, but decreases towards the end of logarithmic growth phase (Fig. 1). After addition of 11β,21-dihydroxy-4,17(20)-pregnadien-3-one for enzyme induction there are significant alterations of the amounts of ATP and GTP in comparison to the nucleotide levels in control cells. Immediately after the start of enzyme induction there is a sudden drop in total RNA content [4] which is due to an increased RNAse

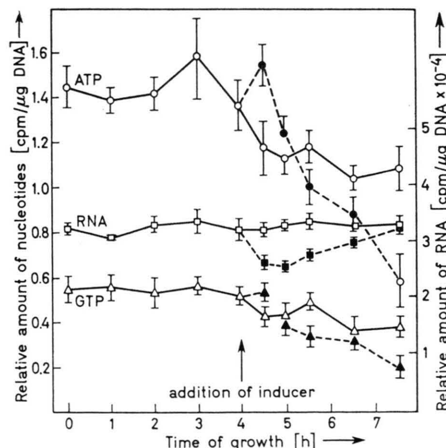

Fig. 1. Relative amount of nucleoside triphosphates and of RNA of *Streptomyces hydrogenans* as a function of growth and after addition of an inducing steroid. Cells were prepared as described under methods. ○, ATP; △, GTP; □, RNA. Open symbols: control cells. Full symbols: induced by 100 µg 11β,21-dihydroxy-4,17(20)-pregnadien-3-one per ml medium.

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that has not yet been studied in detail. So-called stable as well as polysomal RNA are degradated by the new enzyme activity, forming free nucleotides which are mainly transferred into the medium [5]. This may be due to a kind of leaky effect, *e.g.* a damage of the cell membrane caused by the steroids. However, unaffected cell growth and cell multiplication do not support this assumption.

Shortly after addition of the inducer the concentrations of ATP and GTP increase and are higher than in control cells. But later on pool sizes of nucleoside triphosphates in induced cells decrease and never reach the level of control cells. However, the amount of stable RNA recovers from the initial loss. Growth and respiration of the cells are not impaired by the synthetic corticosteroid.

These results are in clear contradiction to the relationship generally believed between enzyme induction and increase of RNA synthesis. To combine the rapid increase of enzyme synthesis with the simultaneous breakdown of cellular RNA and the decrease of nucleotide pools after 1 h, we would like to propose the following hypothesis. Inducing steroids act at least by two different mechanisms: 1. They control the specific synthesis of a messenger RNA coding for 20β-hydroxysteroid dehydrogenase (in the case of testosterone or estradiol as inducing steroid the synthesis of 17β-hydroxysteroid dehydrogenase [EC 1.1.1.63] increases [12]. These enzymes catalyze the NADH resp. NAD^+ dependent transformation of inducing steroids to non-active metabolites (inactivation of the inducer). 2. The steroids change cell membrane permeability and activate (or liberate) a RNase which rapidly decomposes part of the cellular RNA. The degradation of ribosomal and messenger RNA following phosphate starvation has already been reported for *Escherichia coli* [13]. Because of the reduced number of intact polysomal RNA molecules the mRNA coding for 20β-hydroxysteroid dehydrogenase can successfully compete with the remaining mRNA population for factors limiting the translation rate. This may be the cause for the increased mRNA stability determined for mRNA coding for 20β-hydroxysteroid dehydrogenase in induced cells [4]. Competition between different mRNA species for available translation factors is the main reason for the so called superinduction by actinomycin [14].

Both, the specific increase of mRNA synthesis coding for 20β-hydroxysteroid dehydrogenase and the higher stability of this mRNA in the translation machinery as a consequence of partial breakdown of competing cell RNA, cause a fortyfold increase of specific enzyme synthesis. In addition the extent of stabilization of mRNA may be under control of the nucleotide pool. In hepatoma cell cultures a direct relationship between ATP depletion and mRNA stabilization could be shown [15]. Whether specific steroid-binding proteins which could be detected in *Streptomyces hydrogenans* [16, 17], are involved in the alteration of nucleotide pool sizes shown here, is still unknown.

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