Enzymatic Reduction of Purine Ribonucleotides

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The enzymatic formation of pyrimidine deoxyribonucleotides through reduction of the corresponding ribonucleotides has been found in mammalian [1] and bacterial [2] systems. Studies with purified enzymes from *Escherichia coli* [2] demonstrated that the reaction occurred at the diphosphate level and showed requirements for ATP, Mg^{2+}, and reduced lipoic acid. Very little evidence exists for a similar reaction with purine nucleotides.

In the present work it was shown that the same enzyme fraction (Fraction B) from *E. coli*, which catalyzed the reduction of pyrimidine ribonucleotides, also catalyzed an analogous transformation of adenosine and guanosine phosphates to the corresponding deoxyribosyl compounds.

The formation of deoxyribonucleotides was measured by microbiological assays with either *Lactobacillus leichmanni* 313 or *Lactobacillus acidophilus* [3]. For this purpose the reaction was stopped by boiling, and all nucleotides were transformed to nucleosides by treatment with crude snake venom. When *L. leichmanni* 313 was used, the reaction mixture was also boiled for 15 min at pH 12 to destroy all vitamin B_{12} activity.

Fraction B was shown to catalyze the formation of deoxyribonucleotides from ADP. The reaction showed absolute requirements for Mg^{2+} and reduced lipoic acid, and was strongly stimulated by addition of ATP. Attempts to determine the level of phosphorylation for the reductive step were not successful.

A similar synthesis of deoxyribonucleotides from guanosine phosphates was found. Again Mg^{2+}, ATP, and reduced lipoic acid (or Factor S_2 (Ref. [4]) + TPNH) were required. Evidence was obtained that the reduction occurred at the diphosphate level.

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The Effect of Deoxyadenosine on Growing Cultures of *Escherichia coli*

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Deoxyadenosine in concentrations of 0.0025 M is known to inhibit deoxyribonucleic acid synthesis, but not ribonucleic acid synthesis in Ascites tumor cells [1]. At the same time deoxyadenosine triphosphate accumulates in the cells [2].

When deoxyadenosine in the above-mentioned concentration is added to an exponentially growing anaerobic culture of *E. coli* in a ^{32}P-labelled broth medium, a 60—70 % inhibition of ^{32}P-incorporation into ribonucleic acid as well as into deoxyribonucleic acid is seen. This effect is specific for deoxyadenosine among other deoxynucleosides and nucleosides. It may be completely reversed by simultaneous addition of adenosine in equimolar amounts, but addition of other deoxynucleosides, separately or together, does not restore the ^{32}P-incorporation into ribonucleic or deoxyribonucleic acid.

If the cells are grown in a synthetic medium with glucose as carbon source, addition of deoxyadenosine has little or no effect on the incorporation of ^{32}P into nucleic acids. This suggests that the broth-grown cells for nucleic acid synthesis preferentially use the nucleotides or nucleosides already present in the medium and that deoxyadenosine in some way causes an inhibition of this pathway.

The effect of deoxyadenosine on a T^4-infected culture of *E. coli* has also been investigated. An inhibition of ^{32}P-incorporation into deoxyribonucleic acid of 60 % was seen, while incorporation into ribonucleic acid was less affected. Here, also, the effect was completely specific for deoxyadenosine and could be reversed by simultaneous addition of adenosine.

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