FACTORS INFLUENCING THE LEVEL OF COBAMIDE-DEPENDENT RIBONUCLEOSIDE TRIPHOSPHATE REDUCTASE IN LACTOBACILLUS LEICHMANNII

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Lactobacillus leichmannii, a bacterium requiring cyanocobalamin or deoxyribosyl compounds for growth, contains an enzyme system which reduces CMP to deoxycytidine phosphates in the presence of ATP, Mg^{2+}, a thiol and cobamide coenzyme[1]. Dihydrolipoate is the most effective reductant in this system[2] and partial purification of the enzyme permitted the demonstration[3] that the enzyme reduces ribonucleoside triphosphates much more rapidly than the corresponding diphosphates while monophosphates are reduced at a still lower rate.

It was observed during the course of this work that the specific activity of the reductase system varied considerably from one preparation to another. That this variation was due, at least in part, to nutritional factors was clear from our observation that the unfractionated extract from bacteria grown in a medium containing glucose, Tween 80, yeast extract and tryptose contained negligible amounts of reductase. Factors which influence the level of reductase activity in bacteria have therefore been investigated.

The organism used for this work and the growth conditions

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were as previously described[2]. Unless specified otherwise 36 μμM cyanocobalamin was added to the defined medium. Cell-free extracts were prepared as previously described[2] and were treated with magnesium acetate (final concentration 10 mM) and DNAase (0.2-0.3 mg per ml) for a short period at 4°C and then dialysed overnight at 4° against 0.05M potassium phosphate buffer, pH 7.8. Enzyme activity was assayed as previously described[3] with ATP as the substrate. Specific activity is expressed as mμmoles of deoxyATP produced per mg of protein per hour.

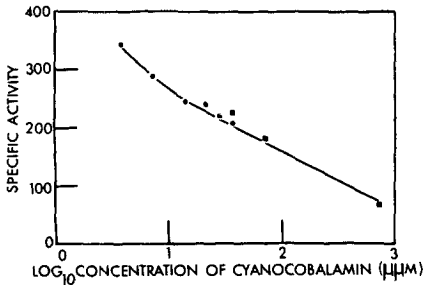

FIG.1: Effect of the concentration of cyanocobalamin in the medium on specific activity of reductase in bacterial extracts. Details are given in the text. The points indicated by the two sets of symbols represent results from two different experiments.

It may be seen from Fig.1 that when the concentration of cyanocobalamin in the growth medium is increased, there is a pronounced decrease in reductase activity of the bacterial extract. This leads us to conclude that cyanocobalamin directly or indirectly causes repression of the synthesis of this enzyme. The optimal concentration of cyanocobalamin in the medium was 36 μμM; this gave a reasonable yield of cells with a good reductase content without production of the filamentous forms which occur in a cyanocobalamin-deficient medium[4].

Since cyanocobalamin can be replaced as growth factor in the medium by deoxyribonucleosides, the effect on reductase synthesis of various concentrations of deoxyribonucleosides was investigated. The following table summarises the results obtained.

TABLE I

Repression of the synthesis of ribonucleotide reductase in L.leichmannii by cyanocobalamin and deoxyribonucleosides.

Growth Supplement	Concentration of deoxyribonucleoside 8 µM	24 µM	72 µM	144 µM
Expt.1				
Deoxycytidine	206	110	65	33
Expt.2				
Thymidine	352			92
Deoxyuridine	570			76
Deoxyadenosine	330			74
Deoxyguanosine	404			76
Expt.3				
Deoxycytidine				55
Deoxyuridine				111
Thymidine				103
Deoxyguanosine				95
Deoxyadenosine				115

Results expressed in specific activity. For Expt.1 cyanocobalamin 36 µµM in place of deoxyriboside resulted in an enzymic specific activity of 323 mµmoles dATP/mg protein/hr.

The results of Expt.1 show that the reductase specific activity is decreased approximately six-fold by an eighteen-fold increase in the concentration of deoxycytidine in the medium. This result conflicts with the suggestion of Abrams and Duraiswami[5] that deoxyribonucleotides cannot act as co-repressors of reductase synthesis. Since these authors appear to base their suggestion on a comparison of the activity of extracts produced from bacteria grown in the presence of a single concentration of cyanocobalamin with the

activity of extracts from bacteria grown at a single concentration of deoxycytidine, their evidence must be regarded as inconclusive. Other deoxyribonucleosides were found to have a similar effect to that of deoxycytidine (Expt.2). Expt.3 shows that deoxycytidine is about twice as active in causing repression as other deoxyribonucleosides tested.

The most plausible explanation of these results is that deoxyribonucleosides (or deoxyribonucleotides) are corepressors in the control of the synthesis of the reductase and that cyanocobalamin is not itself a corepressor, but increases repression indirectly through its ability to increase the intracellular pool of deoxyribosyl compounds[6]. The relative effects on repression of increasing the concentration of cyanocobalamin and of deoxyribonucleosides respectively are consistent with this hypothesis. Thus a 200-fold increase in cyanocobalamin concentration above the minimum level necessary for reasonable growth causes a five-fold decrease in enzyme content of the cells, whereas an increase in deoxyribonucleoside concentration by a factor of only 18 causes a 4 to 8-fold decrease in enzyme level. The observed fall in reductase activity in the experiments cannot be attributed to feedback inhibition of the reductase by metabolites which accumulate in the cells in the presence of cyanocobalamin or deoxyribosyl compounds, since such metabolites would be removed during the period of dialysis before the reductase activity of the extracts was tested.

It was also found that the reductase content of the cells varied considerably during growth of the culture. In these experiments, portions were removed from cultures at intervals, chilled and the cells harvested, washed and lysed. Growth

of cells in the culture was estimated from absorbance at 660 mμ measured with a Cary Model 14 spectrophotometer. The results presented in Fig. 2 show that the specific activity of the reductase in the bacteria starts decreasing as the culture comes to the end of its exponential phase of growth, and that no enzyme can be detected after the stationary phase is reached. Since very little synthesis of DNA is necessary under the latter condition, the bacterial cell is able to dispense with the reductase, perhaps breaking it down to supply amino acids for other needs.

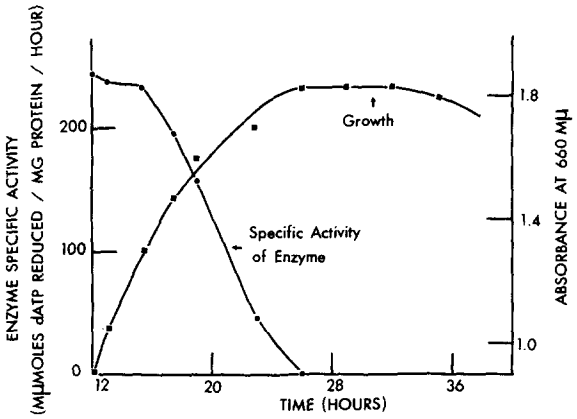

FIG.2: Variation of cellular reductase level during growth of the culture in a medium containing 36 μμM cyanocobalamin.

Fig. 3 shows that when the medium contained 8 μM deoxycytidine instead of cyanocobalamin the reductase content of the cells again declined as the culture approached the stationary phase. It will also be seen that there was an increase in the cellular reductase level during the exponential phase of growth. This was much more marked when the concentration of deoxycytidine in the medium was 72 μM (Fig. 4), although in

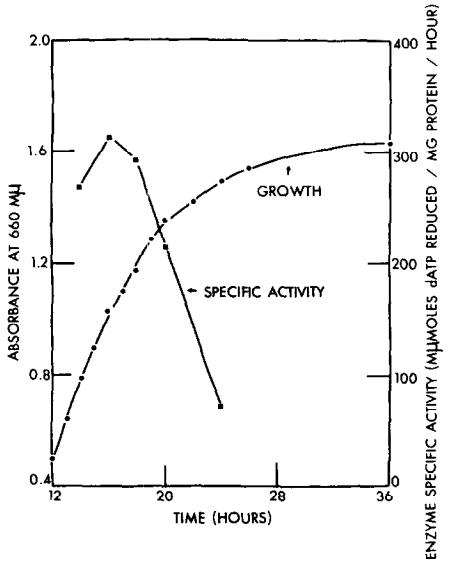

FIG.3: Variation of cellular reductase level during growth of a culture in a medium containing 8 μM deoxycytidine.

this latter case enzyme synthesis is repressed at all stages of growth. The marked rise in reductase level at the end of the exponential phase of growth in Fig. 4 is perhaps due to derepression of enzyme synthesis as deoxycytidine becomes depleted in the medium. This increase in the reductase level did not occur when cyanocobalamin (Fig. 2) was used as a growth supplement, a fact which is also consistent with the view that cyanocobalamin is not the immediate corepressor.

While this work was in progress, Beck and Hardy[7] reported that thymine causes repression of reductase synthesis. We have found, however, that when cells were grown in the defined medium supplemented with both 36 μμM cyanocobalamin and 144 μM thymine or cytosine, reductase specific activity was as high as when cells were grown in medium supplemented with 36 μμM cyanocobalamin alone (Table II).

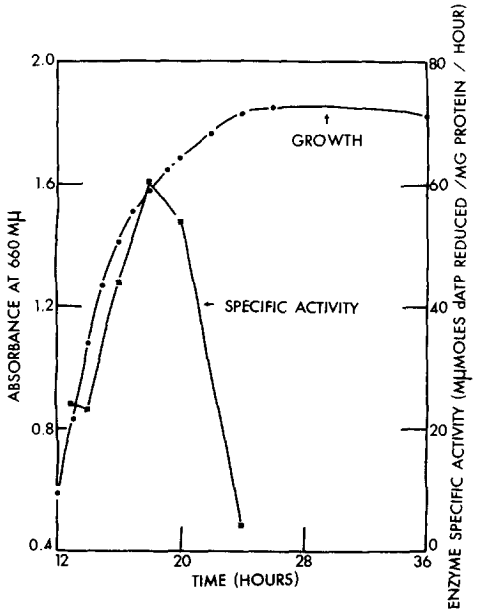

FIG.4: Variation of cellular reductase level during growth of a culture in a medium containing 72 μM deoxycytidine.

TABLE II

Comparison of the effects of thymine, cytosine, thymidine and deoxycytidine on repression of the synthesis of ribonucleotide reductase.

Supplement added to the medium	Enzyme specific activity
Thymidine 144 μM	84
Thymine (144 μM) + cyanocobalamin (36 μμM)	554
Deoxycytidine (144 μM)	46
Cytosine (144 μM) + cyanocobalamin (36 μμM)	498
Cyanocobalamin (36 μμM)	506

In contrast to the lack of repression by thymine and cytosine, 144 μM thymidine or deoxycytidine caused pronounced repression of reductase synthesis. No attempt was made in this experiment to use folate-deficient cells as in the experiment reported by

Beck and Hardy. However, in bacteria replete with folate, the concentration of thymine should be higher than in folate-starved cells. If thymine causes repression of reductase synthesis, the extent of such repression in the presence of exogenous thymine would therefore be even more marked in folate-replete cells. It appears likely from our results that if thymine causes repression of reductase synthesis under certain conditions, the effect must be indirect through conversion of thymine to thymidine. Such a situation would conform more closely to the classical pattern where an end product of a biosynthetic pathway regulates the synthesis of an enzyme in that pathway by acting as a corepressor. The repression by thymine of reductase synthesis in folate-deficient cells reported by Beck and Hardy is surprising since folate-deficiency increases the size of the pool of acid soluble deoxyribosyl compounds[8]. Addition of thymine should decrease this pool size by promoting dTTP synthesis and thus enabling the utilisation of deoxyribonucleotides for DNA synthesis. This should result in derepression of reductase synthesis rather than repression. Although further work will be required to elucidate the significance of the results of Beck and Hardy, the explanation may prove to be related to the fact that under the conditions of their experiment the level of reductase specific activity was low even in the absence of thymine.

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