STIMULATION OF THYMIDYLATE KINASE AND DEOXYCYTIDYLATE DEAMINASE ACTIVITIES OF CHANG CELLS BY DEOXYADENOSINE

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1. Introduction

In a previous report from our laboratory it was shown that treatment of monolayer cultures of Chang human liver cells with deoxyadenosine at concentrations which inhibited cell proliferation induced a marked increase in the specific activity of thymidine kinase [1]. In recent work it has been found that deoxyadenosine has a similar effect on the activity levels of dTMP kinase and dCMP deaminase, two other enzymes of the pathways for production of deoxythymidine triphosphate. The experimental results are reported in the present communication.

2. Methods

Conditions for cultivation of Chang human liver cells and for assay of thymidylate kinase (dTMP kinase) and deoxycytidylate deaminase (dCMP deaminase) have been described previously [2,3]. Under standard incubation conditions the activities of dTMP kinase and dCMP deaminase of the liver cell homogenates were proportional to the amount of protein in the reaction mixture over the range studied, and to the time of incubation up to at least 30 min (fig. 1). In the case of dTMP kinase, 1 unit of enzyme is defined as that amount which phosphorylates 1 mμmole of dTMP per minute. In the case of dCMP deaminase, 1 unit of enzyme is defined as that amount which converts 1 mμmole of dCMP to dUMP per min. Specific activity is expressed as the number of enzyme units per mg of protein. Protein was determined by the Lowry method [4], as modified by Oyama and Eagle [5] with bovine serum albumin as standard. Cell counts were carried out with an automatic particle counter.

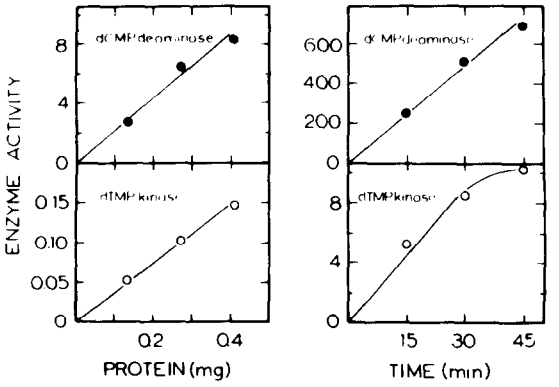

Fig. 1. Effect of protein concentration and time of incubation on dTMP kinase and dCMP deaminase activities. Standard incubation conditions were used, except that the amount of protein in the reaction mixture and the time of incubation were varied as indicated. The results are expressed as mμmoles of substrate converted per minute (left) and per milligram of protein (right).

3. Results and discussion

The effect of deoxyadenosine on the levels of dTMP kinase and dCMP deaminase of Chang liver cell cultures was studied by measuring enzyme activi-

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Table 1
Effect of actinomycin D and deoxynucleosides on dTMP kinase and dCMP deaminase activities of deoxyadenosine-treated cells.

Compounds added	Specific activity			
	18 hours		24 hours	
	dTMP kinase	dCMP deaminase	dTMP kinase	dCMP deaminase
None (control)	0.37	15.05	0.39	15.69
AdR	0.50	20.88	0.57	25.40
AdR and actinomycin D	0.32	14.27	–	–
AdR, GdR, CdR and TdR	–	–	0.54	24.66

Exponentially growing cultures (about 3 X 10^6 cells) were incubated in medium containing AdR (0.5 mM), actinomycin D (1 μg/ml) and deoxynucleosides (0.05 mM) as indicated. Enzyme activities were measured after 18 and 24 hours of incubation.

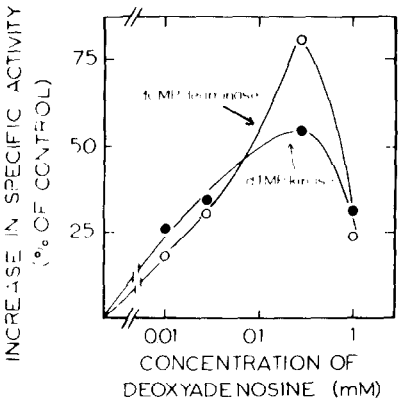

Fig. 2. Effect of deoxyadenosine on dTMP kinase and dCMP deaminase activities. The medium of exponentially growing cultures (about 3 X 10^6 cells) was replaced by fresh medium containing different concentrations of deoxyadenosine as indicated. Enzyme activities were measured after incubation for 24 hours.

ties in homogenates of cells which had been grown on medium containing different concentrations of the deoxynucleoside. From the results in fig. 2 it can be seen that treatment of the liver cells for 24 hours with deoxyadenosine induced a pronounced increase in the specific activity of both dTMP kinase and dCMP deaminase. The stimulating effect on the enzyme levels was found to increase with increasing concentration on deoxyadenosine up to 0.5 mM. Exposure of the cells to higher concentrations of the deoxynucleoside led to a decrease in the enzyme activities. This finding may possibly be due to the fact that in Chang cells deoxyadenosine inhibits not only DNA synthesis, but also RNA and protein synthesis [1].

From the data in table 1 it appears that the deoxyadenosine-induced stimulation of dTMP kinase and dCMP deaminase activities could be prevented by addition of actinomycin D to the culture medium. It is therefore likely that the rise in the enzyme levels caused by deoxyadenosine is due to synthesis of protein, rather than to activation of pre-existing enzyme molecules.

Evidence has been obtained that growth in the presence of deoxyadenosine leads to an accumulation of dATP in the cell [6,7] with subsequent inhibition of the reduction of guanosine and cytidine nucleotides to the corresponding deoxynucleotides [7]. It therefore appeared possible that the deoxyadenosine-induced rise in the activities of dTMP kinase and dCMP deaminase of the liver cells might be due to intracellular depletion of deoxynucleotides, serving as repressors of the enzyme. Such a mechanism, however, seems unlikely in view of the fact (table 1) that addition of deoxynucleosides to the medium did not prevent the stimulating effect of deoxyadenosine. Although the present data do not permit an identification of the repressor or repressors involved, they seem to indicate that both dTMP kinase and dCMP deaminase of Chang liver cells are subject to control by a repression mechanism.

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