FORMATION IN VITRO OF DEOXYADENOSINE TRIPHOSPHATE FROM DEOXYADENOSINE IN EHRLICH ASCITES CELLS.

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It has been shown (Klenow 1959) (Prusoff 1959) that deoxyadenosine in concentrations of about 2 μMoles per ml of cell suspension almost completely inhibits DNA synthesis in vitro in Ehrlich ascites tumor cells. It has furthermore been found that this inhibiting effect may be overcome by simultaneous addition of deoxyguanosine in equimolar amounts (Langer and Klenow 1960). It seemed of interest, therefore, to investigate the deoxynucleotide content of extracts of ascites cells after incubation with these deoxynucleosides. From the results shown below it appears that the addition of deoxyadenosine to a cell suspension causes an accumulation of deoxyATP in the cells, whereas addition of deoxyguanosine results in little or no formation of deoxyguanosine nucleotides. The formation of deoxyATP is, however, enhanced when deoxyguanosine is present in the incubation mixture in addition to deoxyadenosine.

Ehrlich ascites hypotetraploid tumor cells were harvested 5 days after transplantation, collected by centrifugation and suspended in 2 volumes of Tyrode's solution. After incubation with deoxynucleosides (see Table I) the reaction mixture was

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cooled in ice, deproteinized with perchloric acid and centrifuged.The supernatant was neutralized to pH 7.5 with 5 N KOH, the precipitate of $KClO_4$ was removed by centrifugation and the supernatant was then chromatographed on a Dowex-1 Cl^- column (20 cm x 0.51 cm^2).The chromatogram was developed by a linear gradient obtained by leading 250 ml of 0.25 M NaCl in 0.01 N HCl into 250 ml of water in an open mixing vessel. In this chromatographic system a ribonucleotide and its corresponding deoxyribose analogue are eluted as one peak. The mono-, di- and triphosphates of adenosine and guanosine are, however, eluted as separate peaks.

The neutralized fractions (5 ml) were analyzed for ultraviolet absorbing compounds. In the fractions containing the peaks corresponding to ATP and GTP, amounts of ribose (Mejbaum

Table I

Amounts of deoxyATP and deoxyGTP in Ehrlich ascites cells, incubated with deoxynucleosides.

deoxynucleoside added	µMoles deoxyATP	µMoles deoxyGTP	µMoles ATP
deoxyadenosine 35 µMoles	1.75	0	1.14
deoxyguanosine 35 µMoles	0	0	1.0
deoxyadenosine 35 µMoles plus deoxyguanosine 35 µMoles	3.56	0.17	1.49

Incubation mixture contained, besides the deoxynucleosides: 6.65 ml Tyrode's solution, 346 µMoles Na-succinate, 308 µMoles glucose, 2.8 g cells, wet weight, and H_2O to a volume of 14.5 ml. The reaction mixture was incubated with shaking for 90 minutes in a Dubnoff incubator at 37° C.

1939) and deoxyribose (Burton 1956) were determined, and from these values the amounts of nucleoside triphosphate and deoxynucleoside triphosphate, present in each peak, were calculated. Results from a typical experiment are shown in Table I.

The data obtained suggest that, when ascites tumor cells are incubated in the presence of deoxyadenosine, considerable amounts of deoxyATP are formed. These amounts are about twice as high when deoxyadenosine has been incubated with the cells together with deoxyguanosine.

It has not been possible to demonstrate any significant amounts of deoxyribonucleoside triphosphates in control cells, incubated without added deoxynucleoside.

The identity of deoxyATP was further confirmed by paper-chromatographic purification and analysis. An incubation mixture of cells and deoxyadenosine was after deproteinization treated with charcoal (150 mg), which was then washed twice with 6 ml H_2O, eluted with 8 ml 10 per cent pyridine in 50 per cent ethanol and chromatographed on paper in ammonium acetate:ethanol (35:70), saturated with borate (Plesner 1955). In this solvent the ribonucleotides are completely separated from the deoxyribonucleotides. The spot corresponding to deoxyATP was cut out and eluted with H_2O, and the eluate was analyzed for deoxyribose, total phosphorus and 7 minutes acid-hydrolysable phosphorus. The optical density at 240 to 280 mμ was also measured. An aliquot was hydrolyzed for 20 minutes in 0.05 N HCl at 100° C and gave by chromatography in butanol, saturated with water, a spot corresponding to adenine. In Table II are given the analytical data for the isolated compound.

The data are in agreement with those expected for deoxyATP, although the values for the phosphate determination are a little

Table II

Analysis of deoxyATP

	total phosphate	acid-hydrol. phosphate	deoxyribose	adenine (from O.D. at 258 mμ)
μMoles per ml	1.84	1.18	0.76	0.75
	(246)	(158)	(100.5)	(100)

low, probably due to the presence of some deoxyADP in the paper eluate. All values are corrected for paper blank.

Recent experiments in this laboratory (Klenow and Overgaard-Hansen) have shown that when ascites tumor cells are incubated with deoxyadenosine, a small amount of the deoxynucleoside is converted to a compound which inhibits the biosynthesis of DNA and which does not pass through the cell membrane. It was further shown that the effect of deoxyguanosine, when added to the incubation mixture, is to counteract the effect of this inhibitor rather than to prevent the formation of it.

The present finding that deoxyATP accumulates in cells incubated with deoxyadenosine suggests that deoxyATP is identical with the inhibitor formed from deoxyadenosine. The finding that deoxyATP also accumulates when both deoxyguanosine and deoxyadenosine are present in the cell suspension is also in agreement with the experiments mentioned above and may mean, that in the presence of deoxyguanosine the deoxyadenosine-inhibited step is by-passed in the synthesis of DNA.

It has recently been shown (Reichard et al. 1960) that the reduction of cytidine nucleotide to deoxycytidine nucleotide in cellfree extracts of chick embryo is efficiently inhibited by addition of deoxyATP in extremely low concentrations (2×10^{-5} M).

Moreover, other experiments in this laboratory (Overgaard-Hansen 1960) have indicated, that the enzymatic step, which is inhibited by deoxyadenosine, is the reduction of guanosine nucleotide to deoxyguanosine nucleotide (Reichard 1960). This together with the fact that the inhibitory effect of deoxyadenosine can be reversed by the addition of deoxyguanosine exclusively (Langer and Klenow 1960) justifies the conclusion that deoxyATP in ascites cells exerts an inhibitory effect on reduction of guanosine nucleotide to deoxyguanosine nucleotide similar to the effect found by Reichard with the corresponding cytidine compounds.

The increased accumulation of deoxyATP when both deoxyguanosine and deoxyadenosine are present in the incubation mixture may be explained partly by a generally increased nucleotide synthesis and partly by the fact, that deoxyguanosine is known to inhibit the adenosine deaminase activity, present in the cells (Coddington 1960), thus leaving more deoxyadenosine available for conversion to deoxyATP.

REFERENCES

Burton, R.K., Biochem. J., 62, 315 (1956).

Coddington, A., Acta physiol. Scand., 50, suppl. 175, 31 (1960).

Klenow, H., Biochim. Biophys. Acta, 35, 412 (1959).

Klenow, H. and Overgaard-Hansen, K., in preparation.

Langer, L. and Klenow, H., Biochim. Biophys. Acta, 37, 33 (1960).

Mejbaum, W., Z. physiol. Chem., 258, 117 (1939).

Overgaard-Hansen, K., Acta physiol. Scand., 50, suppl. 175, 113 (1960).

Plesner, P., Acta chem. Scand., 9, 197 (1955).

Prusoff, W.H., Biochem. Pharmacol., 2, 221 (1959).

Reichard, P., Biochim. Biophys. Acta, 41, 368 (1960).

Reichard, P., Canellakis, Z.N. and Canellakis, E.S., Biochim. Biophys. Acta, 41, 558 (1960).