DIFFERENTIAL INHIBITION BY 3'-deATP OF NUCLEAR AND CYTOPLASMIC RNA FRACTIONS OF EHRLICH ASCITES TUMOR CELLS IN VITRO

Sune Frederiksen and Hans Klenow

The Fibiger Laboratory
Biochemical Section
Copenhagen, Denmark

Received July 15, 1964

It has previously been found (Klenow, 1963) that 3'-deATP accumulates in Ehrlich ascites tumor cells incubated with low concentrations (about 0.5 μmole/ml) of 3'-deoxyadenosine (Frederiksen et al.). Under such conditions the concentration of acid-soluble ribotides changes to only a small degree. It has, furthermore, been shown (Klenow and Frederiksen, 1964) that the DNA-dependent nucleotidyltransferase isolated from the nuclei of Ehrlich cells is inhibited by very low concentrations of 3'-deATP. It was of interest, therefore, to investigate the possible effect of 3'-deATP on the RNA synthesis in whole cells.

It has been found (Klenow and Overgaard-Hansen, 1964) that the rate of incorporation of $^{32}P_i$ into the ester phosphate of the acid-soluble ribotides is greatly stimulated by low concentrations of 3'-deATP under the experimental conditions used, i.e. cells suspended in Tyrode's solution supplemented with glucose and succinate. The effect of 3'-deATP on the rate of incorporation of $^{32}P_i$ into the nucleic acids would, therefore, under such conditions not reflect the possible effect of this compound on nucleic acid synthesis. In a search for conditions under which the labelling

of the ester phosphate of the acid-soluble ribotides was unaffected of the presence of 3'-deATP it was found that the medium of Harbers and Heidelberger (1959) met this requirement. In this medium about 1.4 µmoles of 3'-deATP accumulate per g of Ehrlich hyperdiploid ascites cells (wet weight), when the cell suspension contains 0.35 µmoles of 3'-deoxyadenosine per ml, and about 2.6 µmoles of 3'-deATP, when the suspension contains 0.7 µmole per ml. In neither of the cases did detectable amounts of 3'-deADP or 3'-deAMP accumulate, and the concentration of the acid-soluble ribotide pool was not different from that of the control cells. With both concentrations of 3'-deoxyadenosine the incorporation of $^{32}P_i$ into RNA was partially inhibited. The maximal inhibition (about 50 %) was obtained after an induction period of about 60 min (see Fig. 1).

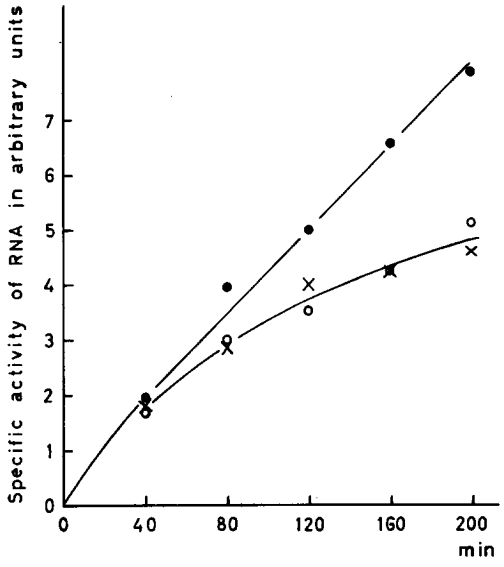

Fig. 1. Effect of 3'-deoxyadenosine on incorporation of $^{32}P_i$ into RNA of Ehrlich ascites tumor cells in vitro. Each vessel contained per ml: Ehrlich ascites tumor cells, 100 mg (wet weight); ascites fluid, 400 µl; Robinson's medium (Robinson, 1949) containing glucose (5.6×10^{-3} M), 500 µl; folic acid, 20 mµmoles; and $^{32}P_i$, 15 µC. Additions: ● - ● , none; o - o , 3'-deoxyadenosine (0.35 µmole); x - x , 3'-deoxyadenosine (0.7 µmole). Specific activity was determined as previously described (Klenow, 1963).

Sibatini et al. (1959) have shown that treatment of aqueous suspensions of mammalian cells with cold phenol separates the RNA into two fractions. After centrifugation one fraction is bound in the interphase, and the other can be recovered from the aqueous phase. The former fraction has been shown to be identical with the nuclear RNA, while the other is cytoplasmic RNA (Georgiev et al., 1961; Harris and Watts, 1962). The nuclear RNA may be separated into several different fractions by treatment of the interphase with phenol at different temperatures. The fractions obtained in this way differ with respect to base composition and rate of labelling with $^{32}P_i$ (Georgiev and Mantieva, 1962). In the experiments presented here the nuclear RNA was separated into two fractions by treatment of the interphase with sodium laurylsulphate and phenol at 70° according to a procedure similar to that of Holland (1963). After subsequent cooling to 0° and centrifugation one nuclear RNA fraction (n-RNA I) may be obtained from the aqueous phase, while another nuclear fraction (n-RNA II) still remains in the interphase.

Fig. 2 shows that n-RNA II obtains the highest specific activity, when cells have been incubated with $^{32}P_i$ and that the rate of labelling of this fraction is not inhibited when the cells contain 3'-deATP. The n-RNA I fraction has a fivefold lower specific activity than that of n-RNA II, and the rate of labelling is about 40 % inhibited by 3'-deATP. This probably means that the two nuclear RNA fractions are metabolically different, and that n-RNA II may be formed independently of the rate of formation of n-RNA I. The RNA present in n-RNA I and n-RNA II amounts to about 9 and 4 % of the total RNA, respectively. The labelling of cytoplasmic RNA (c-RNA) appears to be strongly inhibited in the cells

containing 3'-deATP.

It has previously been found that the DNA-dependent RNA nucleotidyltransferase reaction catalized by an enzyme isolated from the nuclei of Ehrlich ascites tumor cells is immediately and completely inhibited in the presence of 3'-deATP in concentrations equimolar with ATP, and even when the

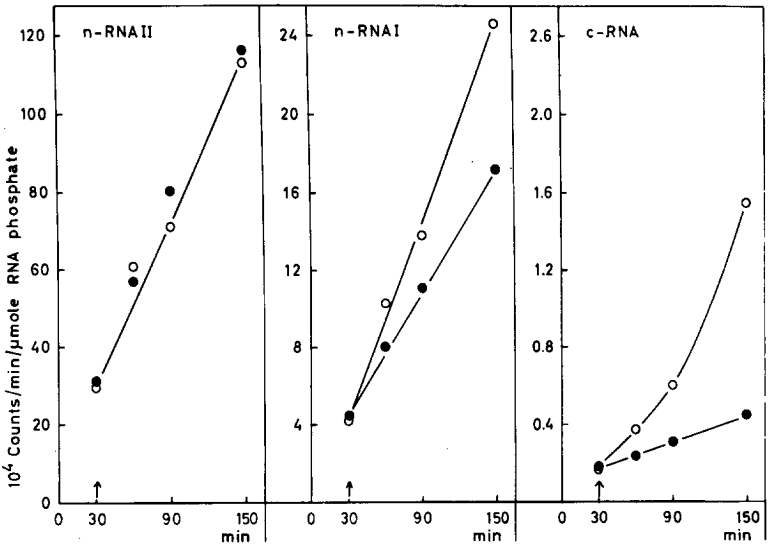

Fig. 2. Effect of 3'-deoxyadenosine on the incorporation of $^{32}P_i$ into different RNA fractions of Ehrlich ascites tumor cells in vitro. Each vessel contained per ml: Ehrlich ascites tumor cells, 85 mg (wet weight); ascites fluid, 415 µl; Robinson's medium (Robinson, 1949) containing glucose (5.6×10^{-3} M), 500 µl; folic acid, 20 mµmoles, and $^{32}P_i$, 20 µC. Additions: o - o , none; • - • , 3'-deoxyadenosine (0.35 µmole).

3-Deoxyadenosine was added to experimental flask after incubation with shaking for 30 min at 37°. Samples were withdrawn at time intervals, the cells were washed with 0.15 M NaCl. Suspensions of washed cells were treated with 1 vol. phenol at 0° for about 10 min. After centrifugation the aqueous phase containing cytoplasmic RNA was made 10 mM with respect to $MgCl_2$, and treated with 2 vol. 96 % ethanol. The RNA precipitate was dissolved and reprecipitated essentially as described by Holland (1963). The interphase was washed several times with phenol-phosphate buffer mixtures, and finally treated with sodium lauryl sulphate and phenol at 70°. After centrifugation of the cooled mixture, n-RNA I was obtained from the aqueous phase by precipitation with $MgCl_2$ and 2 vol. ethanol. The n-RNA II fraction was obtained together with protein as a precipitate from the remaining interphase after addition of about 10 vol. 66 % ethanol. Specific activities of RNA were determined as previously described (Klenow, 1963).

ratio of ATP to 3'-deATP is 25 to 1 the inhibition is complete after a lag period. Since the results presented in Fig. 2 suggest that the synthesis in whole cells of one or more of the RNA fractions of nuclear origin is not inhibited by 3'-deATP it may be concluded that the preparation of nucleotidyltransferase employed catalizes the synthesis of only some of the nuclear RNA fractions and that these fractions are different from n-RNA II. This is further discussed in the following paper concerned with the effect of actinomycin on RNA synthesis (Klenow and Frederiksen, 1964, a).

The cytoplasmic RNA fraction has been analyzed after centrifugation in a sucrose gradient. It was found that the labelling of both the s-RNA and the two ribosomal RNA fractions were strongly inhibited in cells containing 3'-deATP.

Experiments with high speed supernatants of E. coli have shown that the incorporation of ATP into the terminal position of s-RNA is not influenced by the presence of 3'-deATP in concentrations equimolar with ATP (Frederiksen and Klenow, 1964). The pronounced inhibition is, therefore, probably due to inhibition of de novo synthesis and not to inhibition of a possible exchange of the terminal nucleotides.

The present results show that 3'-deATP strongly inhibits the incorporation of $^{32}P_i$ into cytoplasmic RNA. Under these conditions the labelling of one nuclear RNA fraction is inhibited about 40 %, while that of another nuclear RNA fraction is uninhibited. The latter fraction has the highest specific radioactivity, and is more tightly bound to protein than the rest of the RNA.

REFERENCES

Frederiksen, S., Klenow, H., unpublished results.

Frederiksen, S., Malling, H. and Klenow, H., results to be published.

Georgiev, G.P. and Mantieva, V.L., Biochim. Biophys. Acta, 61, 153 (1962).

Georgiev, G.P., Samarina, O.P., Mantieva, V.L. and Zbarsky, Biochim. Biophys. Acta, 46, 399 (1961).

Harbers, E. and Heidelberger, C., J. Biol. Chem., 234, 1249 (1959).

Harris, H. and Watts, J.W., Proc. Roy. Soc.(London), Ser.B., 156, 109 (1962).

Holland, J.J., Proc. Natl.Acad. Sci. U.S., 50, 436 (1963).

Klenow, H., Biochim. Biophys. Acta, 76, 354 (1963).

Klenow, H. and Frederiksen, S., Biochim. Biophys. Acta/SC 93003 (1964).

Klenow, H. and Frederiksen, S., Biochem. Biophys. Res. Comm., this issue (1964, a).

Klenow, H. and Overgaard-Hansen, K., Biochim. Biophys.Acta, 80, 500 (1964).

Robinson, J.R., Biochem. J., 45, 68 (1949).

Sibatini, A., Yamana, K., Kimura, K. and Okagaki, H., Biochim. Biophys. Acta, 33, 590 (1959).