MODE OF INHIBITION OF NUCLEAR POLY(A)POLYMERASE BY 2'-dATP AND 3'-dATP

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1. Introduction

Nontranscriptive addition of poly(A) to hnRNA is catalysed by poly(A)polymerase (EC 2.7.7.19). In vivo, low levels of 3'-deoxyadenosine (Cordycepin) inhibit nuclear poly(A) synthesis and the appearance of polyadenylated mRNA in the cytoplasm. The mechanism of inhibition of poly(A) synthesis by 3'-deoxyadenosine is not yet clear. It has been assumed that 3'-dAMP prevents poly(A) elongation by being incorporated into the growing poly(A) chain. Due to the lack of a 3'-OH group in the analog a new phosphodiester bond cannot be formed with the next incoming AMP (reviewed [1,2]). In contrast to 3'-deoxyadenosine, 2'-deoxyadenosine does not impair poly(A) synthesis if added to a culture of ascites tumor cells [3]. However, if purified poly(A)-polymerase from rat liver nuclei [4] or from HeLa cells [5] is used, poly(A) synthesis is inhibited to the same extent by 3'-dATP and the naturally occurring 2'-dATP.

It will be shown here that 3'-dATP inhibits poly(A)polymerase competitively with respect to ATP while a non-competitive type of inhibition has been found for 2'-dATP. Together with [4] these results indicate that in vitro 3'-dATP is not incorporated into the poly(A) chain but rather inhibits poly(A) synthesis by binding to the active site of poly(A)polymerase. Similarly, 2'-dATP inhibits the enzymatic activity of poly(A)polymerase without being incorporated into poly(A) to a significant extent [2,3].

This paper is dedicated to Professor Dr. Peter Karlson on the occasion of his 60th birthday

2. Methods

Poly(A)polymerase was extracted from purified rat liver nuclei and chromatographed on Bio-Gel A 1.5 m, DEAE-cellulose and CM-cellulose as in [4]. In these experiments only the Mn^{2+}-dependent poly(A)-polymerase corresponding to enzyme A and B [4] was used. Enzyme activity was determined by incubation at 37°C for 60 min in a reaction mixture containing in 0.2 ml: 20 mM Tris–HCl (pH 8.0), 5 mM 2-mercaptoethanol, 2–4 μCi [^{3}H]ATP at 0.1 mM or as indicated, 1 mM creatine phosphate, 5 μg creatine phosphokinase and 0.25 A_{260} units of primer RNA from rat liver nuclei. The radioactivity incorporated into poly(A) was determined as in [4].

3. Results and discussion

Figure 1 shows that poly(A) synthesis is inhibited to the same extent by 2'-dATP and 3'-dATP. At equimolar concentrations of ATP and 2'-dATP or 3'-dATP the poly(A)-synthesizing activity is reduced by 50% and maximum inhibition does not exceed 70% and 75% for 2'-dATP and 3'-dATP, respectively. The fact that poly(A) synthesis cannot be completely suppressed by high inhibitor levels may be due to a differential sensitivity of nuclear poly(A)polymerases towards 2'-dATP and 3'-dATP [6]. The very similar structure of 2'-dATP and 3'-dATP as well as the almost identical extent of inhibition of poly(A) synthesis observed at different concentrations of both inhibitors prompted us to investigate the mode of inhibition involved. In the double reciprocal plot of velocity against ATP concentration [7] in the presence and absence of 3'-dATP (fig.2) only the

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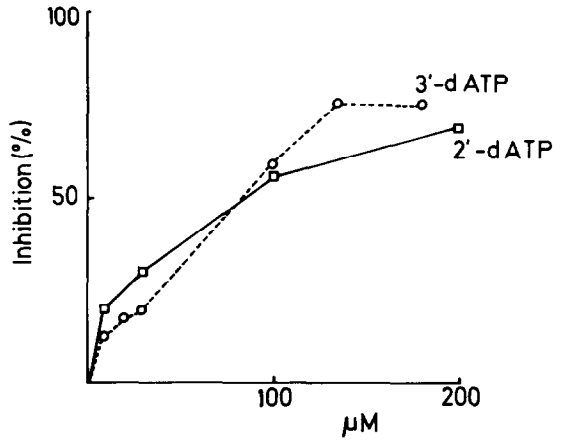

Fig.1. Effect of 2'-dATP and 3'-dATP on poly(A) formation by nuclear poly(A)polymerase.

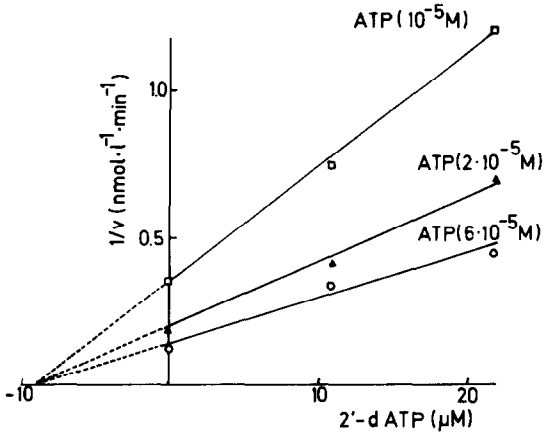

Fig.3. Non-competitive inhibition of poly(A)polymerase by 2'-dATP, plotted according to [9].

slope is affected by the inhibitor while the intercept remains constant. This indicates that the inhibition of poly(A)polymerase by 3'-dATP is competitive with ATP [7,8]. Moreover, at ATP >0.3 mM the reaction velocity slows down drastically which is indicative of substrate inhibition. The K_m and K_i calculated from the kinetics presented in fig.2 are 12 μM and 6 μM, respectively.

In the next experiment, 2'-dATP was used as an inhibitor of poly(A) synthesis instead of 3'-dATP. Figure 3 shows the double reciprocal plot of velocity against 2'-dATP concentration [9]. In this case both, the slope and intercepts are altered by the inhibitor

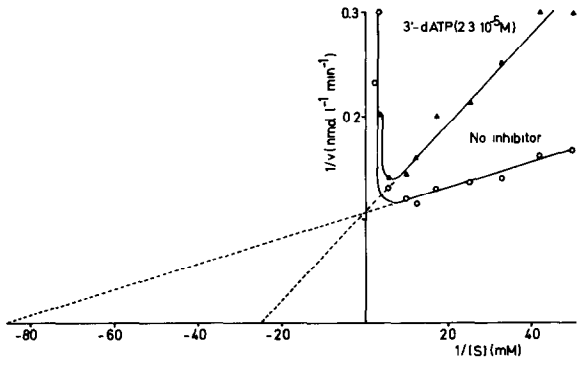

Fig.2. Competitive inhibition of poly(A)polymerase by 3'-dATP, plotted according to [7].

which is characteristic for a non-competitive type of inhibition [8,9]. The K_i values for the 3'-dATP inhibited poly(A) synthesis (9 μM) and the 2'-dATP inhibited reaction (9 μM) are very similar indicating an almost identical inhibitory potency and affinity to the enzyme.

From the results presented in fig.2 and fig.3 we conclude that 2'-dATP inhibits poly(A) synthesis by binding to the enzyme at a site which is not identical with the substrate binding site. On the other hand 3'-dATP competes with ATP for the active site of the enzyme and inhibits poly(A) synthesis by decreasing the rate of product formation. Due to the lack of a 3'-OH group 3'-dATP could also inhibit poly(A) synthesis by being incorporated into the poly(A) chain and subsequently block chain elongation. The role of 3'-dATP to act as a chain terminator in addition to a competitive inhibition has recently been demonstrated for nuclear Mg^{2+}-dependent poly(A)polymerase from quail oviduct [10]. However, using the Mn^{2+}-dependent poly(A)polymerase from rat liver nuclei we have presented evidence that neither 3'-dATP nor 2'-dATP is incorporated into poly(A) [3,4]. We therefore assume that 3'-dATP does not function as a chain terminator but rather inhibits poly(A) synthesis by competing with ATP for the active site of the enzyme.

The biological significance of the inhibition of poly(A) synthesis in vitro observed with the naturally

occurring 2′-dATP is not clear. It remains to be determined in which way impairment of poly(A) synthesis is avoided when high levels of 2′-dATP accumulate in the cell at the beginning of DNA-replication.

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