INHIBITION OF DNA POLYMERASE OF AVIAN MYELOBLASTOSIS VIRUS BY AN ALKALOID EXTRACT FROM NARCISSUS TAZETTA L.

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SUMMARY

An alkaloid extract of the Sacred Lily (narcissus tarzetta L.), a medicinal plant, inhibits the purified DNA polymerase from Avian myeloblastosis virus. The mechanism of action of this inhibitor, differs from that of other known inhibitors. The inhibitor physically combines with the polymerase, it does not affect the binding of the template to the enzyme as demonstrated by classical non-competitive inhibition kinetics and affects either the initiation or elongation phase of the polymerization reaction. The inhibition is the same whether viral 70S RNA or poly d(AT) is used as template.

RNA-dependent DNA polymerase (Reverse transcriptase) is associated with most known oncogenic RNA virus. This enzyme is responsible for the synthesis of DNA chains of the RNA template, giving rise to RNA-DNA hybrid molecules. The DNA chains are released from the RNA template as single stranded DNA molecules and serve as templates for the synthesis of double stranded DNA molecules (1-5). Studies on inhibitors of this enzyme and further understanding their mechanism of action may elucidate the replication cycle of tumor viruses and provide valuable information in the design of new drugs for the treatment of cancer.

We report here inhibition of DNA polymerase of a typical C-type virus, Avian myeloblastosis, by an alkaloid extracted from bulbs of Narcissus tazetta L. "Sacred lily" a medicinal plant grown in the Pacific area. The residual alkaloid used in these studies was the water soluble fraction remaining after removal of two major components, lycorine and pseudolycorine.

This residual alkaloid represents one of the relatively few drugs that exhibit *in vitro* and *in vivo* antiviral and antitumor activity. The narcissus alkaloid has been reported to have marked activity against neurotropic RNA virus infections in mice with EMC, JBE, and LCM[1] (6, 7) and oncogenic viruses *in vitro* (8) and *in vivo* (9).

Methods: Avian myeloblastosis virus (AMV) was obtained from chicken plasma concentrated and purified as described by Riman *et al* (10). DNA polymerase from AMV virus was isolated and purified by chromatography on DEAE and phosphocellulose, 70S viral RNA was extracted by the procedure of Kuff (12).

Results and Discussion: The activity of the purified enzyme was dependent on exogenous substrate. The enzyme reacted with single stranded 70S AMV RNA template, a reation greatly stimulated by the addition of short oligo$(dT)_{10}$ primer (13, 14).

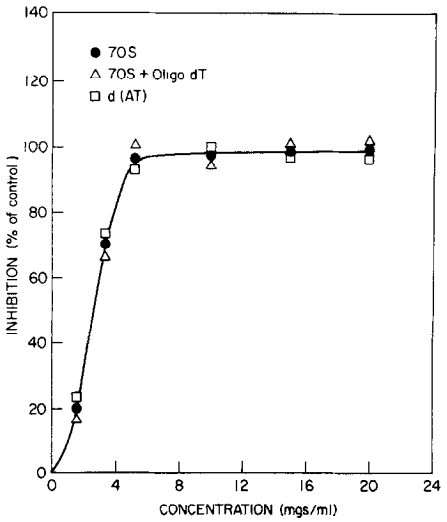
Omission of a single deoxynucleotide triphosphate, when 70S was used as template, markedly reduced DNA synthesis, thus indicating that polymerization was being observed and not terminal addition of triphosphates. The products of the reaction were alkali stable, sensitive to DNase and insensitive to RNase. Initial velocities were obtained by utilizing enzyme concentrations that insured a linear relationship between reaction rates and incubation time.

Increasing the concentration of the residual alkaloid was followed (Fig. 1) by a parallel decrease in enzymatic activity until total inhibition was achieved. No difference was observed when d(AT) or 70S RNA were used as templates.

To understand the complexity of the inhibition and to characterize its nature further, the residual alkaloid was added at zero time and ten minutes after initiation of the polymerization reaction. The inhibitor (Fig. 2) reacts instantaneously with the enzyme and no lag was observed.

To eliminate the possibility that the inhibitor acts by binding to the

[1] Abbreviations: EMC, encephalomycarditis; JBE, Japanese B encephalitis, LCM, lymphocytis choriomeningitis.



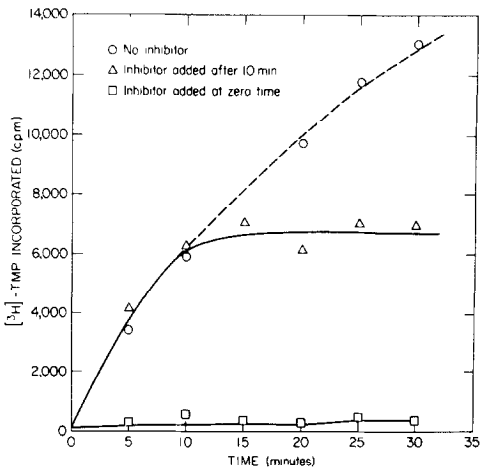


Fig. 1. Effect of residual alkaloid on the AMV DNA polymerase activity. DNA polymerase assays were carried out in 0.1 ml standard reaction mixture composed of 50 mM tris-HCl buffer, pH 8.3; 6 mM $MgCl_2$; 40 mM KCl; 6 mM dithiothreitol; 8.0 µM ^3H [TTP] (17500 count/min/p mole); 10 µgs purified AMV DNA polymerase. Inhibitor was added at zero time, at varied concentrations. In addition to standard reaction mixture each set of experiments contained 30 µg/ml 70S RNA; 0.2 mM each of the three unlabeled nucleotide triphosphate dATP, dGTP, dCTP; ●——● same components with previous experiments with addition of 20 µg/ml oligo $(dT)_{10}$. □——□ 20 µg/ml d(AT); 0.2 mM dATP. The reaction mixtures were incubated at 37° for 5 minutes. The reaction was terminated by addition of 1 ml 10% trichloracetic acid (TCA). The precipitates were collected on Whatman GF/C filters. The filters were washed with 10% TCA and radioactivity determined by scintillation counting.

Fig. 2. Effect of residual alkaloid on the kinetics of polymerization. Standard assay mixture contained the same components as of Fig. 1. The inhibitor was added to the reaction mixture at 0 and 10 minute time intervals. Aliquots (50 µl) taken at the indicated times, precipitated and counted as previously described.

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template we carried out the experiment shown in Fig. 3. The double reciprocal plots of polymerization rate vs. d(AT) concentration shows that the inhibition cannot be abolished at very high template concentrations. This argues that inhibition is due to binding of the inhibitor to the enzyme or to enzyme-template complex but not to template alone. The classical noncompetitive nature of inhibition (binding of template not affected by binding of inhibitor) and the immediate cessation of the polymerization reaction (Fig. 2)

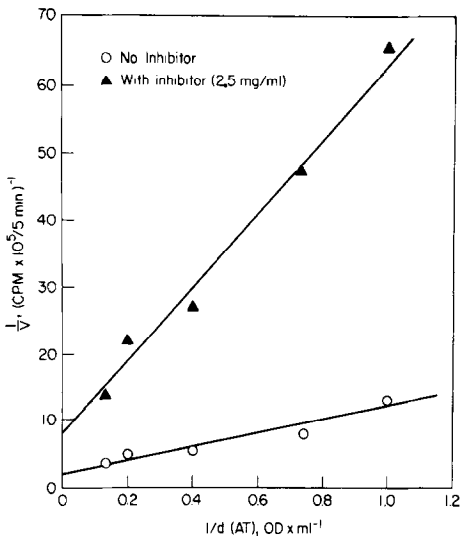

Fig. 3. Effect of residual alkaloid on the kinetics of polymerization. Lineweaver-Burk plot with d(AT) as the variable substrate. The standard reaction mixture (0.1 ml) composed of 50 mM tris-HCl, pH 8.3; 6 mM $MgCl_2$; 40 mM KCl; 6 mM dithiothreitol; 8 mM ^3H [TTP] (17000 count/min/picomole); 10 µgs purified AMV DNA polymerase; 20 µgs/ml d(AT); 0.2 mM dATP. The mixture was incubated at 37° for 5 minutes. The reaction was terminated by addition of 1 ml 10% trichloroactic acid, washed and counted as previously described.

suggested that the inhibitor interfered with the initiation or elongation phase of polymerization process rather than with initial binding of template to enzyme.

Two of the major components of the residual alkaloid, lycorine and pseudolycorine, have been removed. Neither showed polymerase inhibitory activity. Work is now in progress to isolate and characterize the active component(s) of the alkaloid extracts.

The mechanism of action of inhibitors of viral reverse transcriptase such as rifampicins (15, 16), streptovaricins (17), ethidium bromide (18, 19), daunomycin (20), homopolymers of ribonucleotides (21), bis-DEAE-fluorenone (22) has not been studied in detail. These inhibitors seem to interfere with binding of template to the enzyme either by directly interacting with template (23) or by competing with the template for the enzyme site, thus preventing synthesis (21). To our knowledge this is the first report of an

inhibitor of transcriptase which binds to the polymerase and affects steps subsequent to the initial binding of template to enzyme. The importance of such inhibitors which works by binding to enzyme rather than interacting with nucleic acid template has been greatly emphasized in recent reviews (23, 24).

The inhibitory activity of this residual alkaloid coupled with it's reported antiviral activity make this alkaloid a promising therapeutic agent.

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