BIS-DEAE-FLUORENONE: A SPECIFIC INHIBITOR OF DNA POLYMERASES FROM RNA TUMOR VIRUSES

P. CHANDRA*, F. ZUNINO and A. GÖTZ
Institut für Therapeutische Biochemie der Universität Frankfurt/M., Germany

Received 9 March 1972

1. Introduction

The dihydro-chloride salt of 2, 7-bis (2-(diethyl-amino) ethoxy)- fluoren-9-one, referred to as tilorone hydrochloride or bis-DEAE-fluorenone (DEAE-F), is a broad spectrum antiviral compound [1] with anti-tumor activity [2, 3]. Mayer and coworkers [4, 5] have identified this compound as an interferon inducer and established a relationship with the antiviral activity. To our knowledge, nothing is yet known about the molecular mechanism of action of this compound. This report describes the action of DEAE-F on the DNA polymerases from mammalian RNA tumor viruses, FLV (Friend) and MSV (Moloney). We find that in both systems DEAE-F is a very potent inhibitor of DNA polymerases. This inhibition is uniquely and selectively dependent on the type of primer-template used in the assay system. The reactions catalyzed by poly(dA–dT), poly (rA.dT) and poly rA. $(dT)_{12}$ are highly sensitive to DEAE-F inhibition, whereas in the presence of poly (dI.dC) DEAE-F was found to stimulate ^{3}H-dGMP incorporation into DNA.

2. Materials and methods

Labeled deoxynucleoside triphosphates were obtained from NEN-Chemicals GmbH, Germany. Poly (dA–dT) and poly rA were supplied by Miles Laboratories, Elkhart, Indiana, USA. Poly (dI.dC) and poly rA.$(dT)_{12}$ were provided by Dr. G. Weimann of Boehringer Mannheim, GmbH. Bis-DEAE-fluorenone was a gift of Merrel & Co. to Prof. C.L. Fox.

MSV(Moloney), kindly supplied by Dr. J.B. Moloney (NCI, Bethesda, Md.) was passed by intramuscular injection into suckling Swiss mice, and extracted from the tumor tissue. Friend Leukemia Virions (FLV) were isolated from infected spleens (AKR mice) and purified by sucrose-density gradient centrifugation. The viral extracts used to assay for DNA-polymerase activity were stored at –70° before use.

DNA polymerase activity was assayed essentially by the method of Ross et al. [6]. The reaction mixture, regardless of template, was similar to that of Ross et al. [6], except that we used 0.04 M Tris and the end concentration of Nonidet P-40 was 0.2%. The reaction mixture contained 0.25 μg of the template used. Reaction mixtures (vol 0.25 ml) containing virions (12–15 μg protein) were incubated at 37° for 90 min. Acid precipitable material was counted on "Millipore" filters (HAWP 02500) in a liquid scintillation counter. Protein was estimated by the method of Lowry et al. [7].

3. Results and discussion

Munson et al. [3] have recently shown that DEAE-F is effective in inhibiting the established Friend viral leukemia. They believe that interferon induction may not be responsible for the antitumor activity of this compound. This suggests that the virus-associated enzymatic activities, DNA polymerases, may be sensitive towards the action of DEAE-F. Table 1 shows the inhibition of reverse-transcriptase activity from MSV

* Correspondence should be sent to: Professor P. Chandra, Institut für Therapeutische Biochemie der Universität, Ludwig-Rehn Str. 14, Frankfurt/M., Germany

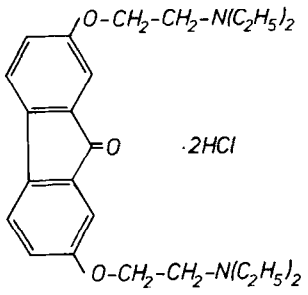

Fig. 1. Chemical structure of bis-DEAE-fluorenone.

(Moloney) and FLV(Friend) by DEAE-F.

Results presented in table 1 show that the *in vitro* system is dependent on the source of enzyme, i.e. virions, and sensitive to RNase. Preincubation of virions with RNase blocks their activity to synthesize DNA. This shows that the endogenic template, viral RNA, is required for the synthesis of DNA. DEAE-F added to the reaction mixture inhibits the DNA-polymerase activity in MSV(M) and FLV. At low concentrations (5 μg/reaction mixt.) of DEAE-F the MSV(M) system is more sensitive than FLV. However, at higher concentrations of DEAE-F the inhibition in both the systems is of the same magnitude. It is interesting to note that concentrations as low as 20 μg/reaction mixt. are able to inhibit approximately 70% of incorporation of ^{3}H-TMP into DNA.

Synthetic polymers containing either deoxyribonucleotide or ribonucleotide strands are known to stimulate the *in vitro* DNA synthesis by RNA tumor viruses [8, 9]. Some inhibitors of the DNA-polymerase reaction in RNA tumor viruses are known to exhibit a template-primer specificity [10–12]. Table 2 shows the inhibition of template-dependent DNA polymerase activity of FLV by DEAE-F at various concentrations.

The reaction catalyzed by poly (dA–dT) is most sensitive to the action of DEAE-F. At 5 μg/reaction mixt. of DEAE-F more than 80% inhibition of ^{3}H-TMP incorporation was seen. Reactions catalyzed by poly (rA.dT) and poly rA.$(dT)_{12}$ are not inhibited at this concentration of DEAE-F. However, at higher concentrations the reactions are very sensitive towards DEAE-F. Compared to poly (rA.dT), the reaction catalyzed by poly rA.$(dT)_{12}$ is more sensitive to DEAE-F action. Surprisingly, the reaction catalyzed by poly (dI.dC) is strongly stimulated by DEAE-F. Thus at 20 μg/reaction mixt. of DEAE-F the incorporation of ^{3}H-dGMP is almost 4 times that of control.

Table 3 shows the inhibition of template-dependent DNA polymerase activity in MSV(M) by DEAE-F at various concentrations. Compared to results on FLV, one observes that the MSV(M) system, regardless of the nature of the template used, is more sensitive to DEAE-F inhibition. Thus, the poly (dA–dT) catalyzed reaction is totally inhibited at 5 μg concentration of DEAE-F. The magnitude of DEAE-F inhibition of poly (rA.dT) catalyzed reaction is the same as observed in FLV system. However, the poly rA.$(dT)_{12}$ catalyzed reaction in MSV(M) system is twice as sensitive towards DEAE-F as the one in FLV system. Even in this system DEAE-F was found to stimulate dGMP incorporation in the presence of poly (dI.dC). However, the stimulation in this system was moderate, and was not observed at higher concentrations

Since the DNA-dependent (poly dA–poly dT) activity was found to be most sensitive to DEAE-F inhibition in both the viral systems, it was of interest to study DEAE-F effect on other DNA-dependent reactions. Table 4 shows the action of DEAE-F on the DNA-dependent RNA polymerase (*E. coli* K-12) reaction.

Levels of DEAE-F that inhibit viral (MSV-M and FLV) DNA polymerase activity, poly (dA–dT)-dependent incorporation of TMP, more than 90% show no inhibition of DNA-dependent RNA synthesis in the *E. coli* system. Even at higher concentrations of DEAE-F it was not possible to achieve this level (more than 90%) of inhibition.

The present results show that the inhibition exerted by DEAE-F against DNA polymerases from RNA tumor viruses is uniquely and selectively dependent on the type of primer-template used in the assay system. The wide differences between the inhibitory concentrations of DNA-dependent enzymatic reactions in RNA tumor viruses and bacteria could be of a significant therapeutic value. However, it would require more studies on the DNA-dependent reactions in animal cells before one may speculate on its therapeutic superiority. Studies on these aspects are in progress in our laboratory.

Table 1
Inhibition of reverse-transcriptase activity of RNA tumor viruses by DEAE-fluorenone.

System	DEAE-F concentration (μg/reaction mixt. (0.25 ml))	^{3}H-TMP incorporation into DNA (cpm/reaction mixt.) MSV (Moloney)	FLV (Friend)
Without virions	–	7 (3.2)	7 (2.6)
Virions + RNase*	–	31 (14.3)	39 (14.8)
Complete	none	216 (100.0)	262 (100.0)
	5	112 (51.8)	189 (72.0)
	10	88 (40.5)	128 (48.8)
	20	57 (26.4)	81 (30.9)

* Virions containing Nonidet P-40 were preincubated at room temp for 25 min with 50 μg/ml of pancreatic RNase. Figures in parentheses are percent of control. The reaction conditions are described under Materials and methods.

Table 2
Inhibition of DNA-polymerase activity from FLV (Friend) by DEAE-fluorenone in the presence of various templates.

DEAE-F concentration* (μg/reaction mixt.)	^{3}H-TMP incorporation into DNA (cpm/reaction mixt.) poly (dA–dT)	poly (rA.dT)	poly rA.$(dT)_{12}$	^{3}H-dGMP incorporation into DNA (cpm/reaction mixt.) poly (dI.dC)
none	2387 (100.0)	5330 (100.0)	572 (100.0)	1797 (100.0)
5	460 (19.2)	5170 (97.0)	493 (86.2)	3707 (206.3)
10	292 (12.2)	3619 (67.9)	317 (55.4)	3962 (220.4)
20	190 (8.0)	2808 (52.6)	212 (36.5)	6842 (380.7)

* μg/0.25 ml of reaction mixt. The figures in parentheses indicate the percent of control (without DEAE-F). The reaction conditions are described under Materials and methods.

Table 3
Inhibition of DNA-polymerase activity from MSV(Moloney) by DEAE-fluorenone in the presence of various templates.

DEAE-F concentration* (μg/reaction mixt.)	^{3}H-TMP incorporation into DNA (cpm/reaction mixt.) poly (dA–dT)	poly (rA.dT)	poly rA.$(dT)_{12}$	^{3}H-dGMP incorporation into DNA (cpm/reaction mixt.) poly (dI.dC)
none	2516 (100.0)	8911 (100.0)	413 (100.0)	2107 (100.0)
5	107 (4.2)	7852 (88.1)	181 (43.8)	3336 (158.3)
10	100 (3.9)	6466 (72.5)	106 (25.7)	3243 (153.9)
20	67 (2.6)	4212 (47.2)	86 (20.8)	2660 (126.2)

* μg/0.25 ml of reaction mixt. The figures in parentheses indicate the percent of control (without DEAE-F). The reaction conditions are described under Materials and methods.

Table 4
Inhibition of DNA-dependent RNA polymerase reaction (*E. coli* K_{12}) by DEAE-fluorenone.

System	DEAE-F concentration* (μg/reaction mixt.)	^{14}C-AMP incorporation into RNA (cpm/reaction mixt.)	% of Control
Without UTP, CTP and GTP	–	14	3.2
Without DNA	–	11	2.5
Complete	none	435	100
	25	420	96.5
	50	315	72.4
	100	130	29.8

*μg/ 0.25 ml of reaction mixt. The reaction conditions are described under Materials and methods.

Acknowledgements

We thank Professor A. Wacker, Dr. G.D. Mayer, Dr. R.F. Krueger and Dr. A. Richardson for encouragement and valuable discussions.

References

[1] R.F. Krueger and S. Yoshimura, Federation Proc. 29 (1970) 635.
[2] R.H. Adamson, J. Natl. Cancer Inst. 46 (1971) 431.
[3] A.E. Munson, J.A. Munson and W. Regelson, Int. Colloq. on Interferone and Interferon Inducers, Leuven, Belgium, Abstract No. 27 (1971).
[4] G.D. Mayer and R.F. Krueger, Science 169 (1970) 1214.
[5] G.D. Mayer and B.A. Fink, Federation Proc. 29 (1970) 635.
[6] J. Ross, E.M. Scolnick, G.J. Todaro and S.A. Aaronson, Nature New Biol. 231 (1971) 163.
[7] O.H. Lowry, N.I. Rosebrough, A.L. Farr and R.J. Randall, J. Biol. Chem. 193 (1951) 265.
[8] S. Spiegelman, A. Burny, M.R. Das, J. Keydar, J. Schlom, M. Travnicek and K. Watson, Nature 228 (1970) 430.
[9] D. Baltimore and D. Smoler, Proc. Natl. Acad. Sci. U.S. 68 (1971) 1507.
[10] P. Chandra, F. Zunino, A. Götz, D. Gericke, R. Thorbeck and A. Di Marco, FEBS Letters 21 (1972) 264.
[11] P. Chandra, F. Zunino, A. Götz, A. Wacker, D. Gericke, A. Di Marco, A.M. Casazza and F. Guiliani, FEBS Letters 21 (1972) 154.
[12] B. Fridlender and A. Weissbach, Proc. Natl. Acad. Sci. U.S. 68 (1971) 3116.