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The inhibition of mitochondrial DNA polymerase γ from animal cells by intercalating drugs

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ABSTRACT

DNA polymerase γ from purified nuclei of EMT-6 cells (mice) seems to be identical to the mitochondrial DNA polymerase from the same source following several criteria. These two enzyme activities are strongly inhibited by ethidium bromide and acriflavin, while proflavin, acridine orange, daunomycin and chloroquine inhibition is less pronounced. In the case of DNA polymerases α and β very little inhibition by ethidium bromide was observed. Intercalation of this dye in a poly dA-dT_{12-18} template-primer was studied spectrophotometrically under conditions similar to those in the in vitro DNA polymerase assay. The inhibition by this drug of the mitochondrial DNA polymerase γ activity was shown to be competitive at varying concentrations of TTP while the inhibition was of the non-competitive type at different concentrations of poly dA-dT_{12-18}. We conclude that the drug, most probably in the intercalated form, is able to interact with the active site (s) of mitochondrial DNA polymerase.

INTRODUCTION

Several lines of evidence indicate that DNA polymerase γ purified from nuclei or the soluble cytoplasm of animal cells is identical to the DNA polymerase from mitochondria of the same organisms (mt DNA polymerase). Both enzymes recognize very efficiently a poly A-dT_{12-18} template in the presence of manganese ions, their size (6-7 S) is very similar, the chromatographic behaviour of the two enzymes on DEAE-cellulose and phosphocellulose columns is identical and the intercalating dye ethidium bromide, inhibits dramatically both enzymes (1-3).

When animal cell cultures are incubated in the presence of the intercalating drug ethidium bromide a preferential inhibition of mitochondrial DNA synthesis is observed (4). It is assumed that the target of the drug is the closed circular double stranded mitochondrial DNA. The fact that purified mitochondrial DNA polymerase is inhibited in vitro by ethidium bromide with a variety of templates (5-7), prompted us to study the

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possibility that the drug was affecting not only the nucleic acid template but also the mitochondrial enzyme.

In the following report we describe the effect of several intercalating drugs on polymerase γ from mitochondria and nuclei of EMT-6 cells. We have focused our attention on the effect of ethidium bromide on the different DNA polymerases purified from EMT-6 cells. The study of the type of inibition of mt DNA polymerase by this drug at varying concentrations of TTP and template and the affinity of ethidium bromide to poly dA-dT_{12-18} determined by spectrophotometrically titration are described.

MATERIAL AND METHODS

Labeled products were provided by the Radiochemical Centre Amersham. Unlabeled nucleoside triphosphates were bought from Sigma and Boehringer. Poly dA, poly dT, poly dC, oligo dT_{12-18} came from Miles, oligo dG_{12-18} from Pabst Laboratories and poly A from Sigma. All intercalating drugs were from Sigma.

Deoxyribonuclease I was from Sigma. *E. coli* DNA polymerase I was a kind gift of Dr. G. Brun (Paris). DEAE-cellulose DE-22 and phosphocellulose P11 were from Whatman, and were prepared following the technique described by Peterson (8).

Growth of EMT-6 cells. EMT-6 cells (9) were grown at 37° C in RPMI 1629 medium supplemented with 10 % fetal bovine serum containing 200 U/ml penicillin and 0.2 mg/ml streptomycin.

Preparation of mitochondria. EMT-6 cells, washed with 0.15 M NaCl, were resuspended by gentle pipetting in 0.6 M mannitol, 10 mM Tris-HCl pH 7.5, 2 mM EDTA and homogenized with a Dounce B homogenizer (crude extract). The extent of cell breakage was followed with a phase contrast microscope. When at least half of the cells were broken, the suspension was centrifuged (1 min at 1500 g) and the pellet submitted to the same treatment as long as nuclei were intact. The various supernatants were mixed, centrifuged 7 min at 1080 g to sediment nuclei and cell debris, and this procedure was repeated until no precipitate could be observed (usua lly 7 times). The supernatant was centrifuged 23 min at 10 500 g, and the pellet washed by an analogous centrifugation. The second pellet (mitochondria) was resuspended in 0.25 M sucrose, 10 mM Tris-HCl pH 7.4 and 2 mM EDTA. No nuclei nor cell debris could be detected by phase contrast microscopy. In electron microscopy, mitochondria are seen under two different configurations : swollen and twisted. Freshly prepared mitochondria were tested for respiratory capacity

(13) using succinate as substrate. A respiratory control ratio (Q_{O_2} with ADP/Q_{O_2} without ADP) of 1.6 was determined suggesting minimal mitochondrial damage.

Sonication of mitochondria. 0.2 % Triton X-100 was added to the mitochondrial suspension in a volume ratio of 1 : 3 and the solution was sonicated three times for 10 sec in an Annemasse ultrasonic disintegrator at maximal intensity.

Partial purification of mitochondrial DNA polymerase. In order to purify mitochondrial DNA polymerase from other polymerases, sonicated mitochondria were chromatographed through a DEAE-cellulose column; the fractions were tested using either poly A-dT_{12-18} or activated DNA as template. The adsorbed enzyme was eluted as a single peak at 0.13 M KPi, either with poly-A-dT_{12-18} or DNA assays. The peak was concentrated against a solution of 50 % (v/v) glycerol in 10 mM Tris-HCl pH 7.5, 0.5 mM DTT.

DNA polymerase α was purified from the mitochondrial supernatant while polymerase β and γ were purified from nuclei of EMT-6 cells. The detailed purification procedure will be published elsewhere.

Protein determination. Protein was determined by the method of Lowry (10) slightly modified by the presence of deoxycholic acid in order to solubilize the proteins bound to membranes.

DNA activation. Calf thymus DNA was activated by treatment with pancreatic DNAse I using the method of Aposhian and Kornberg (11).

Preparation of synthetic double stranded polynucleotides. The oligonucleotides were annealed with the polynucleotides by heating at 65-70° C for 15 min, a solution containing $2^{OD_{260}}$/ml of the template and $0.4^{OD_{260}}$/ml of the primer in 10 mM Tris-HCl pH 7.5, 100 mM NaCl. After heating, the solution was cooled slowly at room temperature.

DNA polymerase assays. The assays of DNA polymerase α, β and γ as described by Knopf et al.(12) contained in a final volume of 50 µl :

a) Mix α : 180 µg/ml activated DNA, 0.05 mM of each dATP, dGTP, dCTP, 0.5 µM to 10 µM of (methyl-^{3}H)Thymidine 5'-triphosphate (specific activity 0.25-2.5 Ci/mmol), 20 mM K phosphate pH 7.2, 8 mM $MgCl_2$, 1 mM 2-mercaptoethanol, 0.1 mM EDTA, 200 µg/ml bovine serum albumin.

b) Mix β : 180 µg/ml activated DNA, 0.05 mM of each dATP, dGTP, dCTP, 0.5 µM to 10 µM of (methyl-^{3}H) Thymidine 5'-triphosphate (specific activity 0.25-2.5 Ci/mmol), 50 mM Tris-HCl pH 8.6, 8 mM $MgCl_2$, 100 mM NaCl, 1 mM 2-

mercaptoethanol, 200 µg/ml bovine serum albumin. 5 mM N-ethylmaleimide (NEM) was preincubated with the enzyme at 0° C for 30 min.

c) Mix γ : 20 µg/ml poly A-dT_{12-18} (5 : 1), 0.5 µM to 10 µM of (methyl-^{3}H) Thymidine 5'-triphosphate (specific activity 0.25-2.5 Ci/mmol), 25 mM Tris-HCl pH 8.3, 50 mM K phosphate pH 8.4, 0.5 mM $MnCl_2$, 100 mM KCl, 2 mM DTT, 200 µg/ml bovine serum albumin.

Incubation was carried out at 37° C for 30 min. The reaction was stopped by addition of 0.1 ml ice cold 20 % TCA with 1 % pyrophosphate. The mixtures were filtered, washed with 5 % cold TCA, dried and counted in a PPO-POPOP-toluene scintillation mixture.

When poly dC-dG_{12-18} was used as template, the complementary substrate deoxy[8-^{3}H]guanosine 5'-triphosphate, replaced (methyl -^{3}H)Thymidine 5'-triphoshate. Two simplified assays for TMP incorporation using activated DNA or poly A-dT_{12-18} as template were used also :

DNA mix : 50 mM Tris-HCl pH 8.0, 10 mM DTT, 10 mM $MgCl_2$, 32 µg/ml activated DNA, 0.05 mM of each dATP, dCTP, dGTP, 0.5 µM to 10 µM of (methyl-^{3}H) Thymidine 5'-triphosphate (specific activity 0.25-2.5 Ci/mmol), 10 µg/ml bovine serum albumin. Poly A-dT_{12-18} mix : 50 mM Tris-HCl pH 8.0, 10 mM DTT, 0.5 mM $MnCl_2$, 100 mM KCl, 20 µg/ml poly A-dT_{12-18}, 0.5 µM to 10 µM of (methyl-^{3}H) Thymidine 5'-triphosphate (specific activity 0.25-2.5 Ci/mmol), 100 µg /ml bovine serum albumin.

Spectrophotometric titration and analysis of binding data. The binding of ethidium bromide to activated DNA and poly dA-dT_{12-18} was performed using a fixed concentration of the drug corresponding to the molarity used in the in vitro DNA polymerase assay (20 µM). A volume of distilled water similar to each nucleic acid aliquot was added to the blank cell with ethidium bromide in order to keep the drug concentration constant. The absorbance for each concentration of added template was recorded between 400 and 600 nm in a Cary 118 spectrophotometer thermoregulated at 37° C. Plots of the optical density at 480 nm of dye solution containing varying concentrations of the polynucleotide were done according to Scatchard (14).

ν is the mean number of moles of ligand bound per mole of macromolecule and L is the concentration of free ligand. When there is only one class of independent, non interacting binding site, a plot of ν/c versus ν gives a straight line with the slope equal to -Ka and the intercept on the abscissa gives the total number of binding sites (14).

However when more than one class of non equivalent sites of binding is involved, this kind of plot yields a composite line and the binding para-

meters must be calculated using the graphical analyses described by Klotz (15,16).

RESULTS

Properties of the mitochondrial DNA polymerase.

The DNA polymerase from purified mitochondria was tested after sonication of the organelles as described under methods. As seen in Table I the main response is obtained using the mix γ.

	Crude extract (pmoles/mg protein)	Mitochondria (pmoles/mg protein)
α-assay	969	741
β-assay	586	280
γ-assay	394	1022

Table I : DNA polymerase activities in crude extract and purified mitochondria.

The α, β and γ assays were done as described under Material and Methods.

The mitochondrial enzyme purified by ion exchange chromatography was assayed with different templates in the presence of magnesium or manganese; as seen in Table II, the mitochondrial DNA polymerase is very active in the presence of manganese ions when the template poly A-dT_{12-18} was used, while the template poly dC-dG_{12-18} was more active in the presence of magnesium ions.

Template	Mn^{++}		Mg^{++}	
	Units	%	Units	%
Poly A-dT_{12-18}	3.25	100	0.24	100
Poly dA-dT_{12-18}	0.85	26	0.73	304
Poly dC-dG_{12-18}	2.93	90	6.68	2783
Activated DNA	1.89	58	2.98	1241

Table II : DNA polymerase activity using different templates.

The enzymatic test was done using the γ-assay as described under Materials and Methods. One unit corresponds to the amount of enzyme able to incorporate 1 nmol of nucleotide during 1 hour at 37° C. In the case of activated DNA, the nanomoles of TMP incorporated are multiplied by 3.5.

Effect of DNA intercalating agents on cellular DNA polymerases.

In Table III is shown the percentage of inhibition of DNA polymerases α, β, γ and mt by six drugs known to inhibit DNA function by intercalation (17).

	Proflavine		Acridine Orange		Cloroquine		Daunomycine		Acriflavine		Ethidium Bromide	
	20 µM	40 µM	20 µM	40 µM	20 µM	40 µM	20 µM	40 µM	20 µM	40 µM	20 µM	40 µM
α	10	32	38	70	5	8	0	0	55	85	18	62
β	23	34	36	75	10	7	41	63	82	96	0	0
γ	49	60	47	55	0	9	48	53	85	94	84	89
mt	37	66	32	46	0	3	30	40	87	98	94	96

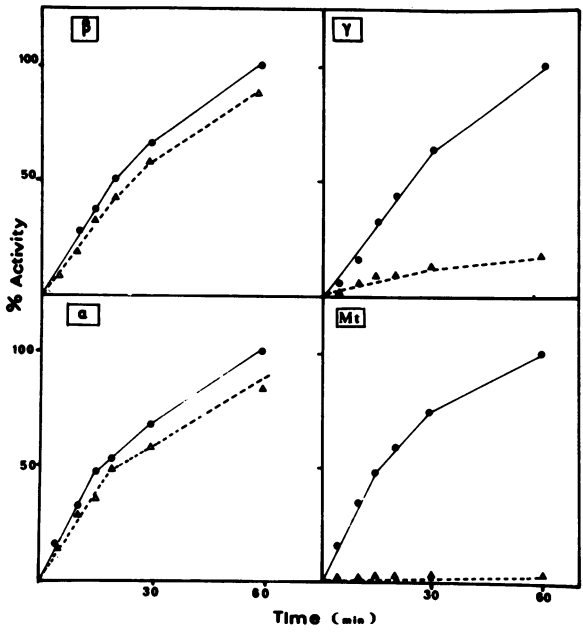
Table III : DNA polymerases inhibition by DNA-intercalating drugs.
The results are given as percent of inhibition. The template used was poly dA-dT_{12-18}. Incubations were carried out at 37° C for 60 min as described in the text. In the case of polymerase α, 100 percent corresponds to 15 pmoles, in the case of polymerase β to 42 pmoles, for polymerase γ and mt was equal to 17 and 58 pmoles respectively.

It can be seen that the effect is dependent on the specific inhibitor used. Enzymes α and β seem less affected than the γ and mt enzymes. Once more the mitochondrial enzyme and polymerase γ give very similar responses to the different inhibitors. Acriflavine and ethidium bromide are the most powerful inhibitors of these last two enzymes. In the case of ethidium bromide this drug inhibits markedly the γ and mt polymerases while DNA polymerase α and β are only slightly affected.

The inhibition by ethidium bromide of mt DNA polymerase being so important we focused our study on the action of this drug. A kinetic study of the inhibition with the four cellular DNA polymerases is shown in Figure 1. While initial velocity was severely affected in the case of DNA polymerases γ and mt this parameter was only slightly changed for the α and β enzymes.

Spectrophotometric titration of ethidium bromide with poly dA-dT_{12-18}

The template most used in our study was poly dA-dT_{12-18}. Because of the short lenght of the primer, one can ask if drug intercalation between both strands of the template-primer complex is operating, or whether only the electrostatic interaction between the dye and the phosphate backbone is valid in this system. The experiment described in Figure 2 was done to answer this



The template used was poly dA-dT_{12-18} and ethidium bromide was used at a concentration of 20 µM.

●——● control
Δ----Δ plus ethidium bromide.

Fig. 1 : Kinetics of DNA polymerase activities in the presence and absence of ethidium bromide.

question. Figure 2B shows the differential absorbance spectrum of ethidium bromide at four concentrations of poly dA-dT_{12-18}. The spectrum is characterized by a maximum at 545 nm and a minimum at 480 nm with an isobestic point at 510 nm. The variation of absorbance at 480 nm was used to measure the amount of complex formed. The Scatchard plot of Figure 2A calculated from the values of Figure 2B and other not shown, shows two classes of binding sites with different affinities. The mathematical analysis was performed considering the two classes of sites as independent and non interacting, by using the formula (15, 16) :

$$\nu = \frac{n_1 K_1 A}{1 + K_1 A} + \frac{n_2 K_2 A}{1 + K_2 A}$$

where n_1 and n_2 are the number of sites of higher and lower affinities respectively, K_1 and K_2 are the association constants for sites of higher and lower affinities and A is the concentration of free inhibitor.

Effect of ethidium bromide on the activity of mitochondrial and γ DNA polymerases at variable TTP concentrations.

Ethidium bromide was tested as an inhibitor of mitochondrial and γ DNA polymerases at various concentrations of TTP using poly dA-dT_{12-18} as template. The plot 1/v versus 1/s is shown in Figure 3. A competitive type

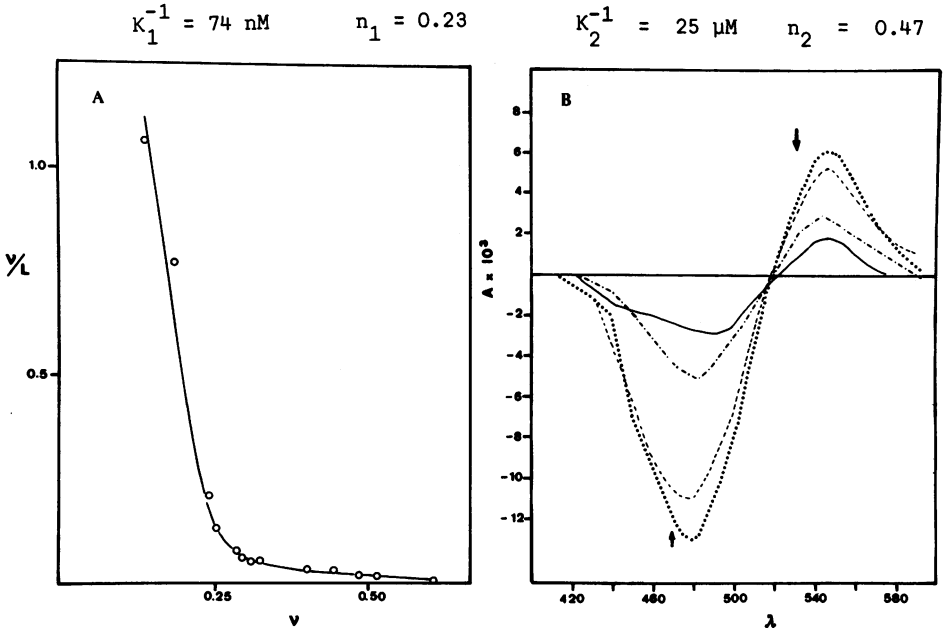


Fig. 2 : Spectrophotometric titration of ethidium bromide with varying amounts of poly dA-dT_{12-18}.
2 A - Scatchard plot. Details are given in the text.
2 B - Differential absorbance spectra of mixtures of ethidium bromide and various concentrations of poly dA-dT_{12-18} in 10 mM Tris-HCl buffer pH 7.9 at 37° C. —— 7 µM, ·-·-·- 17 µM, – --- 32 µM, ····· 53 µM poly dA-dT_{12-18} calculated as polynucleotide-phosphorus.

of inhibition was observed in this kind of experiments, i.e. the Lineweaver-Burk type of analysis gives the same value for Vm in presence or absence of inhibitor. The Michaelis constant (K_M value) and Vm of the enzyme for TTP as well as the K_M, and V_m, values obtained in the presence of inhibitor can be calculated. With these values as well as with the inhibitor concentration (I), the dissociation constant of the (enzyme-inhibitor)complex (Ki) can be calculated according to the equation :

$$Ki = [I] \Big/ \left(\frac{K_M'}{K_M} - 1 \right)$$

The values of K_M, K_M', and Ki are shown in Table IV.

Effect of dye on DNA synthesis by mitochondrial polymerase at variable poly dA-dT_{12-18} concentrations.

A non competitive type of inhibition at a concentration of poly dA-dT_{12-18} higher than 40 µM was obtained when the inhibitory capacity of ethidium bromide was tested with the mitochondrial DNA polymerase as seen in

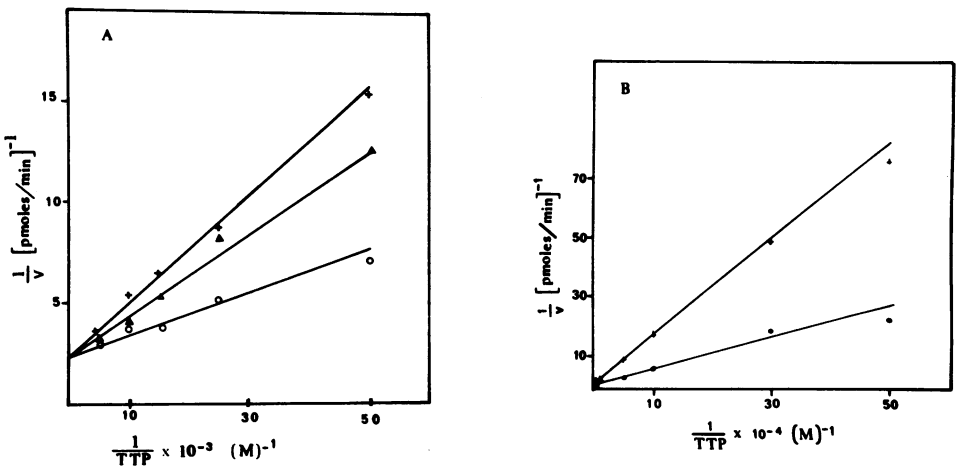


Fig. 3 : Effect of ethidium bromide on the incorporation of TMP into dA-dT_{12-18} by DNA polymerase mt (3A) and DNA polymerase γ (3B) at variable concentrations of TTP.

O—O control, Δ—Δ 10μM ethidium bromide, •—• 20 μM ethidium bromide. Reciprocal plot according to Lineweaver-Burk. The Michaelis and inhibition constants are shown in Table IV.

Figure 4. Different values of Vm were obtained in the presence or absence of the inhibitor, while K_M given by the abscissa intersection, are the same in both cases as seen in Table IV.

At concentration of template lower than 40 μM an uncompetitive inhibition is observed in the biphasic double reciprocal plot of Figure 4.

DISCUSSION

Our work was originally concerned with the purification and characterization of the mitochondrial DNA polymerase from mouse cells cultivated in vitro. While this work was under progress, γ polymerases obtained from the post mitochondrial supernatant of HeLa cells (1), nuclei of chick embryos (2) and rat brain nuclei (3) were reported to be identical to the mitochondrial DNA polymerase from the same sources.

In this article we describe some properties of EMT-6 mitochondrial DNA polymerase. Not shown are some experiments indicating that γ-polymerase from purified nuclei and the mitochondrial enzyme, both from EMT-6 cells, are the same. By chromatographic criteria (DEAE-cellulose and Phosphocellulose), effect of KCl and phosphate, molecular weight determination under high ionic strength, template specificity (unpublished results) and the results shown in Tables I and II we concluded that the enzymes isolated from nuclei and mito-

A - For varying amounts of TTP	K_M	$K_{M'}$	Ki	(I)
DNA polymerase γ (nuclei)	45 µM	125 µM	11 µM	20 µM
DNA polymerase γ (mt)	47 µM	91 µM	21 µM	20 µM

B - For varying amounts of poly dA-dT_{12-18}	K_M	Vm	Vm'	Ki	I
DNA polymerase γ (mt)	40 µM	0.13 $\frac{pmol}{min}$	0.019 $\frac{pmol}{min}$	3.46 µM	20 µM

Table IV : Kinetic constants of DNA polymerase γ.

chondria of exponentially grown EMT-6 cells are the same.

When the "γ incubation mixture" was used (50 mM phosphate and poly A-dT_{12-18} template) the DNA polymerase activity from purified organelles was higher than the one from the crude extract. The partially purified enzyme from EMT-6 mitochondria is very active with a poly A-dT_{12-18} template in the presence of manganese ions as shown in Table II. This is common property to all DNA polymerases γ described up to now (7). As in the case of the enzyme of HeLa cells, it recognizes very efficiently a poly dC-dG_{12-18} template in the presence of magnesium. The relatively low activity found for poly dA-dT_{12-18} in the presence of both cations, can be explained by the presence of 50 mM phosphate, which inhibits the activity of the polymerase to 20-30 % of the control activity (12).

The enzyme used in the experiments described in this report is not homogenous. After DEAE-cellulose and Phosphocellulose chromatography only one peak of DNA polymerase activity was found. Under the same conditions and starting with purified nuclei, polymerases α, β and γ can be well separated on DEAE-cellulose and phosphocellulose columns. We conclude that most probably only one type of DNA polymerase is present in the solubilized fraction of EMT-6 cells mitochondria.

The conclusion that γ and mitochondrial polymerases are one and the same enzyme is confirmed by the experiments shown in Table III. The pattern of inhibition with several nucleic acid intercalating drugs is very similar for the two polymerases. Acriflavin and ethidium bromide are the most powerful inhibitors of these enzymes, the effect being almost total at 20 µM. Clo-

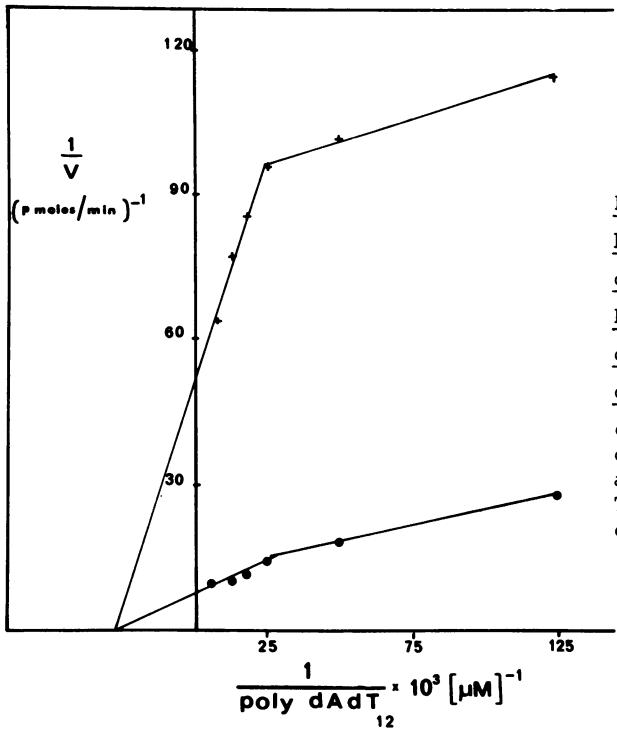


Fig. 4 : Effect of ethidium bromide on the incorporation of TMP into poly dA-dT_{12-18} by DNA polymerase mt at variable concentration of template and constant concentration of TTP. ●—●control, ♦—♦ 20 μM ethidium bromide. Reciprocal plot according to Lineweaver-Burk. The Michaelis and inhibition constants are shown in Table IV

roquine a drug structurally unrelated to the other dyes used in this experiment, does not affect DNA polymerase activity at the concentration used. DNA polymerase α and β are less affected in general by the drugs as compared with γ and mitochondrial polymerases. It is important to point out that proflavin, which has a structure very similar to acriflavin, inhibits to a lesser extent the enzymatic activity of mt and γ polymerases. As seen in Figure 5 the only difference between proflavine and acriflavin is the presence in the latter of a quaternary ammonium substituted with a methyl group. In the case of ethidium bromide a quaternary ammonium is also found but substituted with an ethyl group We can only speculate on the possible role of this group on the interaction with DNA polymerases. The partial inhibition observed in Table III in the case of DNA polymerase α and β may be explained by the intercalating properties of the dyes used, while the pronounced effect on mitochondrial and γ polymerases may be ascribed to an interaction of ethidium bromide or acriflavin (free or bound to the template) with the enzyme itself.

The inhibition by ethidium bromide is extremely specific for γ and mitochondrial polymerases as shown in Figure 1. The inhibition is more complete in the case of the mitochondrial enzyme, but this may be explained by

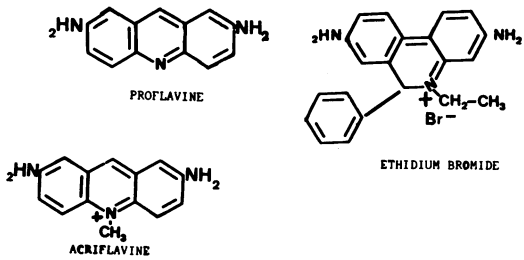


Fig. 5

the fact that DNA polymerase γ from nuclei is slightly contaminated by polymerase α which is poorly inhibited by ethidium bromide.

The template poly dA-dT_{12-18} represents a special type of double stranded nucleic acid, since one of the strands is made up of relative short oligonucleotide fragments. As we were unable to find any information on ethidium bromide intercalation with this type of template, we performed the experiments described in Figure 2 in order to show that the drug was able to interact strongly with this template. As in the case of DNA (18), two types of binding are observed, one with a very strong affinity ($K1^{-1}$ = 74 nM) and the other with a weaker affinity ($K2^{-1}$ = 25 μM). An interesting feature is the shift of maximum and minimum absorbance when the spectra of DNA is compared with that of poly dA-dT_{12-18}. The arrows in Figure 2B indicate the values obtained with DNA.

From the data of Figure 2 we were able to calculate that under the usual DNA polymerase assay conditions and 20 μM ethidium bromide, about 80% of the drug was intercalated and more than 90 % of the enzyme activity was inhibited. Under the same conditions but using activated DNA as template we found a similar degree of inhibition with more than 99 % of the drug intercalated (not shown). We conclude that most probably the drug interacts with the enzyme in the intercalated form.

When the nucleic acid-ethidium complex is cooled from 37° C to 20° C an important increase in the absorbance at 545 nm was observed suggesting an increase of the intercalation process (not shown).

That the drug (free or intercalated) can interact with the enzyme is shown in Figure 3, where both polymerases, mitochondrial and γ, are inhibited competitively by ethidium bromide in the presence of increasing amounts of TTP. This effect may be tentatively explained by a competition between TTP and the drug.

The latter could then occupy the TTP site in the enzyme. A recent article (19) describes the non competitive inhibition of DNA polymerase I

from E.coli by the intercalating dye 9-aminoacridine when DNA was used as template and the concentration of the four nucleosides triphosphates was varied. The different type of inhibition obtained in both cases may be explained by the structural differences of both dyes, as well as the difference that may exist in the polymerization mechanism of both enzymes, since the bacterial enzyme also catalyses the exo and endonucleolytic cleavage of polynucleotides (20), while such activities have not been found in highly purified mitochondrial polymerase (1). In the case of E.coli DNA polymerase I, the dye 9-aminoacridine inhibits the polymerizing activity only when intercalated in the template, since no effect was observed when single stranded DNA was used as template (19). In the case of mt polymerase no polymerization is observed in the presence of single stranded polynucleotides or DNA. As shown in Table IV-A, the K_M, K_M' and Ki of the γ and mitochondrial polymerases are similar; this is an added proof that the two enzymes are the same.

When the inhibition by ethidium bromide was studied at varying concentrations of poly dA-dT_{12-18} the results shown in Figure 4 were obtained. In the absence of inhibitor the biphasic pattern obtained would indicate that two sites with different affinities for the template were present in the enzyme molecule. We assume in this case that only one type of DNA polymerase activity is present in our partially purified preparation. With ethidium bromide the biphasic pattern is preserved and for low concentrations of template an uncompetitive type of inhibition was obtained, while the inhibition was non competitive at higher template concentrations.

Our results indicate that intercalating drugs such as ethidium bromide may interact with the DNA polymerase found in the mitochondria of animal cells. The dramatic decrease of DNA synthesis when the drug is administered in vivo can be partially explained as a direct effect at the enzyme level by the free or intercalated dye, although the structural modifications of the circular mt DNA by the drug can also account for the organelle specific inhibition of DNA synthesis.

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