# TEMPLATE ACTIVITY OF BROMINATED DNA FOR DNA POLYMERASE

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## 1. Introduction

Bromination of DNA affects only guanine and cytosine which are converted into 8-bromoguanine (8-BrG) and 5-bromocytosine (5-BrC) respectively [1]. If the Br:nucleotide ratio is below 1, the percentage of brominated bases is proportional to the amount of bromine added. The brominated DNA molecules are more flexible than native DNA [2] and electron-microscope photographs of material brominated to a low degree reveal that they are composed of two species: clusters resembling the denatured DNA and extended chains simulating native molecules [3]. The presence of two distinct species is also revealed by the melting curve profile of partially brominated DNA and these two species can be separated by fractionation on a hydroxylapatite column.

DNA isolated from antibiotic-resistant strains of *Haemophilus influenzae* looses some of its transforming activity after bromination. A similar inactivation is observed in brominated *Bacillus subtilis* DNA; however, in both cases, a significantly different degree of inactivation is found for various markers [4].

In the course of this study, the template activity of calf thymus DNA, brominated to different extents, was tested with two DNA polymerases: *E. coli* DNA polymerase, insensitive to the secondary structure of the primer; and regenerating rat liver replicative DNA polymerase requiring preferentially denatured DNA [5].

The action of both polymerases is inhibited by the presence of 8-BrG and 5-BrC in the template DNA and for the mammalian enzyme, modifications of the secondary structure of DNA are not responsible for the inhibition.

## 2. Methods

*E. coli* DNA polymerase (Fraction IV) prepared according to Richardson et al. [6] was obtained from Worthington. Mammalian DNA polymerase was extracted and purified from regenerating rat liver as reported earlier [5]. Unlabeled deoxyribonucleoside-5′-triphosphates were purchased from Schwartz Bioresearch Inc. and thymidine-5′-triphosphate-$\alpha$-$^{32}$P from CEA (France). Replicative DNA polymerase activity was measured by incorporation of the four deoxyribonucleoside triphosphates (dGTP-, dCTP-, dATP- and TTP-$\alpha$-$^{32}$P). The radioactivity incorporated in the acid-insoluble product is determined by the glass filter method [6, 7] using a Packard liquid scintillation counter. Assay for terminal transferase activity is performed in the same conditions as with labelled TTP alone.

Four different samples of calf thymus DNA were used as template:

a) *Native DNA*: extraction was according to Kay et al. [8]. According to physico-chemical criteria, the DNA obtained by this method was a double stranded native DNA: the sedimentation constant extrapolated at zero concentration in water was equal to 21.6. The weight average molecular weight measured by light scattering was $7.5 \times 10^6$ daltons; $\epsilon(P) = 6800$. The protein content was less than 1% and no single strand breaks could be detected by the method of Studier [9].

b) *Heat denatured DNA*: denaturation was achieved by heating for 10 min at 100° and rapid cooling at 0°.

c) *Brominated DNA*: bromide was added to a dimethylformamide (DMF) solution of the quaternary

ammonium salt of DNA. The sodium salt of the brominated DNA was then regenerated on addition of a saturated sodium bromide solution in DMF. Four samples of brominated DNA were studied: $Br_1$DNA containing 1 modified base for 200 nucleotides, $Br_2$DNA (2 modified bases for 200 nucleotides), $Br_3$DNA (3 modified bases for 200 nucleotides), and $Br_4$DNA (4 modified bases for 200 nucleotides).

d) *'regenerated' DNA*: The sodium salt of DNA was regenerated from the DMF solution of the quaternary ammonium salt of native DNA. The secondary structure of this DNA is slightly different from native DNA [2]. Therefore, this 'regenerated' DNA provided a control for irreversible structural modifications introduced in the template by DMF.

## 3. Results

In a first set of experiments, template activity was studied by measuring the rate of DNA synthesis at increasing concentrations of DNA in the presence of a constant amount of enzyme. Template saturation curves for native and regenerated DNAs were virtually identical with *E. coli* DNA polymerase (fig. 1). This result was expected since this polymerase is not sensitive to the secondary structure of the primer. Nevertheless, the priming function of the brominated DNA was substantially reduced, the inactivation increasing with increasing degree of bromination (fig. 1).

The DNA polymerase from regenerating rat liver is more efficient with denatured DNA and, in fact, in this system, the non-brominated regenerated DNA appears to be more active than native DNA, although less active than denatured DNA (fig. 2). This is in good agreement with the physico-chemical properties of this regenerated DNA [2]. However, it should be pointed out that high concentrations of regenerated as well as heat denatured DNA inhibit the reaction, probably by excess single stranded template [10, 11].

Again, in this system, bromination reduces the template activity of primer DNA (fig. 2) and its activity is not restored by thermal denaturation (fig. 3).

Kinetic studies with regenerating rat liver enzyme and four deoxyribonucleotides or TTP alone indicate that the replicative reaction is inhibited while the terminal addition reaction is not affected. The polymerisation reaction proceeds for a few minutes and

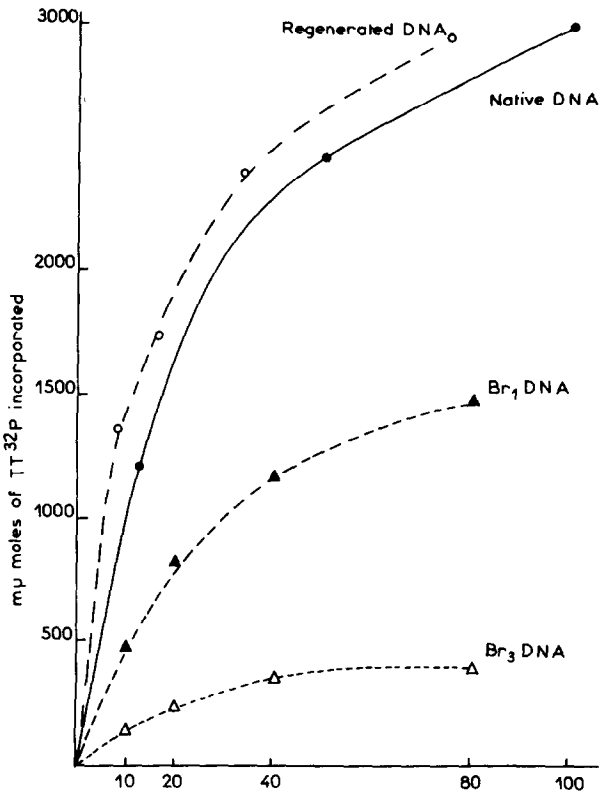


Fig. 1. Template activity of brominated DNA for *E. coli* DNA polymerase. Reaction mixtures (total volume 0.25 ml) contain: tris-HCl 30 mM (pH 7.2); 2 mercaptoethanol 0.4 mM; $MgCl_2$ 3 mM; KCl 2.5 mM; dGTP, dCTP, dATP 0.2 mM, TTP-$\alpha$-$^{32}$P 0.2 mM (3 $\mu$Ci/$\mu$mole); 0.1 ml of enzyme and varying concentrations of DNA. Incubation was carried out at 38° for 60 min. Over this time interval the time course of DNA synthesis is linear with untreated DNA as template. Activity is expressed as nmoles of TTP-$\alpha$-$^{32}$P incorporated per ml of enzyme in 1 hr. Each point represents the average of duplicate experiments. BrDNA: see Methods.

then stops at a plateau level inversely related to the ratio of brominated bases:nucleotides. This suggests that template activity is restricted to the free portions of the primer DNA (fig. 4).

## 4. Conclusions

Even a small percentage of 8-bromoguanine or 5-bromocytosine in a DNA template drastically

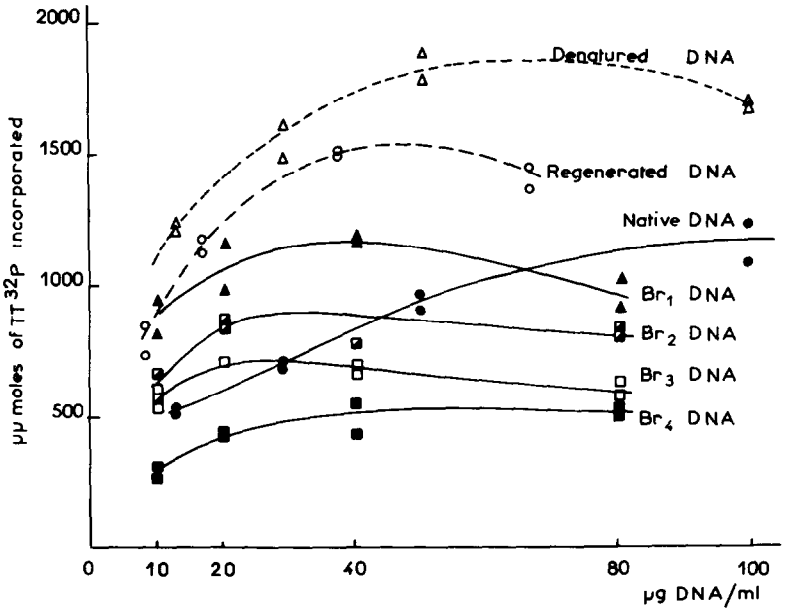


Fig. 2. Template activity of brominated DNA for regenerating rat liver DNA polymerase. The assay system is described in the legend to fig. 1. Activity is expressed as pmoles of TTP-α-$^{32}$P incorporated per ml of enzyme per 1 hr. BrDNA: see Methods.

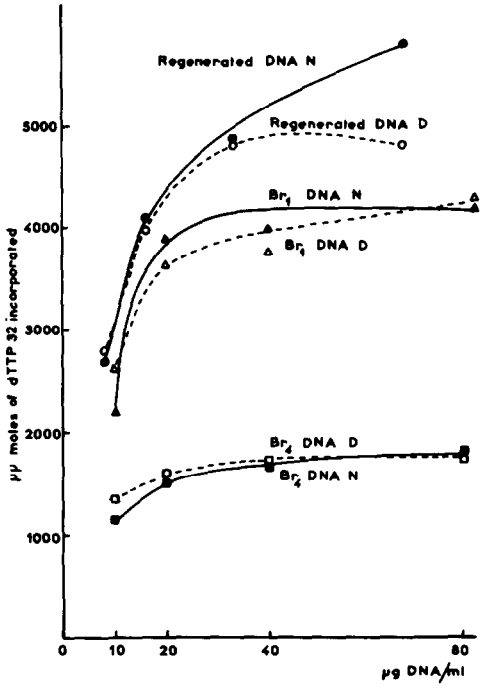


Fig. 3. Regenerating rat liver DNA polymerase: Inefficiency of thermal denaturation on template activity of brominated DNA. N = native DNA, D = heat denatured DNA.

decreases the priming capacity of *E. coli* or mammalian DNA polymerase, although similar concentrations of potassium bromide in the reaction mixture have no inhibitory effect on the polymerisation reaction.

For regenerating rat liver enzyme, which requires as primer a single stranded DNA, template activity of brominated DNA before and after thermal denaturation is identical, suggesting that the decrease in template capacity is not due to modifications of secondary structure of DNA.

For a constant concentration of DNA, the decrease in priming function is proportional to the number of brominated bases in the template. The inhibitory effect of the brominated bases may be due to a lack of recognition of the modified regions by the polymerase, to a lack of pairing with the complementary non-brominated base or to an irreversible binding of the polymerase to the modified nucleotides of the template.

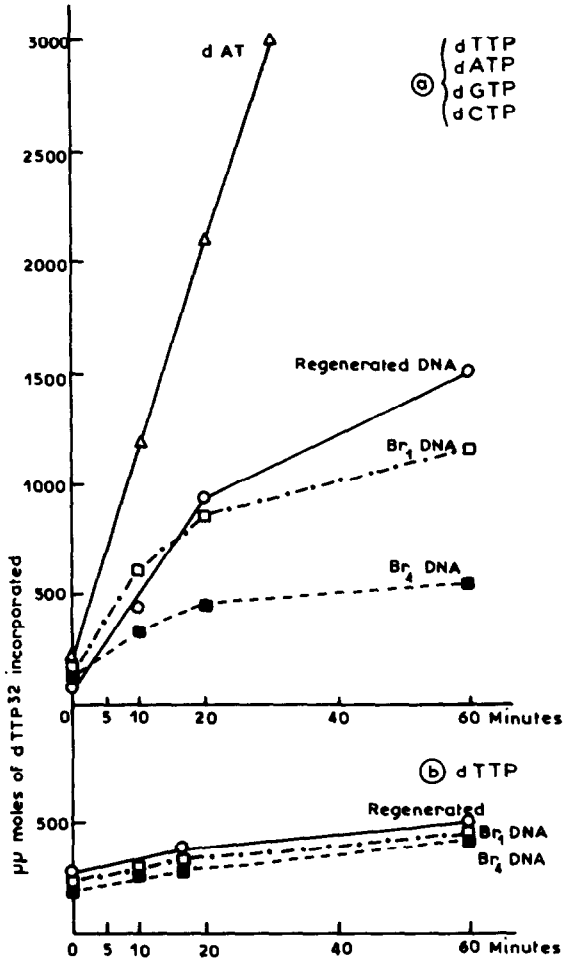


Fig. 4. Regenerating rat liver DNA polymerase: Effect of template bromination on replicative DNA polymerase activity (a) and terminal transferase activity (b). DNA concentration 40 μg/ml. A reference kinetic with synthetic dAT* is included.

* (Alternating copolymer purchased from Miles Laboratories Inc.).

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