Cys-90 and Cys-129. The sequence from residues 60 to 80 of the recA protein is very similar to the sequence of the ATP binding protein (Walker et al., 1982).

The reduced reactivity of cysteinyl residues at a high ionic strength (Figure 6) showed a conformational change around the cysteinyl residues; nevertheless, the gross conformation of the recA protein is not altered. The decrease in the extent of aggregation of the recA proten was observed with an increase in ionic strength (Kuramitsu et al., 1981). These results suggest that the change in the aggregation state of the recA protein at high ionic strength affects the reactivity of the cysteinyl residues with DTNB, the details of which will be reported elsewhere.

Acknowledgments

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Registry No. ATP, 56-65-5; ADP, 58-64-0; ATPase, 9000-83-3; L-cysteine, 52-90-4.

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Molecular Mechanisms of Chemical Mutagenesis: 9-Aminoacridine Inhibits DNA Replication in Vitro by Destabilizing the DNA Growing Point and Interacting with the DNA Polymerase†

Michael D. Topal

ABSTRACT: 9-Aminoacridine was found to inhibit dNTP incorporation into DNA homopolymer duplexes by phage T4 DNA polymerase in vitro. Systematic variation of the molar ratio of 9-aminoacridine to DNA, to DNA polymerase, and to DNA precursors demonstrated that this inhibition at 9-aminoacridine concentrations below 10 μM was mainly due to interaction of 9-aminoacridine with the DNA and suggested that the basis for the preferential inhibition of incorrect precursor incorporation was destabilization of the DNA growing point. Consistent with destabilization, 9-aminoacridine stimulated the hydrolysis of correctly base paired DNA by the 3′–5′ exonuclease activity of phage T4 DNA polymerase. This is the first indication to my knowledge that an intercalating dye destabilizes the DNA growing point, whereas it raises the overall T_m of the DNA. At 9-aminoacridine concentrations above 10 μM overall incorporation of dNTPs was inhibited by 9-aminoacridine interaction with the DNA polymerase. A possible explanation for the induction of both deletion and addition frameshift mutations by 9-aminoacridine during DNA biosynthesis is discussed in light of growing-point destabilization.

Planar aromatic dyes such as 9-aminoacridine (9-AA)[1] have been shown to inhibit DNA replication in vitro (Goodman et al., 1974) and to enhance the occurrence of substitution mutations (Goodman et al., 1974; Shearman et al., 1983) as well as frameshift mutations (Crick et al., 1961; McCann et al., 1975). The aromatic dyes intercalate into double-strand DNA (Lerman, 1961, 1963; Sakore et al., 1977; Hogan et al., 1979), and this action has been suggested as the basis for both their

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[1] Abbreviations: 9-AA, 9-aminoacridine; Tris, tris(hydroxymethyl)-aminomethane; HPLC, high-pressure liquid chromatography.

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frameshift mutation (Streisinger et al., 1966) and their substitution mutation (Goodman et al., 1974; Shearman et al., 1983) enhancing properties.

This investigation studies the inhibitory effect of 9-AA on DNA replication (Goodman et al., 1974) in detail. The results indicate that 9-AA inhibits DNA precursor incorporation via two effects. First, 9-AA destabilized the DNA growing-point terminus even while it raised the melting temperature of the DNA duplex. This new result offers the possibility that the induction of frameshift mutations by 9-AA may be the result of increased searching by the template and primer at the growing point, in the presence of 9-AA, for the correctly annealed conformation. Second, 9-AA interacted directly with the DNA polymerase.

Materials and Methods

Nucleotides. Tritium-labeled dNTPs were purchased from ICN and purified by ion-exchange chromatography (DEAE-Sephadex A-25) using a 0.01–0.5 M linear gradient of ammonium acetate buffer, pH 7.0. Peak fractions were pooled and lyophilized to dryness and then redissolved in 10 mM Tris/acetate buffer, pH 7.8. In this way, monophosphate contamination was kept below 0.4%.

Unlabeled dNTPs, dNDPs, and dNMPs were purchased from Sigma and dissolved in 10 mM Tris/acetate buffer, pH 7.8. All were chromatographed on poly(ethylenimine)–cellulose thin-layer plates containing fluorescent indicator (Baker) with 1.0 M lithium chloride as solvent to check for impurities and were found to be greater than 95% pure by this technique.

Nucleic Acid Polymers. Homopolynucleotides were purchased from Miles Laboratories and were identified by their ultraviolet spectra. They were shown to be free (>99%) of contaminating residues by HPLC (C_{18} reverse-phase column, Varian) of their acid hydrolysis (6 M HCl) products. To ensure production of double-stranded poly(dA)·poly(dT) duplexes, equimolar amounts of poly(dA) and poly(dT) were incubated together for 2 weeks at 4 °C in 20 mM Tris/acetate buffer, pH 7.0. The poly(dA) and poly(dT) were reported by Miles Laboratories to have s_{20} values of 6.8 and 8.3 S, respectively, in 0.05 M phosphate buffer, pH 7.0, and 0.1 M sodium chloride. These s values correspond to molecular weights of approximately 2.7×10^5 for poly(dA) and 4.3×10^5 for poly(dT) (Fresco & Doty, 1957). Poly(dG)·poly(dC) (Miles Laboratories) was the product of de novo synthesis by *Escherichia coli PolI* using a small amount of poly(dG)·poly(dC) as primer. The homopolymer nature of this duplex was confirmed by sedimentation in alkaline cesium sulfate (pH 12.0) according to the method of Inman & Baldwin (1964). This duplex (s_{20} = 10.5 S in 0.05 M phosphate buffer, pH 7.0, and 0.1 M NaCl) supported good DNA synthesis by phage T4 DNA polymerase. In contrast, the product formed by heating and then slow cooling an equimolar mixture of poly(dG) and poly(dC) failed to support DNA synthesis by the same enzyme.

Nucleotide Incorporation Assay. Reaction mixes (15 μL), prepared at 4 °C, contained 0.5 mM dithiothreitol, 39 mM Tris/acetate buffer, pH 7.8, 10 mM magnesium acetate, 67 mM potassium acetate, 200 μg of bovine serum albumin/mL, 0.4% glycerol, and phage T4 DNA polymerase (a gift from Navin Sinha) at concentrations indicated in the figure legends. The reaction mix also contained deoxyhomopolymer template-primer and dNTPs as indicated in the figure legends and approximately 1×10^6 cpm of tritium-labeled dNTP (greater than 10 Ci/nmol). A 9-AA (Sigma) stock solution (0.5 mM 9-AA in 20 mM Tris/acetate, pH 7.8) was prepared, kept cold and dark, and added to reactions to give the concentrations indicated in the figures. Reactions were initiated by addition of dNTPs followed by incubation at 37 °C. At intervals noted in the figures, aliquots were removed and applied to PEI–cellulose thin-layer plates atop a previously applied mixture of dNTP, dNDP, and dNMP identical with the labeled nucleotide in the reaction. Plates were developed in 1.0 M LiCl for pyrimidine–nucleotide analysis and 1.2 M LiCl for purine–nucleotide analysis. Plate areas containing marker nucleotides were identified by UV absorbance and were removed by scraping. Origin, which contained polymer, was similarly removed. Nucleotides and polymer were eluted from the scrapings by incubation at 55 °C in 1.0 M $MgCl_2$. Radioactivity was determined in a Mark II Nuclear Chicago scintillation counter with ScintiVerse (Fisher) scintillation fluid. Both eluent and scrapings were analyzed. Nucleotide incorporation into polymer also was monitored by application of aliquots of reaction mix onto glass fiber filters and precipitation of radiolabeled DNA using a cold solution (4 °C) of 5% trichloroacetic acid and 10% saturated sodium pyrophosphate (10 mL/filter). Filters were batch washed 2× with cold 1 M HCl and then 3× with cold 95% ethanol and dried for 15 min at 90 °C. Radioactivity was determined with Econofluor (Fisher) scintillation fluid. All radioactivity determinations were corrected for quenching.

Results and Discussion

9-AA Inhibits the Incorporation of Precursors into DNA. The fates of incorrect precursors dATP and dTTP and correct precursor dGTP were determined during replication of poly(dG)·poly(dC) in the presence of increasing concentrations of 9-AA (Figure 1). DNA homopolymer template-primers, the most repetitive DNA sequences available, were used because of the dependence of frameshift mutations on repetitive sequence (Streisinger et al., 1966; Okada et al., 1972; Farabaugh et al., 1978). Phage T4 DNA polymerase was used because the substitution errors made by this enzyme when homopolymer template-primers were replicated have been reported (Topal et al., 1980). The incorporation of dNTP, irrespective of the activity of the proofreading activity of the DNA polymerase, was measured from the relationship incorporation equals amount of precursor turned over plus amount of precursor polymerized [incorporation = dXMP + $(pdX)_N$]. Polymerization of incorrect residues was below the level of detectability [<0.3 pmol min^{-1} (μg of polymerase)$^{-1}$] in all experiments, but turnover of incorrect precursors (dNTP → dNMP), the result of proofreading newly incorporated base pairs by the 3′–5′ exonuclease activity of the phage T4 DNA polymerase (Brutlag & Kornberg, 1972), was easily detected.

Incorrect precursor incorporation has been shown to occur mainly at the end of the purine strands of poly(dA)·poly(dT) and poly(dG)·poly(dC) (Topal et al., 1980). The results in Figure 1, therefore, are mainly the incorporation of precursors at the end of the poly(dG) strand of poly(dG)·poly(dC). Each data point represents the percentage difference in the linear rate of incorporation of dNTP in the presence of 9-AA compared to that in its absence. The incorporation of incorrect precursors into poly(dG)·poly(dC) appeared more sensitive to 9-AA than that of correct precursor dGTP; at 5 μM 9-AA incorporations of the incorrect precursors, dATP and dTTP, were both inhibited about 75%, whereas that of the correct precursor, dGTP, was inhibited about 20%. However, this difference could possibly be related to the fact that incorrect incorporation was measured in the absence of DNA synthesis while correct precursor incorporation was measured concomitant with DNA synthesis. Note that the 9-AA concentration is plotted logarithmically in this and subsequent figures to

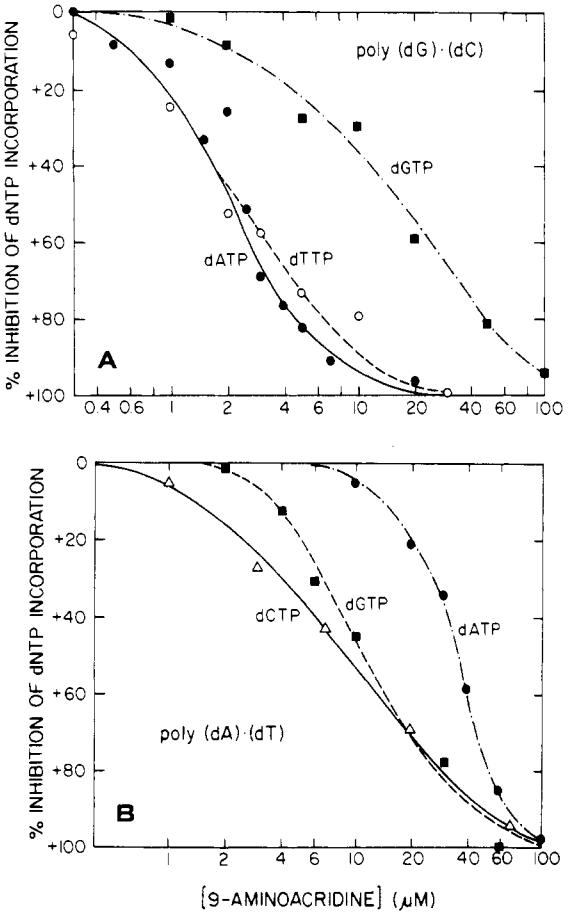

FIGURE 1: Effect of 9-AA on precursor selection by bacteriophage T4 DNA polymerase during DNA synthesis in vitro. Reactions contained the following: (A) 0.25 mM poly(dG)·poly(dC), 0.11 mM dATP, dTTP, or dGTP as the only dNTP substrate, and 4 μg of DNA polymerase/mL in reactions containing dATP and dGTP and 8 μg of DNA polymerase/mL when dTTP was substrate; (B) 0.26 mM poly(dA)·poly(dT), 0.10 mM dATP or dGTP or 0.12 mM dCTP as the only dNTP substrate, and 4 μg of DNA polymerase/mL in reactions containing dATP and dGTP and 8 μg of DNA polymerase/mL when dCTP was substrate. Each point in the two plots represents a comparison of the linear rate of dNTP incorporation (picomoles per minute per microgram of polymerase), determined as described under Materials and Methods and in Topal et al. (1980) in the presence of the indicated concentrations of 9-AA. No turnover was detected in any experiments in the absence of template-primer.

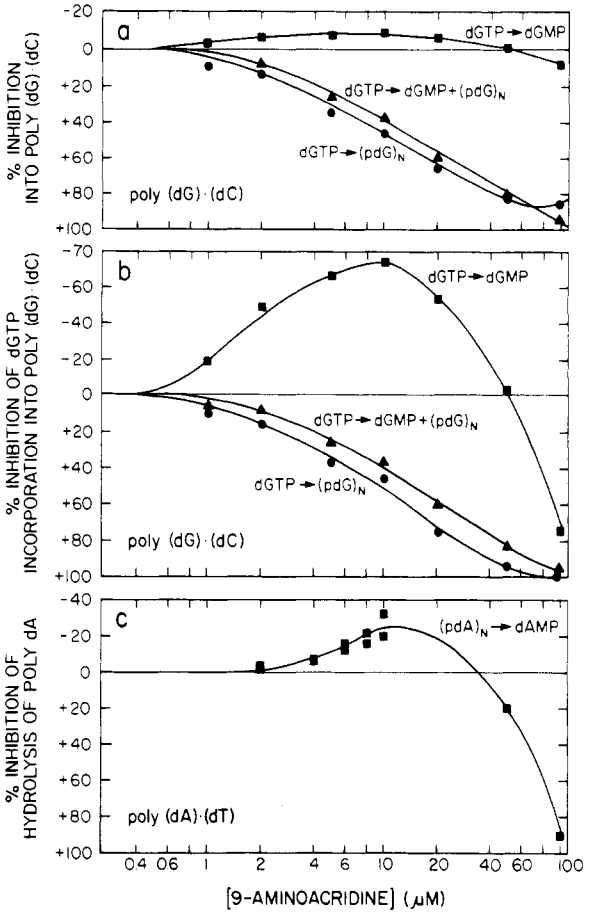

FIGURE 2: Incorporation vs. proofreading activity of DNA polymerase at increasing concentrations of 9-AA. (a) The conversion of dGTP (0.10 mM) to either dGMP or poly(dG) was determined as described under Materials and Methods during the synthesis of poly(dG)·poly(dC) (0.24 mM) by DNA polymerase (4 μg/mL) in the presence of increasing concentrations of 9-AA as indicated. The conversion of dGTP to dGMP (■) and polymer (●) in the presence of 9-AA relative to that in the absence of 9-AA is reported as a percentage of the total incorporation: dGTP → dGMP + $(pdG)_N$ (▲). (b) The data for conversion of dGTP to either dGMP or polymer in the presence of 9-AA are expressed as a percentage of the conversion in the absence of 9-AA irrespective of the total conversion. (c) The effect of 9-AA on the hydrolysis of the poly(dA) strand of poly(dA)·poly(dT) (0.4 mM) by DNA polymerase (2 μg/mL) in the absence of dNTPs was also determined.

display the wide range of 9-AA concentrations studied.

Incorporation of incorrect precursors dGTP and dCTP and correct precursor dATP into poly(dA)·poly(dT) was similarly quantitated (Figure 1). Once again, 9-AA preferentially inhibited incorrect precursor incorporation. At 10 μM 9-AA incorporation of the incorrect precursors dGTP and dCTP was inhibited about 45%, whereas incorporation of the correct precursor dATP into poly(dA) was inhibited only 5%.

Effect of 9-AA on the Fate of Correct Base Pairs. The fate of dGTP during poly(dG)·poly(dC)-dependent DNA synthesis in the presence of 9-AA was resolved into amounts of incorporation, dGTP → [dGMP + $(pdG)_N$], turnover, dGTP → dGMP, and polymerization, dGTP → $(pdG)_N$ (Figure 2a). Incorporation of dGTP was inhibited by 9-AA because of the large effect of 9-AA on polymerization of dGTP into poly(dG). Although not a major contributor to the decrease in polymerization of dGTP, the turnover of dGTP → dGMP was significantly enhanced by 9-AA. The amount of enhancement is easily seen when the results of Figure 2a are replotted at each concentration of 9-AA as the percentage of their values in the absence of 9-AA. Plotted in this way 10 μM 9-AA is seen to have enhanced the turnover of dGTP → dGMP by about 75% (Figure 2b).

A study of the incorporation of dATP into poly(dA)·poly(dT) also showed enhancement of turnover and inhibition of incorporation at 9-AA concentrations less than 10 μM (not shown).

Mechanisms that increased turnover may also be manifested as an increase in "chewing back" of the daughter strand in the absence of precursor. Therefore, poly(dA)·poly(dT) was prepared in which the poly(dA) strand was ^3H labeled. Incubation of this duplex with phage T4 DNA polymerase and increasing concentrations of 9-AA demonstrated increasing 3′–5′ exonucleolytic cleavage of poly(dA) at up to 10 μM 9-AA (Figure 2c).

Interaction of 9-AA with Reaction Components. At least three possible interactions can account for the inhibition by 9-AA of precursor incorporation. First, 9-AA may interact with DNA polymerase to affect its polymerizing function. Second, 9-AA, through its cationic or its intercalating properties, may interact with DNA to affect the DNA's conformation and stability. Third, 9-AA, an aromatic molecule that mimics the stacking properties of the nucleic acid bases, may

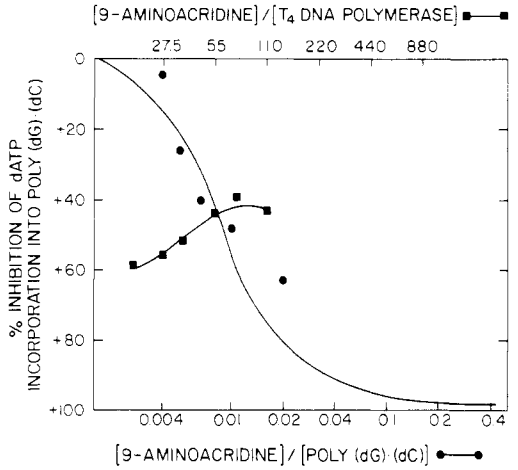

FIGURE 3: Effect of the 9-AA/DNA polymerase (■) and 9-AA/poly(dG)·poly(dC) (●) molar ratios on the incorporation of incorrect precursor dATP during DNA synthesis in vitro. Reactions contained 0.11 mM dATP, 20 μM 9-AA (a concentration which inhibits dATP incorporation by 50% under the conditions of Figure 1), 0.25 mM DNA, 4 μg of DNA polymerase/mL, and either 4 μg of DNA polymerase/mL with poly(dG)·poly(dC) varying from 0.1 to 0.5 mM or 0.25 mM poly(dG)·poly(dC) with DNA polymerase varying from 2 to 12 μg/mL. Each point represents a comparison of the incorporation of dATP at the given concentrations of DNA and DNA polymerase in the absence and presence of 2 μM 9-AA.

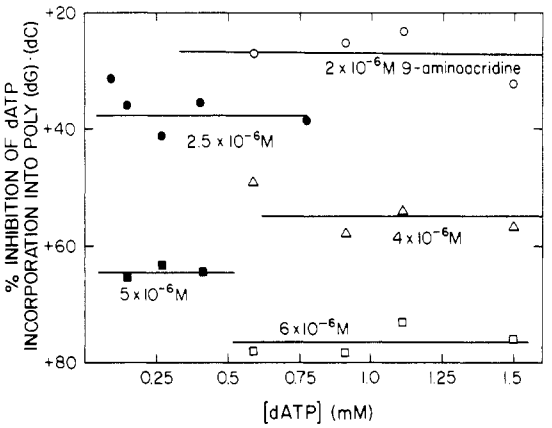

FIGURE 4: Effect of the 9-AA/dATP molar ratio on the incorporation of incorrect precursor dATP during DNA synthesis in vitro. Reactions contained 0.5 mM poly(dG)·poly(dC), 8 μg of DNA polymerase/mL, and dATP and 9-AA as indicated. Each line represents a different 9-AA/DNA molar ratio: (○) 0.004; (●) 0.005; (△) 0.008; (■) 0.010; (□) 0.012.

compete with the DNA precursors for binding at the active site of the polymerase–DNA complex. To determine which of these mechanisms was responsbible, the 9-AA/DNA, 9-AA/DNA polymerase, and 9-AA/dNTP molar ratios were systematically varied. The effect of varying the 9-AA concentration relative to all reaction components (Figure 1) on incorrect dATP incorporation into poly(dG)·poly(dC) is replotted in Figure 3. Superimposed over this curve are the effects on dATP incorporation of varying the 9-AA/DNA and 9-AA/DNA polymerase molar ratios. Varying the 9-AA/DNA molar ratio mimicked the effects of varying the molar ratio of 9-AA against all reaction components. Varying the 9-AA/DNA polymerase molar ratio, however, did not.

By the same method, 9-AA was shown not to compete with dNTPs for binding at the growing strand terminus. The concentration of dATP was varied while keeping the 9-AA concentration constant at levels shown to significantly inhibit the incorporation of dATP into poly(dG)·poly(dC) (Figure 1A). This varying of the 9-AA/dATP molar ratio while

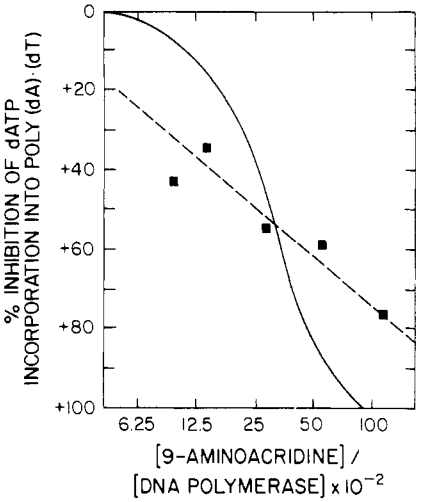

FIGURE 5: Effect of the 9-AA/DNA polymerase molar ratio on the poly(dA)·poly(dT)-dependent incorporation of dATP by phage T4 DNA polymerase in vitro. Reactions contained dATP (0.10 mM), poly(dA)·poly(dT) (0.25 mM), 9-AA (40 μM), and DNA polymerase from 0.4 to 4.8 μg/mL. The variation between the results from this experiment (■---■) and the results from Figure 1B (—) may be due to the difference in the way the ratios were generated in the two different experiments. For the data of Figure 1B, 9-AA was varied relative to all other components while in this experiment the DNA polymerase alone was varied relative to 9-AA. The results were corrected for changing DNA polymerase concentrations by running proper controls at each concentration.

keeping the 9-AA/DNA molar ratio constant (Figure 4) did not change incorporation of dATP. Varying the 9-AA/DNA molar ratio while keeping the 9-AA/dATP molar ratio constant, however, had an effect on dATP incorporation consistent with that shown in Figure 3.

Inhibition of dATP and dTTP incorporation into poly(dG)·poly(dC) was complete by 10 μM 9-AA (Figure 1A), whereas inhibition of dATP incorporation into poly(dA)·poly(dT) did not start until 10 μM 9-AA (Figure 1B). These results suggest that the mechanism for 9-AA inhibition of precursor incorporation below 10 μM may be different from that above 10 μM. This suggestion is consistent with the 9-AA-stimulated turnover of correct precursors (Figure 2b). 9-AA at 10 μM gave maximum enhancement of poly(dG)·poly(dC)-dependent turnover of dGTP. Turnover of dGTP declined at 9-AA concentrations greater than 10 μM until, at about 100 μM 9-AA, all indications of incorporation ceased. A similar maximum was exhibited for the 9-AA concentration dependence of hydrolysis of the poly(dA) strand of poly(dA)·poly(dT) (Figure 2c). These results suggest that, at 9-AA concentrations above 10 μM, 9-AA may be interacting with the DNA polymerase as well as the DNA. Indeed, variation of the 9-AA/DNA polymerase molar ratio was found to have a significant effect on dATP incorporation into poly(dA)·poly(dT) (Figure 5).

The effects of 9-AA on dNTP incorporation and hydrolysis of the growing strand in the absence of precursors suggested destabilization of the DNA growing point by 9-AA. Therefore, the T_m of poly(dA)·poly(dT) in the same solvent condition used for DNA synthesis studies was determined (Figure 6) to see if the overall stability of the DNA was similarly affected. The T_m of poly(dA)·poly(dT) was found to be 72.7 °C (uncorrected for single-strand melting). The addition of 75 μM 9-AA *raised* the overall T_m of the DNA to 74.5 °C, an increase of 1.8 °C. This increase is consistent with measurements reported by Lerman (1964).

9-AA interacts with the DNA duplex through a combination of intercalating and electrostatic binding properties. K^+ was

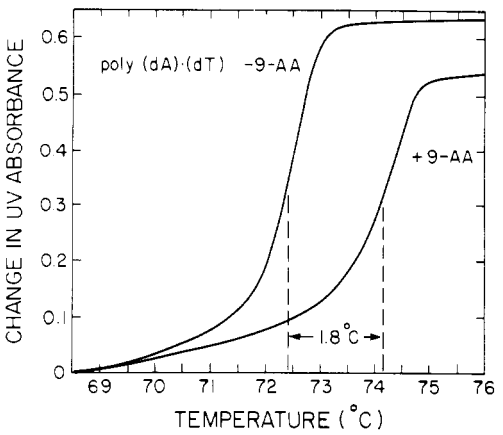

FIGURE 6: Effect of 9-aminoacridine on melting temperature (T_m) of poly(dA)·poly(dT). Melting temperatures were determined on solutions (400 μL) prepared to contain 0.25 mM poly(dA)·poly(dT), 0.5 mM dithiothreitol, 39 mM Tris/acetate buffer, pH 7.8, 10 mM magnesium acetate, 67 mM potassium acetate, 0.4% glycerol, and 0 or 7.5 mM 9-AA as indicated. Blanks were prepared identically except without 9-AA and DNA. Absorbances were measured on a Perkin-Elmer 320 double-beam analytical spectrophotometer, which has low stray light characteristics and is accurate to greater than 3.0 absorbance units at the wavelengths used (checked by dilution of high absorbance standards). Temperature was ramped (0.5 °C/min) by using thermoelectric cell holders and electronic temperature controller (Perkin-Elmer). Starting absorbances (1.6 for DNA with 9-AA at λ 275 nm and 2.1 for DNA without 9-AA at λ 265 nm) were subtracted electronically so that absorbance changes could be detected with high sensitivity. Absorbance changes were monitored continuously with time, whereas readings of the temperature in the cell holder (corrected to the temperature in the cell) were recorded at 5-min intervals.

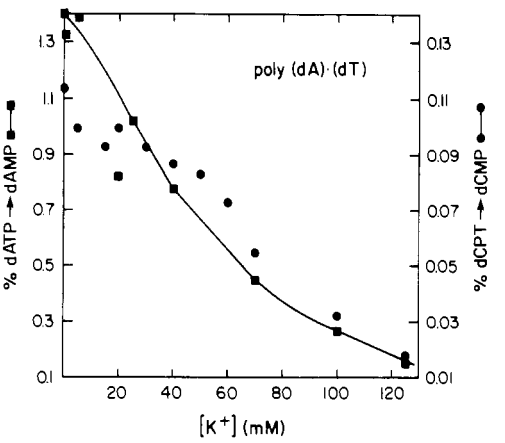

FIGURE 7: Effect of increasing [K+] on the incorporation of dATP and dCTP into poly(dA)·poly(dT) by DNA polymerase. Reactions contained 0.26 mM poly(dA)·poly(dT), 8 μg of DNA polymerase/mL, and either 0.10 mM dATP (■) or 0.12 mM dCTP (●).

used to mimic the cationic properties of 9-AA. Increasing the K^+ concentration of a DNA synthesis reaction from 0 to 120 mM had essentially no effect on the poly(dA)·poly(dT)-dependent incorporation (as determined by measurements of turnover) of incorrect precursor, dCTP, relative to that of correct precursor, dATP (Figure 7). The absolute amount of incorporation was affected, being higher at low K^+ concentrations, but the ratio of dATP to dCTP incorporation remained almost constant. This suggested that intercalation of 9-AA into DNA is responsible for the selective inhibition of precursor incorporation (Figure 1) rather than charge neutralization of the DNA phosphate backbone.

Conclusions

9-AA strongly inhibited the incorporation of incorrect precursors into homopolymer duplexes as measured by a decrease in their turnover. Turnover is the net result of *incorporation* followed by *excision* of precursors at the growing-strand terminus. A reduction in turnover could result from inhibition of either of these steps. However, inhibition of the excision step would result in an equivalent buildup of polymerized precursors, whereas inhibition of the incorporation step would result in an equivalent decrease in turnover of the precursor. In the experiments described above the turnover of incorrect precursors was greatly inhibited by 9-AA. However, no concomitant increase in polymerization of incorrect precursors was detected. This suggests that the significant inhibition by 9-AA of incorrect precursor turnover (Figure 1) is due to the effect of 9-AA on the incorporation step of DNA synthesis. Polymerization of correct precursors was also inhibited by 9-AA. This decrease was also caused predominantly by inhibition of the incorporation step of DNA synthesis since it was not associated with a buildup of turned-over residues. It is clear from Figure 2a that the decrease in incorporation of dGTP into poly(dG)·poly(dC) is accounted for almost entirely by the decrease in polymerization.

The interaction of 9-AA with the DNA also enhanced proofreading by the DNA polymerase. Enhancement of turnover of incorrect precursors was not possible because the preponderance of incorporated incorrect precursors was already turned over in the absence of 9-AA. Enhancement of the turnover of correct precursors, however, was significant (Figure 2b). Consistent with the 9-AA enhanced turnover of correct precursors by DNA polymerase was the 9-AA-enhanced hydrolysis of the 3′ end of the DNA primer strand by DNA polymerase in the absence of dNTPs (Figure 2c). All of these results are consistent with 9-AA-induced destabilization of the DNA growing point. Such destabilization inhibits the incorporation step of DNA synthesis by decreasing base pairing between template residues and dNTP. It enhances the proofreading step of DNA synthesis by increasing the frequency of melting of already incorporated base pairs at the growing-point terminus. The 3′–5′ exonuclease proofreading activity recognizes and removes unpaired residues (Brutlag & Kornberg, 1972). Preferential inhibition of incorrect compared to correct precursor incorporation (if not due to comparisons without and with DNA synthesis, respectively) noted in Figures 1 and 2 may also be consistent with growing-point destabilization. The weakest base pairs are "melted out" first so that incorrectly bound precursors do not base pair long enough to be incorporated covalently.

That intercalation and not charge neutralization properties are responsible for 9-AA's effects on precursors selection is suggested by the lack of effect on precursor selection of increasing [K^+]. Varying K^+ concentration affected the amount of DNA synthesis but not its fidelity.

The DNA-destabilizing effects of 9-AA suggest a mechanism for the induction of frameshift mutations during DNA replication. 9-AA is one of a general class of aromatic dyes shown to intercalate into double-stranded DNA (Lerman, 1961, 1963; Sakore et al., 1977; Hogan et al., 1979) with a resulting rise in melting temperature and therefore overall stability of the DNA duplex (Lerman, 1964). These dyes are potent frameshift mutagens (Crick et al., 1961; McCann et al., 1975) and have been suggested to be active during DNA replication (Brenner et al., 1961; Newton et al., 1972), recombination (Lerman, 1963), and repair (Streisinger et al., 1966). Frameshift mutations have been found to occur at repeating DNA sequences (Streisinger et al., 1966; Okada et

al., 1972; Farabaugh et al., 1978), and so it was proposed that the role of intercalating dye in frameshift mutagenesis is to *stabilize* short regions of non-Watson–Crick base pairing that border unpaired "bulges" formed by strand slippage (Streisinger et al., 1966). Increased strand slippage, however, would appear to be incompatible with increased helix stability.

The results presented here suggest that 9-AA destabilizes the growing end during DNA biosynthesis. Such destabilization would increase "searching" by the template and growing strand at the growing point through the misaligned partially annealed structures that are possible at regions of redundancy. This increased searching is likely to result in trapped misaligned structures that can be extended by DNA polymerase. Destabilization of the growing point is able to explain how the aromatic dyes would increase slippage and predicts that factors contributing to 9-AA-induced frameshift mutations may be the number of possible metastable misalignments possible at a given sequence and their relative stabilities. By use of the strand-slippage model, it can explain addition mutations by slipping of the growing strand and deletions by slippage of the template strand.

9-AA, at concentrations greater than 10 μM, inhibited both the exonuclease and incorporation functions of the polymerase (Figure 2b,c). The effect of varying DNA polymerase concentration relative to 9-AA on precursor incorporation (Figure 5) suggested that interaction directly with the DNA polymerase as well as the DNA caused such inhibition. The interactions of 9-AA with DNA polymerase as well as with DNA may have interesting implications in light of 9-AA's suggested causing of substitution (Goodman et al., 1974; Shearman et al., 1983) as well as frameshift mutations (Crick et al., 1961; McCann et al., 1975).

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Registry No. 9-AA, 90-45-9; DNA polymerase, 9012-90-2.

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