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Mechanism of Inhibition of Deoxyribonucleic Acid Synthesis by 1-β-D-Arabinofuranosyladenosine Triphosphate and Its Potentiation by 6-Mercaptopurine Ribonucleoside 5′-Monophosphate†

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ABSTRACT: The mechanism of inhibition of DNA synthesis by 1-β-D-arabinofuranosyl-ATP (*ara*-ATP) and the potentiation of this inhibition by 6-mercaptopurine ribonucleoside 5′-monophosphate (6-MPR-P) have been investigated with mammalian DNA polymerase δ by using poly(dA-dT) as the template. The inhibition of DNA synthesis by *ara*-ATP correlates with incorporation of *ara*-AMP into poly(dA-dT). Nearest-neighbor analysis indicates that *ara*-AMP does not act as an absolute chain terminator but rather that chains with 3′-terminal arabinosyl nucleotides are extended slowly. The inhibition of DNA synthesis by *ara*-ATP is markedly enhanced by the addition of the nucleotide derivative of 6-mercaptopurine, 6-mercaptopurine ribonucleoside 5′-monophosphate. The increased inhibition of DNA synthesis in the presence of 6-MPR-P is due to increased incorporation of *ara*-AMP. The mechanism by which 6-MPR-P increases the incorporation of *ara*-AMP is by selective inhibition of the 3′ to 5′ exonuclease activity of DNA polymerase, thereby preventing the removal of newly incorporated *ara*-AMP at 3′ termini of DNA chains.

The purine nucleoside analogue 1-β-D-arabinosyladenine (*ara*-A) and the pyrimidine nucleoside analogue 1-β-D-arabinosylcytosine (*ara*-C) inhibit the growth of a variety of mammalian cells and viruses. The active metabolites of these nucleoside analogues are their corresponding 5′-triphosphates, *ara*-ATP and *ara*-CTP, which are potent inhibitors of DNA synthesis in vitro. Kinetic studies have shown that *ara*-ATP and *ara*-CTP are competitive inhibitors of DNA synthesis when the corresponding deoxynucleoside triphosphate, dATP or dCTP, is the variable substrate (Furth & Cohen, 1968; Müller et al., 1975). However, the exact mechanism by which *ara*-CTP inhibits DNA synthesis remains uncertain. Studies with partially purified DNA polymerases have shown that nearly all of the incorporated *ara*-CMP is present at the 3′-hydroxyl termini of DNA chains (Momparler, 1969, 1972; Waqar et al., 1971). These findings led to the proposal that *ara*-C inhibits DNA synthesis by acting as a chain terminator, either preventing or markedly slowing the addition of deoxynucleotides (Momparler, 1969, 1972; Waqar et al., 1971; Magnusson et al., 1974; Burgoyne, 1974). However, studies with intact cells and cell lysates have indicated that *ara*-CMP and *ara*-AMP are incorporated primarily in internucleotide linkage of DNA. It was also found that inhibition of DNA synthesis by low concentrations of *ara*-C is readily reversible in vivo; i.e., removal of the inhibitor leads to the rapid re-

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sumption of DNA replication at a normal rate. These results, together with the observations that *ara*-ATP and *ara*-CTP are competitive inhibitors of DNA synthesis, led to the suggestion that the inhibition of DNA synthesis by arabinosyl nucleotides is not due to their incorporation into DNA but rather to inhibition of DNA polymerase activity (Furth & Cohen, 1968; Graham & Whitmore, 1970; Hunter & Francke, 1975; Müller et al., 1975; Manteuil et al., 1974; Wist et al., 1976; Dicioccio & Srivastava, 1977).

The effectiveness of *ara*-C as a chemotherapeutic agent in the treatment of acute myelogenous leukemia is significantly enhanced when used in combination with the purine analogues 6-thioguanine (6-TG) or 6-mercaptopurine (6-MP) (Gee et al., 1969; Carey, 1970; Buchanan et al., 1973). The mechanism of this synergism is unknown. We have recently shown that the active metabolite of 6-MP, 6-mercaptopurine ribonucleoside 5′-monophosphate (6-MPR-P), selectively inhibits the 3′ to 5′ exonuclease activity associated with mammalian DNA polymerase δ while the DNA polymerase activity is not inhibited (Byrnes et al., 1976, 1977). Since the proposed function of the 3′ to 5′ exonuclease is to excise mismatched nucleotides or nucleotide analogues incorporated at the primer terminus during DNA synthesis (Brutlag & Kornberg, 1972; Bessman et al., 1974), the selective inhibition of the proofreading exonuclease activity by 6-MPR-P might be expected to result in an increased net incorporation of nucleotide analogues. In this study we have investigated the effects of metabolites of *ara*-A and 6-MP on DNA synthesis using purified DNA polymerase δ and poly(dA-dT). We shall present data showing that (1) the inhibition of DNA synthesis by *ara*-ATP is a consequence of *ara*-AMP incorporation into the 3′-hydroxyl termini of DNA chains, (2) the inhibitory effect of *ara*-ATP is potentiated by 6-MPR-P, (3) the potentiation of *ara*-ATP inhibition of DNA synthesis by 6-MPR-P is due to an increased net incorporation of *ara*-AMP into DNA, and (4) the mechanism by which 6-MPR-P increases incorporation of *ara*-AMP is by selective inhibition of the 3′ to 5′ exonuclease activity of DNA polymerase δ, thereby preventing hydrolysis of incorporated *ara*-AMP.

Materials and Methods

^{3}H-Labeled deoxyribonucleoside triphosphates were obtained from New England Nuclear. [^{3}H]-*ara*-ATP was custom synthesized by ICN, and its purity was examined by thin-layer chromatography on poly(ethylenimine)–cellulose (PEI–cellulose) as described by Cashel et al. (1969). Less than 1.5% contamination by nucleoside diphosphates or monophosphates was detected. To rule out the possibility that [^{3}H]-*ara*-ATP was contaminated by [^{3}H]dATP, [^{3}H]-*ara*-ATP was enzymatically converted to [^{3}H]-*ara*-AMP by the template-dependent conversion of deoxyribonucleoside triphosphate to monophosphate reaction, catalyzed by purified DNA polymerase δ with poly(dA-dT) as the template. The products of the reaction were examined by PEI–cellulose thin-layer chromatography as described by Waqar et al. (1971), and no contamination by [^{3}H]dAMP was detected (<1%). Unlabeled deoxyribonucleoside triphosphates were obtained either from P-L Biochemicals or Calbiochem. The barium salt of 6-mercaptopurine ribonucleoside 5′-monophosphate was obtained from P-L Biochemicals and converted to the sodium salt before use. Poly(dA-dT) was obtained from Biopolymers, Inc., and was dialyzed against two changes of 0.06 M KCl and 0.01 M Tris-HCl buffer, pH 7.4 (DNA buffer), before use. PEI–cellulose thin-layer chromatography plates were purchased from Brinkman Instruments, Inc., and were prerun with distilled water and stored at 0 °C before use. Salmon sperm DNA, micrococcal nuclease, and bovine spleen phosphodiesterase II were obtained from Worthington Biochemical Corp.

Preparation of Enzyme. DNA polymerase δ (step VI) was prepared from rabbit erythroid hyperplastic bone marrow as previously described (Byrnes et al., 1976). One unit of DNA polymerase catalyzes the incorporation of 1 nmol of dTMP in 1 h at 37 °C.

Preparation of 3′-Terminus [^{3}H]-ara-AMP-Labeled Poly(dA-dT). Poly(dA-dT) labeled at the 3′ terminus with [^{3}H]-*ara*-AMP was prepared by incubating poly(dA-dT) with DNA polymerase δ and [^{3}H]-*ara*-ATP. The reaction mixture contained in a final volume of 2.5 mL the following: 66 mM Hepes (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) buffer, pH 6.5; 0.79 A_{260} unit of poly(dA-dT); 66 mM KCl; 0.13 mM $MnCl_2$; 1.2 mM 6-MPR-P; 1.5 μM [^{3}H]-*ara*-ATP, 6800 cpm/pmol; 3.4 units of DNA polymerase δ. The reaction mixture was incubated at 37 °C for 15 min and stopped by the addition of 12.5 μmol of EDTA (ethylenediaminetetraacetic acid), 1.25 mmol of sodium perchlorate, and sodium dodecyl sulfate to a final concentration of 1% (w/w). The solution was deproteinized twice with chloroform–isoamyl alcohol (24:1), and poly(dA-dT)·[^{3}H]-*ara*-AMP was separated from unincorporated nucleotides by dialysis against two changes of DNA buffer. The final specific activity of poly(dA-dT)·[^{3}H]-*ara*-AMP was 6.34×10^4 cpm/A_{260} unit.

DNA Polymerase Assay. Each reaction mixture contained in a final volume of 0.25 mL the following: 80 mM Hepes buffer, pH 7.0; 125 mM KCl; 0.6 mM $MnCl_2$; 25 μM poly(dA-dT); 0.5 unit of DNA polymerase δ. The concentration of the deoxynucleoside triphosphate substrates is given for each experiment. After 15 min at 37 °C the reaction was terminated by the addition of 2 mL of cold 5% trichloroacetic acid and 20 μL of 20 mM dATP. The precipitate was collected, washed, and counted as previously described (Byrnes et al., 1976).

Results

Correlation of Inhibition of DNA Synthesis with Incorporation of ara-AMP into DNA. The effects of increasing concentrations of [^{3}H]-*ara*-ATP both on [^{3}H]-*ara*-AMP incorporation and on poly(dA-dT) synthesis, as measured by the incorporation of [^{32}P]dTMP, are shown in Figure 1. Increasing concentrations of [^{3}H]-*ara*-ATP result in increased [^{3}H]-*ara*-AMP incorporation but decreased [^{32}P]dTMP incorporation into poly(dA-dT). The correlation between the degree of inhibition of DNA synthesis and the amount of *ara*-AMP incorporated into DNA suggests that the inhibition of DNA synthesis by *ara*-ATP is the result of its incorporation into poly(dA-dT).

Potentiation of ara-ATP Inhibition of DNA Synthesis by 6-MPR-P. The effects of 6-MPR-P on the inhibition of poly(dA-dT) synthesis by *ara*-AMP is shown in Figure 2. In the absence of 6-MPR-P, poly(dA-dT) synthesis is inhibited 50% at an *ara*-ATP concentration of 4×10^{-6} M, whereas in the presence of 8×10^{-5} M 6-MPR-P, the concentration of *ara*-ATP required to inhibit 50% is reduced to 1.5×10^{-6} M. Higher 6-MPR-P concentrations (1.2×10^{-3} M) resulted in a further reduction in the concentration of *ara*-ATP required to inhibit poly(dA-dT) synthesis. Thus, the inhibition of DNA synthesis by *ara*-ATP is potentiated by 6-MPR-P. No inhibition of DNA synthesis is observed in the presence of 6-MPR-P alone (data not shown).

Effect of 6-MPR-P on Incorporation of [^{3}H]-ara-AMP into Poly(dA-dT). To determine whether the potentiation of *ara*-ATP inhibition of DNA synthesis by 6-MPR-P is due to

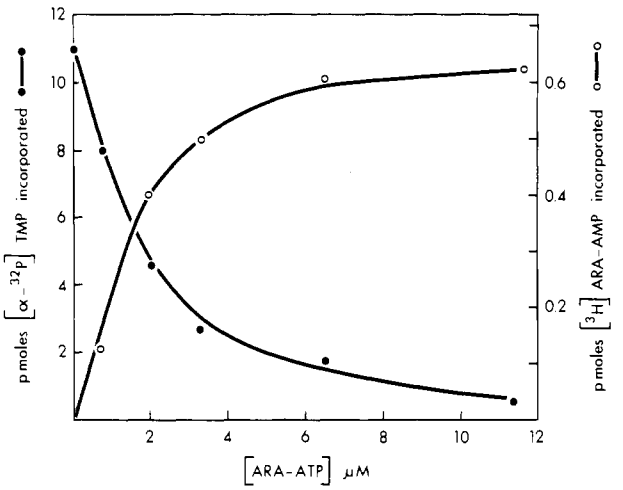

FIGURE 1: Effect of *ara*-ATP concentration on [^{3}H]-*ara*-AMP and [^{32}P]dTMP incorporation. Assays were carried out as described under Materials and Methods except that the KCl concentration was 45 mM. The concentration of [α-^{32}P]dTTP was 4.6 μM, 1707 cpm/pmol, dATP was present at 0.4 μM, and the concentration of [^{3}H]-*ara*-ATP (1917 cpm/pmol) was varied from 0 to 11.2 μM. The samples were counted in toluene–Omnifluor scintillant in a Packard Model 33AT liquid scintillation counter. The ^{32}P spillover was 0.7% and the ^{3}H spillover was less than 0.04%.

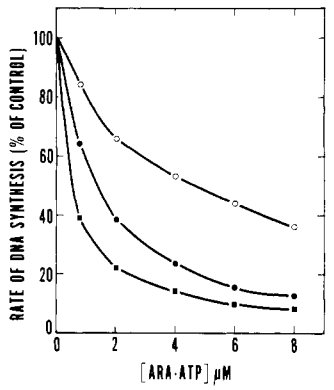

FIGURE 2: Effect of 6-MPR-P on the inhibition of DNA synthesis by *ara*-ATP. Assays were carried out as described under Materials and Methods. The concentration of [^{3}H]dATP was 0.45 μM, 7787 cpm/pmol, and the concentration of dTTP was 4 μM. *ara*-ATP was varied as indicated. No 6-MPR-P (○); 8×10^{-5} M 6-MPR-P (●); 1.2×10^{-3} M 6-MPR-P (■).

an increased incorporation of *ara*-AMP into poly(dA-dT), we have investigated the effects of 6-MPR-P on the incorporation of [^{3}H]-*ara*-AMP into poly(dA-dT) (Figure 3). Increasing concentrations of 6-MPR-P increase the incorporation of [^{3}H]-*ara*-AMP into poly(dA-dT); the incorporation of [^{3}H]-*ara*-AMP is increased 11-fold at 1×10^{-4} M and 14-fold at 4×10^{-4} M 6-MPR-P. These results are also consistent with the proposal that the inhibition of poly(dA-dT) synthesis by *ara*-ATP is due to its incorporation into DNA.

Increased Incorporation of ara-AMP is Due to Inhibition of 3′ to 5′ Exonuclease Activity of DNA Polymerase δ. We have previously shown that the 3′ to 5′ exonuclease activity associated with both mammalian and bacteria DNA polymerases can be selectively inhibited by nucleoside 5′-monophosphates, whereas the DNA polymerase activity is not affected (Byrnes et al., 1977; Que et al., 1978). Thus, it is likely that the increase in the incorporation of [^{3}H]-*ara*-AMP into poly(dA-dT) in the presence of 6-MPR-P is due to the inhibition of hydrolysis of newly incorporated [^{3}H]-*ara*-AMP. That this is the case is seen in Figure 4, which shows the effects of increasing concentrations of 6-MPR-P both on the incorporation of [^{3}H]-*ara*-AMP into polynucleotide and on the template-dependent conversion of [^{3}H]-*ara*-ATP to [^{3}H]-*ara*-AMP. The template-dependent generation of [^{3}H]-*ara*-AMP represents the successive incorporation and hydrolysis of [^{3}H]-*ara*-AMP at the 3′-hydroxyl termini of the template/primer by the DNA polymerase and 3′ to 5′ exonuclease activities of DNA polymerase δ. Increasing concentrations of 6-MPR-P result in decreased hydrolysis of newly incorporated [^{3}H]-*ara*-AMP and, thus, in an increased net incorporation of [^{3}H]-*ara*-AMP into poly(dA-dT).

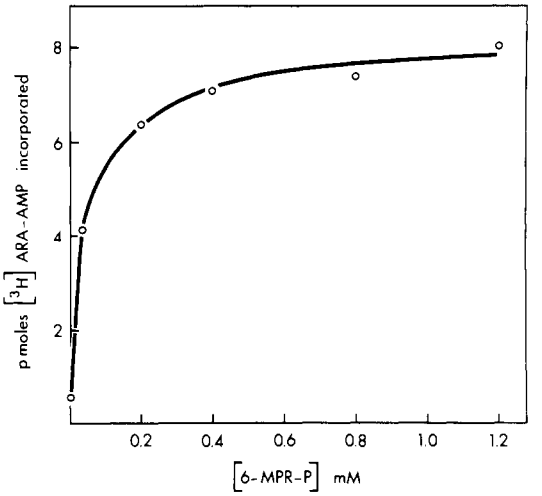

FIGURE 3: Effect of 6-MPR-P on the incorporation of [^{3}H]-*ara*-ATP into DNA. Assay conditions were as described under Materials and Methods. The concentration of [^{3}H]-*ara*-ATP was 1.7 μM, 4230 cpm/pmol; 6-MPR-P was present as indicated.

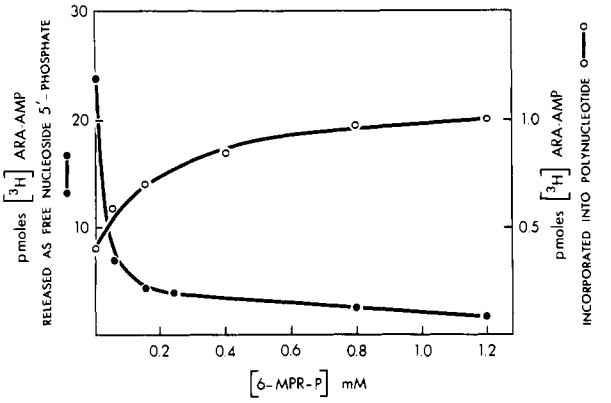

FIGURE 4: Effect of 6-MPR-P on the incorporation and hydrolysis of [^{3}H]-*ara*-AMP. The reaction mixtures were as described under Materials and Methods except that the KCl concentration was 85 mM and the poly(dA-dT) concentration was 6 μM. The [^{3}H]-*ara*-ATP concentration was 1.7 μM, 4230 cpm/pmol. The reaction was incubated at 37 °C for 15 min and stopped by rapid chilling and the addition of 20 μL of 0.1 M EDTA. An aliquot of the reaction mixture (0.1 mL) was pipetted into a tube containing 2 mL of 5% trichloroacetic acid. The precipitate was collected, washed, and counted as previously described (Byrnes et al., 1976). A second aliquot of the reaction mixture (0.12 mL) was spotted on a thin-layer plate marked into 1.2-cm lanes. The plate was predeveloped with absolute methanol, and, after drying, the plate was developed with 1 N acetic acid to 3 cm above the origin, followed by 0.3 M LiCl (Randerath & Randerath, 1965). Each lane was subsequently cut into 18 1-cm sections and counted. *ara*-ATP and *ara*-AMP standards were run in parallel as markers and identified by ultraviolet absorption at 254 nm. Minus enzyme, minus template, and minus incubation controls were run with the experimental assays.

It is interesting to note that, when the exonuclease is active, most of the *ara*-AMP that is incorporated is hydrolyzed and released as free *ara*-AMP, suggesting that most of the *ara*-AMP that has been incorporated remains at 3′ termini and

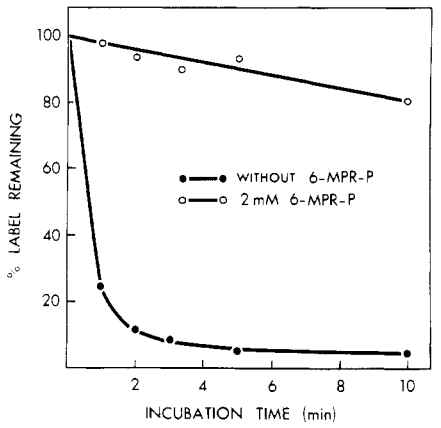

FIGURE 5: Effect of 6-MPR-P on the hydrolysis of terminally labeled poly(dA-dT)·[^{3}H]-*ara*-AMP. Each reaction mixture contained in a final volume of 0.15 mL the following: 133 mM Tris-HCl, pH 7.8; 0.1 A_{260} unit of poly(dA-dT)·[^{3}H]-*ara*-AMP, 6.34×10^4 cpm/A_{260} unit; 0.33 mM $MnCl_2$; 0.5 unit of DNA polymerase δ; 2 mM 6-MPR-P as indicated. The reaction was incubated at 37 °C for the indicated time and stopped by the addition of 2 mL of cold 5% Cl_3AcOH. After the mixture was allowed to stand for 10 min at 0 °C, the mixture was filtered on Millipore HAWP filter disks and washed with 30 mL of distilled water. The disks were dried and counted as previously described (Byrnes et al., 1976). Similar results were obtained when assayed under DNA polymerase assay conditions in the absence of deoxyribonucleoside triphosphates (unpublished observation).

is not extended. This is consistent with the hypothesis that *ara*-ATP inhibition of DNA synthesis is a consequence of its incorporation into DNA and that incorporation of *ara*-AMP slows the rate of DNA chain extension. Also consistent with this hypothesis is the observation that inhibition of the 3′ to 5′ exonuclease activity results in a 10-fold inhibition of *ara*-AMP turnover but only a 2-fold stimulation of its incorporation into poly(dA-dT). If the incorporation of ara-AMP at 3′ termini slows the rate of further DNA chain growth, then the rate of *ara*-AMP incorporation would be limited by the availability of template/primer (which is limiting in the present case) whereas the generation of free *ara*-AMP would not be dependent on the availability of template/primer since the hydrolysis of 3′-terminal *ara*-AMP regenerates effective 3′ termini.

To further demonstrate that the inhibition of 3′ to 5′ exonuclease activity by 6-MPR-P is the mechanism responsible for increased incorporation of *ara*-AMP into DNA, the effect of 6-MPR-P on the hydrolysis of 3′-terminally labeled poly(dA-dT)·[^{3}H]-*ara*-AMP by the 3′ to 5′ exonuclease activity of DNA polymerase δ was investigated (Figure 5). In the absence of 6-MPR-P, the rate of release of [^{3}H]-*ara*-AMP is very rapid; approximately 90% is released after 2 min of incubation and complete removal is attained after 5 min. However, in the presence of 6-MPR-P very little of the poly(dA-dT)·[^{3}H]-*ara*-AMP is hydrolyzed; after 10 min of incubation only 20% of the [^{3}H]-*ara*-AMP had been released.

Extension of Chains with 3′-Terminal ara-AMP. To determine whether DNA polymerase δ can extend DNA chains with 3′-terminal *ara*-AMP, we have analyzed the enzymatic digest of poly(dA-dT) synthesized in the presence of *ara*-ATP and [α-^{32}P]dTTP (Table I). The transfer of ^{32}P to *ara*-AMP would indicate that *ara*-AMP is incorporated into internucleotide linkage and that chains with 3′-terminal arabinosyl nucleotides can serve as primers for chain extension. In the absence of 6-MPR-P, very little ^{32}P originally present in dTMP is transferred to *ara*-AMP. However, in the presence of 6-MPR-P, which inhibits the 3′ to 5′ exonuclease activity of DNA polymerase δ, the transfer is stimulated fourfold. The

Table I: Transfer of ^{32}P from [α-^{32}P]dTTP to *ara*-AMP or dAMP[a]

substrates	6-MPR-P	[^{32}P]-*ara*-AMP (pmol)	[^{32}P]dAMP (pmol)
dATP, [^{32}P]dTTP	−	0	38
dATP, [^{32}P]dTTP	+	0	152
ara-ATP, [^{32}P]dTTP	−	1.1	1.6
ara-ATP, [^{32}P]dTTP	+	4.0	6.0
ara-ATP, [^{32}P]dTTP, dATP	−	6.5	28
ara-ATP, [^{32}P]dTTP, dATP	+	15.8	85

[a] Assay conditions were as described under Materials and Methods except that the concentration of KCl was 105 mM and 3.08×10^{-3} A_{260} unit of poly(dA-dT) was used. The concentration of [α-^{32}P]dTTP was 0.6 μM (900 cpm/pmol). The concentrations of *ara*-ATP and dATP were 4 μM. The concentration of 6-MPR-P, when present, was 1.2×10^{-3} M. The mixtures were incubated at 37 °C for 60 min and terminated by the addition of 0.5 mL of 20% trichloroacetic acid, 50 μL of Salmon sperm DNA, 2 mg/mL, and 0.1 mL of 0.3 M sodium pyrophosphate. Samples were hydrolyzed by the procedure of Wu & Kaiser (1968). The DNA product was washed 3 times by repeated suspension and precipitation, extracted 3 times with ether, and dried under vacuum. The dried DNA was redissolved in 0.3 mL of 0.1 M Tris-acetate buffer, pH 8.4, and digested by the addition of 10 μL of 0.05 M $CaCl_2$ and 20 μL of micrococcal DNase (500 μg/mL), followed by incubation for 1 h at 37 °C. The incubation mixture was adjusted to pH 6.4 by the addition of 2 μL of 0.2 M acetic acid and 10 μL of 0.1 M Tris-acetate, pH 6.4. The reaction mixture was also made 1.5 mM in potassium phosphate, pH 6.4, to inhibit phosphatase activity. The mixture was lyophilized to dryness, 50 μL of H_2O and 20 μL of spleen phosphodiesterase, 11 units/mL, were added, and the mixture was incubated for 90 min at 37 °C. The mixture was then heated at 100 °C for 3 min. Samples and standards were spotted on PEI-cellulose thin-layer plates and developed twice in isobutyric acid–1 M NH_4OH–0.1 M EDTA (100:60:1 by volume) (Waqar et al., 1971). The R_f of dAMP was 0.76 and that of *ara*-AMP was 0.59.

transfer of ^{32}P to *ara*-AMP is further stimulated by the addition of dATP. In the presence of both dATP and *ara*-ATP, the transfer of ^{32}P to *ara*-AMP is only 20% of that transferred to dAMP, suggesting that, although DNA primers with 3′-terminal arabinosyl nucleotides can serve as sites for chain elongation, they are inefficient primer termini compared to chains with 3′-terminal deoxyribosyl nucleotides. The amount of ^{32}P transferred to dAMP when no dATP was present in the reaction mixture represents transfer of ^{32}P to dAMP present at the primer termini of the poly(dA-dT) primer/template.

Discussion

Although it has been over a decade since it was first shown that *ara*-C and *ara*-A are potent inhibitors of DNA synthesis, there is still a lack of unanimity as to the mechanism of inhibition of DNA synthesis by arabinosyl nucleotides. There are two main hypotheses. Momparler (1968) has suggested that inhibition of DNA synthesis is a consequence of the incorporation of arabinosyl nucleotides into 3′ termini of DNA chains, thereby preventing further chain extension. This hypothesis is based upon in vitro results obtained with a partially purified DNA polymerase. A modified version of this hypothesis which is gaining wider acceptance suggests that arabinosyl nucleotides do not act as absolute chain terminators, but rather the incorporation of arabinosyl nucleotides at 3′ termini markedly slows the addition of subsequent deoxynucleotides and this is the basis of inhibition of DNA synthesis (Magnusson et al., 1974; Burgoyne, 1974; Cozzarelli, 1977). The other hypothesis is that the direct inhibition of DNA polymerase activity and not the incorporation of arabinosyl

nucleotides into DNA is the cause of inhibition of DNA synthesis. This hypothesis is based upon results of studies with intact cells in which it was found that (1) there is no correlation between the amount of arabinosyl nucleotide incorporated and the degree of lethality induced by this analogue, (2) most of the *ara*-CMP incorporated into DNA is found in internucleotide linkage and only a small percentage is located at 3′ termini, and (3) inhibition of DNA synthesis is readily reversible—removal of the inhibitor leads to rapid resumption of DNA replication at normal rate. It was assumed that incorporation of arabinosyl nucleotides into DNA would result in irreversible inhibition and that no mechanism exists in the cell to excise nucleotide analogues blocking DNA chain extension (Graham & Whitmore, 1970; Wist et al., 1976).

Previous interpretation of the above data has not taken into consideration the possibility that mammalian DNA polymerases might be similar to microbial enzymes in being associated with a proofreading 3′ to 5′ exonuclease activity whose function is to remove mismatched nucleotides or nucleotide analogues that are incorporated during DNA synthesis (Brutlag & Kornberg, 1972; Bessman et al., 1974; Byrnes et al., 1976). The observations with intact cells that the amount of *ara*-CMP incorporated into DNA is very small and that it is present primarily in internucleotide linkage may be due to preferential hydrolysis of 3′-terminal arabinosyl nucleotides by the 3′ to 5′ exonuclease activity of mammalian DNA polymerase during the isolation of the cells. Similarly, the reversibility of the inhibition of DNA synthesis by arabinosyl nucleotides may also be explained by the efficient hydrolysis of incorporated arabinosyl nucleotides at the 3′ termini of growing DNA chains.

The present study indicates that inhibition of DNA synthesis by *ara*-ATP is consequent to incorporation of *ara*-AMP into DNA and not to inhibition of DNA polymerase activity. This is based on the finding that there is a correlation between the degree of inhibition of DNA synthesis and the amount of *ara*-AMP incorporated into DNA. Increasing concentrations of *ara*-ATP result in increased incorporation of *ara*-AMP but decreased incorporation of dTMP.

We have further demonstrated that *ara*-AMP is not an absolute chain terminator and that *ara*-AMP is incorporated into internucleotide linkage in DNA chains, since ^{32}P can be transferred from [^{32}P]dTMP to *ara*-AMP with poly(dA-dT) as primer/template. However, the small amount of ^{32}P transferred to *ara*-AMP as compared to dAMP is consistent with the hypothesis that chains terminated with arabinosyl nucleotides are extended very slowly (Magnusson et al., 1974; Burgoyne, 1974; Cozzarelli, 1977).

We have observed that 6-MPR-P potentiates the inhibition of DNA synthesis by *ara*-ATP. We have demonstrated that this potentiation is due to increased net incorporation of *ara*-AMP into the 3′ termini of poly(dA-dT) and that selective inhibition of the 3′ to 5′ exonuclease activity of DNA polymerase δ by 6-MPR-P is the mechanism by which 6-MPR-P increases incorporation of *ara*-AMP into DNA. The demonstration that selective inhibition of the 3′ to 5′ exonuclease activity of DNA polymerase by 6-MPR-P prevents the removal of arabinosyl nucleotides incorporated at the 3′ termini of DNA chains and that this results in increased inhibition of DNA synthesis is also consistent with the hypothesis that inhibition of DNA synthesis by *ara*-ATP is due to the incorporation of arabinosyl nucleotides into DNA.

Studies on the metabolism of purine analogues in animal models suggest that the in vivo concentrations of 6-MPR-P may be sufficient to inhibit the exonuclease activity of DNA polymerase. 6-MP and 6-TG are good substrates for adenylic pyrophosphorylase and inosinic, guanylic pyrophosphorylase, respectively, and are readily converted to the nucleoside 5′-monophosphates. Since 6-MPR-P and 6-TGR-P are poorly converted to the diphosphate or triphosphate, the concentration of the nucleoside 5′-monophosphate derivatives of these purine analogues can reach very high levels in vivo, i.e., greater than 10^{-4} M (Paterson, 1959; Moore & LePage, 1958). At this concentration 6-MPR-P inhibits the 3′ to 5′ exonuclease activity of mammalian DNA polymerase δ (Byrnes et al., 1977).

The enhanced effectiveness of *ara*-C in the treatment of acute myelogenous leukemia when used in combination chemotherapy with 6-TG or 6-MP is well established. The present studies provide a mechanism to account for this synergism and a rational basis for this combination of chemotherapeutic agents.

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