Escherichia coli DNA Polymerases II and III: Substrate Specificity†

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ABSTRACT: *Escherichia coli* DNA polymerases II and III have a greater specificity for the 2′-deoxy configuration of substrate than has been found previously for DNA polymerase I. Neither ribosyl nor arabinosyl derivatives appreciably substitute in DNA polymerase II and III reactions, even in the presence of Mn^{2+} ions. The derivative 2′-fluorodeoxycytidine 5′-triphosphate partially substitutes for dCTP in DNA polymerase II reactions, and not at all in DNA polymerase III catalyzed reactions. The corresponding dUTP analogue, 2′-fluorodeoxyuridine 5′-triphosphate, has negligible substrate activity for either enzyme. Both DNA polymerases II and III catalyze considerable synthesis in the absence of one of the four deoxyribonucleoside triphosphates. This activity is inhibited by the respective arabinonucleoside triphosphate in the case of DNA polymerase II, but there is no inhibition of DNA polymerase III. Reactions catalyzed by both enzymes, either Mg^{2+}- or Mn^{2+}-activated, show a sigmoidal dependence on dCTP concentration, but a nonsigmoidal dependence on the concentrations of the other dNTPs, when each concentration is varied singly. DNA polymerases II and III have an absolute specificity for the 5′-triphosphate derivative of substrate, since neither deoxyribonucleoside 5′-monophosphates nor diphosphates serve as substrates even in the presence of ATP. Both enzymes apparently require the anti conformation of nucleotide, since the syn derivative, 8-bromodeoxyguanosine 5′-triphosphate, does not support DNA polymerase II or III activity. In contrast, the base analogues dUTP and dITP, derivatives having an anti conformation, substitute nearly completely for dTTP and dGTP, respectively, in the DNA polymerase II and III reactions.

DNA polymerases have specificity for at least three structural features of each nucleotide substrate: (i) the configuration of the sugar moiety, especially at the 2′ and 3′ carbon atoms; (ii) the nature of the base, especially the C-5 position of thymine and the syn or anti conformations which are determined by a rotation about the glycosidic bond; and (iii) the 5′-triphosphate group. Each of these interactions with the enzyme could be, in addition, modifiable by the environment of the reaction, conditions such as pH, temperature, and divalent cation.

Manganous ions have been shown to permit utilization of the unnatural ribonucleoside triphosphates by DNA polymerase I of *E. coli* (Berg et al., 1963; Van de Sande et al., 1972). Perhaps this incorporation of ribonucleotides relates to the mutagenic effects of Mn^{2+} in *E. coli* (Demerec & Hanson, 1951; Roberts & Aldous, 1951) and in bacteriophage T4 (Orgel & Orgel, 1965). Alternatively, Mn^{2+} ions may modify the base or template specificity of the polymerase (Hall & Lehman, 1968). That mutagenesis could arise from a defect in a replicative DNA polymerase, i.e., unlike DNA polymerase I, was first suggested by Speyer (1965). With the discovery of the replicative capacity of the *polC* gene product, DNA polymerase III, in *E. coli* (Gefter et al., 1971; Nüsslein et al., 1971), it seemed more likely that the mutagenic event derived from a modification of the base, template, or 2′-deoxyribose specificity of *E. coli* DNA polymerase III.

This paper describes the effects of the individual deoxyribonucleoside 5′-triphosphates, several C-2′ sugar analogues, and base analogues upon the activity of Mn^{2+}-activated and Mg^{2+}-activated *E. coli* DNA polymerases II and III. DNA polymerase III exhibits a higher specificity for the 2′-deoxy configuration of the substrate molecules than does DNA polymerase II, and both are more exacting in this regard than is DNA polymerase I. Substitution of ribo-, arabino-, and 2′-deoxy-2′-fluoronucleotides by DNA polymerases II and III, in contrast to DNA polymerase I, is not appreciably increased by replacement of Mg^{2+} with Mn^{2+} ions. This is in contrast to the modification of template specificities which we observed previously (Helfman et al., 1976).

Experimental Procedures

Materials

Unlabeled deoxyribonucleoside 5′-triphosphates (dNTPs),[1] ribonucleoside 5′-triphosphates (rNTPs), deoxyribonucleoside 5′-diphosphates (dNDPs), deoxyribonucleoside 5′-monophosphates (dNMPs), 2-mercaptoethanol, and spermidine were purchased from Calbiochem. Deoxyuridine 5′-triphosphate (dUTP) was purchased from P-L Biochemicals and deoxyinosine 5′-triphosphate (dITP) was obtained from Sigma. Calf thymus DNA was purchased from Calbiochem. Poly[d(A-T)] and bovine serum albumin (fraction 5) were purchased from Miles. 8-Br-dGTP was a generous gift of Dr. Alan Kapuler. [^{3}H]dTTP (17 Ci per mmol), [^{3}H]dATP (17.7 Ci per mmol), [^{3}H]dCTP (30 Ci per mmol), and [^{3}H]dTDP (39.8 Ci per mmol) were obtained from Schwarz BioResearch. [^{3}H]dCMP (19 Ci per mmol) was purchased from New England Nuclear Corp. The purity of each dNTP was determined

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[1] Abbreviations used: 2′-fl-dCTP, 1-β-(2′-fluoro-D-2-deoxyribofuranosyl)cytosine 5′-triphosphate; 2′-fl-dUTP, 1-β-(2′-fluoro-D-2-deoxyribofuranosyl)uracil 5′-triphosphate; *ara*-CTP, 1-β-D-arabinofuranosylcytosine 5′-triphosphate; *ara*-UTP, 1-β-D-arabinofuranosyluracil 5′-triphosphate; *ara*-ATP, 9-β-D-arabinofuranosyladenine 5′-triphosphate; 8-Br-dGTP, 8-bromo-9-β-D-2-deoxyribofuranosylguanine 5′-triphosphate; 7-methyl-dGTP, 7-methyl-9-β-D-2-deoxyribofuranosylguanine 5′-triphosphate; dUTP, 1-β-D-2-deoxyribofuranosyluracil 5′-triphosphate; dITP, 9-β-D-2-deoxyribofuranosylhypoxanthine 5′-triphosphate; dNTP, deoxyribonucleoside 5′-triphosphate; rNTP, ribonucleoside 5′-triphosphate; dNDP, deoxyribonucleoside 5′-diphosphate; dNMP, deoxyribonucleoside 5′-monophosphate.

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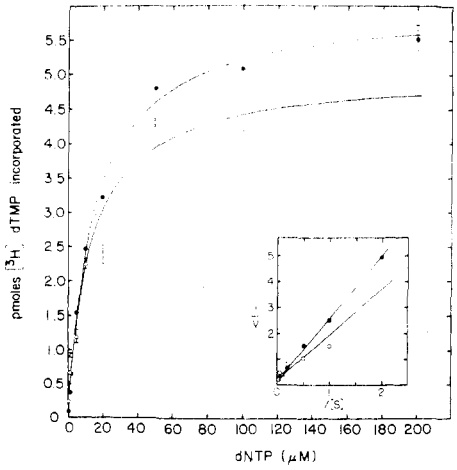

FIGURE 1: Dependence of DNA polymerase II activity on the concentration of deoxyribonucleoside 5′-triphosphates. Substrate concentrations were varied collectively and abscissa represents individual values; i.e., the total dNTP concentration would be fourfold higher than indicated. Reactions were as described in Methods except for the inclusion of either Mg^{2+} (○) or Mn^{2+} (●). Error bars represent the deviation in duplicate reactions. The curved lines represent the optimal Michaelis–Menten curves determined by $1/v$ vs. $1/[S]$, $[S]/v$ vs. $[S]$, and $v/[S]$ vs. v plots. Each of these plots gave similar values for V and K_m. The inset shows the $1/v$ vs. $1/[S]$ (Lineweaver–Burke) plot.

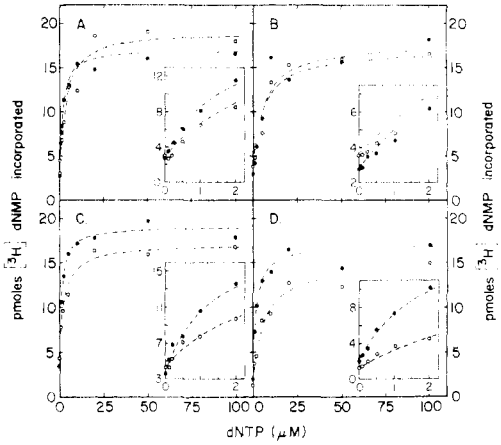

FIGURE 2: Effect of concentration of individual dNTP substrates on the activity of DNA polymerase II. The remaining three dNTPs, including the labeled dNTP substrate, were present in reactions at 50 μM each. [^{3}H]dTTP at 1200 cpm per pmol was used for all experiments except when the dTTP concentration was varied, in which case [^{3}H]dCTP at 1100 cpm per pmol was the label. Reactions were as described in Methods in the presence of Mg^{2+} (○) or Mn^{2+} (●). (A) dATP; (B) dCTP; (C) dGTP; (D) dTTP. The dashed lines are optimal Michaelis–Menten curves for the substrate-dependent reaction as calculated using V obtained from $v/[S]$ vs. v (Eadie–Hofstee) plots and K_m obtained as the value of [S] for which v was $V/2$. The curves and data presented in the insets are those for low substrate concentrations.

using two-dimensional thin-layer chromatography on PEI-cellulose (Randerath & Randerath, 1967). All compounds were >95% pure and the impurities were the corresponding di- and monophosphates exclusively.

The arabino- and 2′-deoxy-2′-fluoronucleosides were synthesized as previously described (Shannahoff & Sanchez, 1973). The corresponding 5′-phosphorodichloridates were prepared by direct phosphorylation of the unprotected nucleosides (Yoshikawa et al., 1967). The arabinonucleoside 5′-triphosphates were obtained by treatment of the 5′-phosphochloridate derivatives with tetraethylammonium pyrophosphate, whereas the 2′-deoxy-2′-fluoronucleoside 5′-triphosphates were prepared by an analogous reaction with tetrabutylammonium pyrophosphate (Sowa et al., 1971). DNA polymerases II and III from *E. coli* p3478 *polA1* (DeLucia & Cairns, 1969) were purified by phase separation, DEAE-cellulose, and phosphocellulose chromatography as described previously (Helfman et al., 1976).

Methods

DNA polymerase II reactions (0.10 mL) contained: 0.09 mM "gapped" calf thymus DNA; 0.067 M Tris-acetate, pH 7.6; 0.25 mg per mL bovine serum albumin; 3.0 mM 2-mercaptoethanol; and 50 mM KCl. "Gapped" calf thymus DNA was prepared as previously described (Helfman et al., 1976). Deoxyribonucleoside 5′-triphosphate (dNTP) solutions were neutralized and added in variable amounts as described in Results. Mg^{2+} reactions (Mg^{2+}-activated enzyme) contained 5.0 mM $MgCl_2$ and 0.5 mM spermidine, whereas Mn^{2+} reactions (Mn^{2+}-activated enzyme) contained 0.10 mM $MnCl_2$ and 1.0 mM spermidine. All reactions contained 0.5 μL of enzyme per 0.10 mL of reaction mixture and incubations were for 30 min at 37 °C.

DNA polymerase III reactions (0.10 mL) contained: 0.09 mM "gapped" calf thymus DNA; 33 mM morpholinopropanesulfonic acid–10 mM KOH (pH 7.0); 50 mM 2-mercaptoethanol; and 0.25 mg per mL bovine serum albumin. Deoxyribonucleoside 5′-triphosphate (dNTP) solutions were neutralized and concentrations varied as described in Results. Mg^{2+} reactions contained 13 mM $MgCl_2$ and 10% (v/v) ethanol, whereas Mn^{2+} reactions contained 5.0 mM $MnCl_2$ and 50 mM KCl, for reasons previously described (Helfman et al., 1976). All reactions contained 0.5 μL of enzyme per 0.10 mL of reaction mixture and incubations were for 1 h at 21 °C. Under these conditions, both enzyme reactions demonstrated linear kinetics even at subsaturating levels of substrate.

Mixtures which tested incorporation of dNMPs or dNDPs contained 5.0 μM [^{3}H]dCMP or [^{3}H]dTDP and 20 μM each of the other dNMPs or dNDPs.

Results

Dependence on Deoxyribonucleoside 5′-Triphosphate Concentration. DNA polymerase II activity is saturated with about 50 μM in each dNTP when either Mn^{2+} or Mg^{2+} is the divalent cation (Figure 1). Saturation is not due to a limitation in divalent cation concentration (Helfman et al., 1976). Apparent K_M values determined from reciprocal plots were 13 μM and 15 μM in the presence of Mg^{2+} and Mn^{2+}, respectively (Figure 1, inset). Similar studies have been reported for the Mg^{2+}-activated DNA polymerase III, which has K_M values of 20 μM for each dNTP (Kornberg & Gefter, 1972).

The dNTP concentrations were also varied singly, while the other three dNTPs were maintained at approximately saturating concentrations (50 μM). Comparison with calculated Michaelis–Menten curves (dashed curves) shows that when the dCTP concentration is varied, both for DNA polymerase II (Figure 2B) and for DNA polymerase III (Figure 3B), whether activated by Mg^{2+} or by Mn^{2+}, a significant deviation from the dashed curves occurs. The experimental points follow a sigmoidal curve, with the points lying below the dashed curves at low dCTP concentrations (Figures 2B and 3B, insets), and above the dashed curves at high dCTP concentrations. This deviation from the Michaelis–Menten curves is not seen when the other dNTP concentrations are individually varied, with the possible exception of the Mg^{2+}-activated DNA polymerase II reaction when the dATP concentration is varied (Figure 2A, inset).

Considerable DNA polymerase II (Figure 2) and DNA

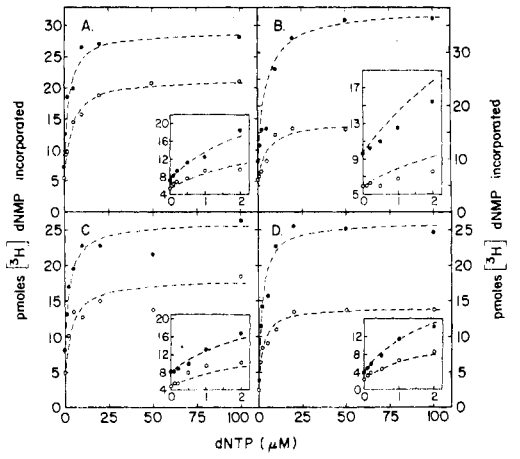

FIGURE 3: Effect of concentration of individual dNTP substrates on the activity of DNA polymerase III. The remaining three dNTPs, including the labeled dNTP substrate, were present in reactions at 130 μM each. [^{3}H]dTTP at 500 cpm per pmol was used for all experiments except when dTTP was varied and [^{3}H]dATP at 200 cpm per pmol was the label. Reactions were as described in Methods in the presence of Mg^{2+} (○) or Mn^{2+} (●). (A) dATP; (B) dCTP; (C) dGTP; (D) dTTP. The dashed lines are optimal Michaelis–Menten curves for the substrate-dependent reaction calculated as described in Figure 2 legend. The curves and data presented in the insets are those for low substrate concentrations.

TABLE I: DNA Polymerase Activity in the Absence of One Deoxyribonucleoside 5′-Triphosphate.[a]

Omitted dNTP	DNA polymerase II		DNA polymerase III	
	Mg^{2+}	Mn^{2+}	Mg^{2+}	Mn^{2+}
−dATP	15.8	16.5	25.6	25.2
−dCTP	21.2	15.7	35.3	25.3
−dGTP	25.3	19.0	27.8	31.6
−dTTP	8.7	11.8	17.9	15.4

[a] Reactions were as described in the legends to Figures 2 and 3. Results are expressed as percentages of activity relative to saturating concentrations of the missing dNTP.

TABLE II: Apparent K_m Values for the Individual Deoxyribonucleoside 5′-Triphosphates.[a]

Substrate	DNA polymerase II		DNA polymerase III	
	Mg^{2+}	Mn^{2+}	Mg^{2+}	Mn^{2+}
dATP	3.3	1.5	4.1	2.4
dCTP	6.0	5.7	4.8	5.0
dGTP	2.6	1.2	4.0	2.8
dTTP	6.0	1.8	2.0	2.0

[a] Apparent K_m values were determined as described in the legend to Figure 2.

TABLE III: Specificity of DNA Polymerases II and III for Deoxyribonucleoside 5′-Triphosphates.[a]

Added substrate	Omitted dNTP	DNA polymerase II		DNA polymerase III	
		Mg^{2+}	Mn^{2+}	Mg^{2+}	Mn^{2+}
None	−dCTP	23	21	26	23
rCTP	−dCTP	20	22	23	21
ara-CTP	−dCTP	16	6	23	23
2′-fl-dCTP	−dCTP	36	21	26	26
None	−dATP	18	20	18	18
rATP	−dATP	16	18	18	18
ara-ATP	−dATP	6	2	14	15
None	−dGTP	26	22	26	32
rGTP	−dGTP	17	22	28	31
dITP	−dGTP	60	76	65	90
8-Br-dGTP	−dGTP	17	25	24	28
None	−dTTP	9	12	15	15
dUTP	−dTTP	50	74	57	71
rUTP	−dTTP	7	13	16	16
ara-UTP	−dTTP	6	2	18	17
2′-fl-dUTP	−dTTP	11	16	14	16

[a] Reaction mixture contained 50 μM added substrate as indicated; other reaction conditions were as described in legends to Figures 2 and 3. Results are expressed as percentages of activity relative to activity observed in reactions containing 50 μM of the missing dNTP.

polymerase III (Figure 3) activity is present in the absence of any one of the dNTPs. In contrast, using identical reaction conditions, purified *E. coli* DNA polymerase I has only 2% of the control activity when dCTP alone is omitted from the reaction mixture (data not shown). Quantitation of the activity observed when each of the dNTPs is singly omitted from the reaction is summarized in Table I. Of the four dNTPs, both DNA polymerases show greatest dependence on dTTP.

Apparent K_M values for each of the dNTPs determined from calculated Michaelis–Menten curves (Figures 2 and 3, dashed curves) are summarized in Table II. Wickner et al. (1972a,b) previously reported that Mg^{2+}-activated DNA polymerase II activity is half-saturated at 2.5 μM dTTP, a value somewhat lower than our determination. Both DNA polymerases exhibit the least affinity for dCTP, the dNTP for which the resulting saturation curves are sigmoidal (see above). With the exception of dCTP, the K_M values are lower for the Mn^{2+}-activated reaction than for the Mg^{2+}-activated reactions, for both DNA polymerases, suggesting that Mn^{2+} ions increase the affinities of both enzymes for these substrates.

Requirement for the Triphosphate Form of Substrate. Activity with dNMPs or dNDPs was less than 1% for both DNA polymerases II and III (data not shown) under various ionic conditions of monovalent and divalent cations used previously (Helfman et al., 1976). Addition of 1.0 mM ATP had negligible effect on the incorporation of mono-, di-, or triphosphate forms of deoxyribonucleotides by either enzyme (data not shown).

Substitution of dCTP with rCTP, ara-CTP, and 2′-fl-dCTP. Substitution of 2′-fluorodeoxycytidine 5′-triphosphate (2′-fl-dCTP) for dCTP results in stimulation of net DNA synthesis but only to an extent of about 25–30% of that observed in the presence of dCTP, in either the Mg^{2+}-activated or Mn^{2+}-activated DNA polymerase II reactions (Table III). Essentially negligible stimulation or inhibition of DNA synthesis is found when rCTP is substituted for dCTP in the DNA polymerase II reactions. However, *ara*-CTP inhibits DNA polymerase II catalyzed DNA synthesis even in the absence of dCTP. This inhibition, also observed with the full complement of dNTP substrates (Helfman et al., submitted for publication), is greater with the Mn^{2+}-activated reaction.

The requirement for dCTP is even more stringent in the case of DNA polymerase III catalyzed reactions (Table III). *ara*-CTP, rCTP, and 2′-fl-dCTP neither stimulate nor inhibit net DNA synthesis in the absence of dCTP for either the Mg^{2+}-activated or Mn^{2+}-activated reactions.

Substitution of dATP with rATP and ara-ATP. Neither *ara*-ATP nor rATP stimulate net DNA synthesis by DNA polymerase II in the absence of dATP, and *ara*-ATP inhibits the dATP-independent reaction (Table III). This inhibition is greater for the Mn^{2+}-activated reaction than for the Mg^{2+}-activated reaction. Neither *ara*-ATP nor rATP stimulate or inhibit net DNA synthesis by DNA polymerase III,

activated either with Mg^{2+} ions or with Mn^{2+} ions.

Substitution of dGTP with rGTP, 8-Br-dGTP, and dITP. When rGTP is substituted for dGTP, some inhibition is observed of the Mg^{2+}-activated DNA polymerase II reaction, but no inhibition of net DNA synthesis occurs in the Mn^{2+}-activated DNA polymerase II reaction (Table III). There is similar inhibition when the syn conformation analogue, 8-Br-dGTP, is substituted for dGTP in the Mg^{2+}-activated DNA polymerase II reaction. However, 8-Br-dGTP causes no inhibition of net DNA synthesis in the Mn^{2+}-activated DNA polymerase II reaction. Replacement of dGTP with dITP stimulates DNA synthesis in both the Mg^{2+}-activated and the Mn^{2+}-activated DNA polymerase II reactions. This stimulation is presumably due to substitution of dITP for dGTP as a substrate for DNA synthesis, although incorporation of dIMP into DNA is not directly demonstrated in these experiments.

When substituted for dGTP in the DNA polymerase III reactions, rGTP has negligible effect on net DNA synthesis. However, 8-Br-dGTP causes slight inhibition of net DNA synthesis when DNA polymerase III is activated with either Mg^{2+} or Mn^{2+}. As observed for the DNA polymerase II reactions, when substituted for dGTP, dITP stimulates DNA synthesis in both Mg^{2+}-activated and Mn^{2+}-activated DNA polymerase III reactions.

Substitution of dTTP with dUTP, rUTP, ara-UTP, and 2′-fl-dUTP. When rUTP or 2′-fluorodeoxyuridine 5′-triphosphate is substituted for dTTP, negligible net stimulation or inhibition of either the Mg^{2+}-activated or Mn^{2+}-activated DNA polymerase II reactions is observed (Table III). *ara*-UTP inhibits net DNA synthesis in the DNA polymerase II reactions in the absence of dTTP and is more pronounced for the Mn^{2+}-activated reaction than for the Mg^{2+}-activated reaction. When substituted for dTTP, dUTP stimulates DNA synthesis in both DNA polymerase II reactions.

Substitution of rUTP, 2′-fl-dUTP, or *ara*-UTP for dTTP has little effect, either stimulation or inhibition, on net DNA synthesis in either Mg^{2+}-activated or Mn^{2+}-activated DNA polymerase III reactions. Thus, on the basis of fluoronucleotide substitution, the requirement for dTTP by DNA polymerase III is as stringent as the requirement for dCTP. Replacement of dTTP with dUTP, however, does stimulate net DNA synthesis in both the Mg^{2+}-activated and Mn^{2+}-activated DNA polymerase III reactions. Stimulation of net DNA synthesis by dUTP in the Mn^{2+}-activated DNA polymerase III reaction is even more extensive when poly[d(A-T)] is the template (data not shown).

Discussion

DNA polymerases II and III from *E. coli* catalyze extensive synthesis in the absence of any one of the dNTPs. The purity of the dNTPs and the inability of *E. coli* DNA polymerase I to catalyze this reaction exclude the possibility of contaminating dNTPs in the reaction mixture. Similar activity in the absence of a full complement of dNTPs is probably template directed with *B. subtilis* DNA polymerases II or III (Gass & Cozzarelli, 1973). Evidence presented for the *B. subtilis* DNA polymerases II and III makes misincorporation or terminal addition an improbable cause of this activity. However, the experiments presented here do not exclude these possibilities for the *E. coli* enzymes.

On the other hand, *E. coli* DNA polymerases II and III reportedly fill in only short gaps in DNA of less than 100 nucleotides (Wickner et al., 1972b; Livingston et al., 1975). Thus, the enzyme may possibly jump to a new primer terminus when the missing nucleotide is reached. An excess of primer sites in the "gapped" thymus DNA substrate was present in these reactions at the DNA concentrations used. Synthesis in the absence of one or more dNTPs, then, could represent the initial phase of the normal DNA polymerase II or III reaction. Also, the possibility of incorporation of incorrectly paired nucleotides cannot be excluded.

Our results show that *E. coli* DNA polymerase II, but not DNA polymerase III, is inhibited by arabinonucleotides in the absence of the appropriate dNTP substrate (Table III). *B. subtilis* DNA polymerase II, however, is unaffected by *ara*-CTP under these conditions (Gass & Cozzarelli, 1973).

The stringent specificity of *E. coli* DNA polymerase III for the 2′-deoxy configuration of nucleotide substrate is very significant. Since this enzyme appears to be indispensable for the replication of the bacterial chromosome (Gefter et al., 1971; Nüsslein et al., 1971), such specificity would assure exclusion of ribonucleotides in the newly synthesized DNA. Furthermore, although a less strict specificity for the 2′-deoxy configuration is required in the DNA polymerase II reactions, there is little or no difference between Mg^{2+} and Mn^{2+} ion activation. Thus, if Mn^{2+}-induced mutagenesis is due to misincorporation of ribonucleotides into DNA, it appears more likely to be due to DNA polymerase I catalyzed reactions. The mutagenic event, however, could also be due to an error in *base specificity* in DNA polymerase II or DNA polymerase III catalyzed reactions. The studies presented here have not determined the extent of correct base pairing.

In this study, 2′-fluoronucleoside derivatives were used as sensitive indicators of the enzymatic specificity toward the 2′-deoxy configuration. Considering both the charge and effective size of the fluorine atom, the 2′-fluoronucleosides are expected to have properties which are intermediate between deoxyribo and ribo compounds. For example, although no significant substitution of rCTP for dCTP is observed in either DNA polymerase II or III reactions, there is about 25–30% substitution of 2′-fl-dCTP in the DNA polymerase II reactions and very little substitution in the DNA polymerase III reactions (Table III). However, this stimulation of net DNA synthesis in the DNA polymerase II reactions is markedly less when 2′-fl-dUTP is substituted for dTTP (Table III). Similar studies with Rous sarcoma virus DNA polymerase, *E. coli* RNA polymerase, and *E. coli* DNA polymerase I suggest that these enzymes have less specificity for the preferred 2′ configuration (data not shown).

These fluoro compounds are also of possible utility in cancer chemotherapy. Thus, 2′-deoxy-2′-fluorocytidine is a differential inhibitor of the growth of certain lymphoblastic cell lines, especially Raji cells (Brox et al., 1974). The inhibition may be correlated with the extensive incorporation of 2′-fl-dCMP residues into DNA which these authors observed. There was also extensive deamination of 2′-fl-CdR to 2′-fl-UdR in certain cells (Brox et al., 1974). We would predict, however, that even less 2′-fl-dUMP would be incorporated into DNA based on the relative inability of 2′-fl-dUTP to substitute for dTTP in either DNA polymerase II or III reactions.

Both DNA polymerases II and III substitute dUTP or dITP more efficiently than does DNA polymerase I (Bessman et al., 1958). Although dUTP is incorporated into DNA by the three DNA polymerases, it inhibits DNA synthesis in permeabilized ϕX174-infected cells (Geider, 1972). Furthermore, the 5′ to 3′ exonuclease of DNA polymerase I is capable of specific endonucleolytic cleavage of DNA at dUMP residues (Wovcha & Warner, 1973). This could account for the absence of deoxyuridine in natural DNA molecules, with the exception of the *B. subtilis* phage PBS2 (Takahashi & Marmur, 1963). The extensive replacement of dGTP with dITP may indicate

a less stringent requirement of DNA polymerases II and III for the formation of a stable GC base pair.

In contrast, the requirement for the anti conformation of nucleotide is strictly observed for DNA polymerases II and III, as also is true for RNA polymerase (Kapuler & Reich, 1971) and DNA polymerase I.[2] Thus, 8-Br-dGTP does not support synthesis by any of these enzymes, but acts as an inhibitor when dGTP or rGTP are present (data not shown).

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