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Ribonucleoside and Deoxyribonucleoside Triphosphate Pools during 2-Aminopurine Mutagenesis in T4 Mutator-, Wild Type-, and Antimutator-infected *Escherichia coli**

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Ribonucleoside and deoxyribonucleoside triphosphate pools have been measured in *Escherichia coli* infected with bacteriophage T4 DNA polymerase mutator, wild type, and antimutator alleles during mutagenesis by the base analogue 2-aminopurine. ATP and GTP pools expand significantly during mutagenesis, while CTP and UTP pools contract slightly. The DNA polymerase (gene *43*) alleles and an *r*II lesion perturb normal dNTP pools more than does the presence of 2-aminopurine. We find no evidence that 2-aminopurine induces mutations indirectly by causing an imbalance in normal dNTP pools. Rather, it seems likely that, by forming base mispairs with thymine and with cytosine, 2-aminopurine is involved directly in causing bidirectional A•T ⇄ G•C transitions. The ratios for 2-aminopurine deoxyribonucleoside triphosphate/dATP pools are 5–8% for *ts*L56 mutator and 1–5% for *ts*L141 antimutator and 43⁺ alleles. We conclude that the significant differences observed in the frequencies of induced transition mutations in the three alleles can be attributed primarily to the properties of the DNA polymerases with their associated 3′-exonuclease activities in controlling the frequency of 2-aminopurine•cystosine base mispairs.

Base substitution mutation frequencies are affected by replication and repair enzymes (1–7), base context surrounding a mutated site (8–17), and both relative (13, 14, 18–24) and absolute (13, 21) concentrations of deoxyribonucleoside triphosphate pools. AmPur,[1] a base analogue of adenine, induces bidirectional A·T → G·C and G·C → A·T base substitution transition mutations in *Escherichia coli* and bacteriophage T4 at frequencies substantially above spontaneous backgrounds (for a review, see Ref. 25). The molecular basis for AmPur's mutagenicity is its ability to form relatively stable base mispairs with Thy and with Cyt when present either as a template base on DNA (15) or as a deoxyribonucleoside triphosphate substrate, dAmPurTP (21, 26, 27).

In this paper, we report measurements of deoxyribonucleoside triphosphate pools during AmPur mutagenesis in T4 *ts*L56-*r*UV199 mutator-, 43⁺-*r*UV199-, and *ts*L141-*r*UV199 antimutator T4-infected *E. coli*. These pool size determinations serve three primary purposes with regard to mutagenesis. First, knowledge of relative magnitudes of dAmPurTP/dATP allows one to assess the potential for dAmPurTP to be inserted into DNA opposite Thy; this step is required for *direct* involvement of AmPur in causing A·T → G·C transitions. Similarly, a measurement of the dAmPurTP/dGTP ratio relates to AmPur's direct mutagenic potential in the G·C → A·T pathway where dAmPurTP is inserted opposite template Cyt in competition with dGTP. Second, a measurement of dAmPurTP pools for each of the three T4 gene *43* alleles will allow us to determine if the widely different AmPur-induced mutagenic rates observed in mutator, wild type, and antimutator backgrounds (see *e.g.* Ref. 28) might be attributed to differences in the metabolism of the analogue in the three genetic backgrounds. Alternatively, differences in AmPur mutagenesis in the T4 gene *43* alleles may be caused primarily by differences in the insertion and proofreading properties of the mutator, wild type, and antimutator DNA polymerases. Finally, the pool size measurements should allow us to determine whether AmPur exerts an indirect effect on mutagenesis by perturbing pools of the four common dNTPs.

EXPERIMENTAL PROCEDURES AND RESULTS[2]

DISCUSSION

A measurement of the pool size of 2-aminopurine deoxyribonucleoside triphosphate allows one to estimate the mutagenic potential of the base analogue, provided that the mutations occur as a direct result of AmPur incorporation into DNA. The first step in the induction of an A·T → G·C transition by 2-aminopurine presumably requires the replacement of Ade by AmPur opposite a template Thy site. In general, the rates of insertion into DNA for any two competing nucleotide substrates should be in proportion to their relative dNTP pools. A multienzyme deoxyribonucleotide biosynthetic complex encoded by T4 (see *e.g.* Ref. 31) may act to concentrate dNTPs at the replication fork. Although replication fidelity can be influenced by absolute dNTP concentrations (13, 21), it is the ratio of competing dNTP substrates

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[1] The abbreviations used are: AmPur, 2-aminopurine; HPLC, high pressure liquid chromatography; HMdCTP, hydroxymethyl deoxycytidine triphosphate; dAmPurTP, 2-aminopurine deoxyribonucleoside triphosphate; rAmPurTP, 2-aminopurine ribonucleoside triphosphate.

[2] Portions of this paper (including "Experimental Procedures," "Results," Figs. 1–3, and Tables I–III) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 84 M-3324, cite the authors, and include a check or money order for $3.60 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.

which is expected to play a dominant role in mutagenesis (21).

For the special case of AmPur and Ade deoxyribonucleotides competing for insertion opposite Thy,

$$\frac{\text{I(AmPur)}}{\text{I(Ade)}} = \frac{[\text{dAmPurTP}]}{[\text{dATP}]} e^{-\frac{\Delta G_1}{RT}} \quad (1)$$

where I(AmPur)/I(Ade) is the ratio of inserting dAmPurTP and dATP into DNA, [dAmPurTP]/[dATP] is the ratio of the pool concentrations for the two competing substrates, and ΔG_1 is a measure of the average free energy difference between A·T and AmPur·T base pairs in DNA. Estimates of ΔG *during* DNA replication are in the range 1–1.3 kcal/mol (21, 32).

Pool concentrations of dAmPurTP appear to be similar, within a factor of 2, in T4 *ts*L56 mutator, 43$^+$, and *ts*L141 antimutator backgrounds (Table I). dATP concentrations also appear to be similar in 43$^+$ and *ts*L141 antimutator backgrounds, but may be about 5-fold lower in *ts*L56 mutator-infected cells (Table I). Although we strongly suspect that greater levels of AmPur-induced mutagenesis in L56 mutator backgrounds (see *e.g.* Ref. 28) are attributable primarily to a reduction in the 3′-exonuclease proofreading activity of L56 DNA polymerase in comparison to the proofreading capabilities for the active 43$^+$ and highly active L141 polymerases (3, 21, 26), the increased [dAmPurTP]/[dATP] ratio might be responsible for perhaps as much as a 5-fold greater mutation rate in *ts*L56-infected cells.

The second step in the A·T → G·C pathway involves the insertion of HMdCTP in place of dTTP opposite template AmPur. From Table I we note that the pool of HMdCTP is about 1.5-fold larger in *ts*L141 antimutator-infected cells compared to 43$^+$ and about 5-fold larger in *ts*L141 compared to *ts*L56 mutator. However, these differences would not be expected to have a significant effect on AmPur mutagenesis since the frequency of HMdCTP insertion opposite AmPur should be much more dependent on the HMdCTP/dTTP *ratio* than on the absolute magnitude of HMdCTP concentration (21). The free energy difference, ΔG_2, for inserting HMdCTP *versus* dTTP opposite AmPur is estimated to be about 1.8 kcal/mol (15).

The mutation frequency at each individual site on DNA depends on nearest-neighbor base-stacking partners and surrounding base composition (8–17). However, one can utilize the dNTP pool size ratios and measurements of base-pairing free energy differences to estimate the average mutation frequency, neglecting site-specific effects. This type of estimate is instructive in the case of mutagenesis by 2-aminopurine as it allows an evaluation to be made of the relative importance of proofreading in controlling the relative mutation frequencies in *ts*L56 mutator, 43$^+$, and *ts*L141 antimutator genetic backgrounds.

We will now calculate two extreme cases to characterize AmPur-induced A·T → G·C transition rates, *i.e.* with and without proofreading, using Equation 1 with dNTP pool data and ΔG estimates for each step in the mutational pathway. In the absence of proofreading, the only available nucleotide discrimination step occurs during insertion. For each nucleotide insertion in the AmPur-induced A·T → G·C pathway, the formation of AmPur·T and AmPur·C mispairs, the mutation rate is given by the product of the pool ratios [dAmPurTP]/[dATP] and [HMdCTP]/[dTTP] multiplied by exp $-(\Delta G_1 + \Delta G_2)/RT$ (see Equation 1). Taking the average pool size ratios [dAmPurTP]/[dATP] = 0.02 and [HMdCTP]/[dTTP] = 1 and ΔG_1 = 1.3 kcal/mol and ΔG_2 = 1.8 kcal/mol (15, 21, 32), we obtain an AmPur-induced A·T → G·C mutation frequency of 10^{-4} for a system without proofreading.

A frequency of 10^{-4} is similar to the AmPur-induced reversion of *r*UV199 in the *ts*L56 mutator background (2×10^{-4}; Table III). Perhaps *ts*L56 mutator represents an "ideal" one-step discrimination enzyme. *In vitro* studies on purified L56 polymerase strongly indicate that the mutant enzyme has a diminished 3′-exonuclease activity (3) and exhibits a reduced proofreading capability for AmPur (21, 26).

We now consider a second extreme case where proofreading is maximized. We will assume that the same ΔG discrimination value used in the insertion step is valid for the proofreading step; there is experimental evidence which supports the idea that nucleotide discrimination-free energy differences, governing the frequency of forming AmPur·T *versus* A·T base pairs, are similar during insertion and excision for purified L56, 43$^+$, and L141 DNA polymerases (21). Using the same parameters as before, except that the base pair stabilities ΔG_1 and ΔG_2 are each sampled twice (first during insertion and then during excision), we calculate a mutation rate of 6.5×10^{-7}. This value is of the same order as the *ts*L141 antimutator-induced reversion of *r*UV199 (1×10^{-7}; Table III). Perhaps the L141 antimutator polymerase is behaving similarly to an ideal two-step discrimination enzyme. Reversion of the *r*UV199 marker to *r*$^+$ in 43$^+$-infected cells (4×10^{-5}; Table III) falls between the two extremes of ideal one- and two-step discrimination.

We conclude from the pool size measurements that the metabolism of AmPur appears similar in L56 mutator-, 43$^+$-, and L141 antimutator-infected cells, resulting in a pool of substrate dAmPurTP which is between 1 and 5% of the concentration of dATP pools. However, as we have shown previously (28), AmPur substitution for Ade in T4 L56, 43$^+$, and L141 DNA is at a level which is much less than 1–5%; the actual *in vivo* dAmPurMP/dAMP incorporation ratios are 1:1100 for L56, 1:1800 for 43$^+$, and 1:8500 for L141. The reduction in dAmPurMP incorporation by more than an order of magnitude below the pool ratio is primarily due to the instability of AmPur·T as compared to A·T base pairs (33, 34). Regarding nucleotide insertion, we would expect this reduction to be roughly similar for the three polymerase alleles. At the excision or proofreading step, a further reduction in the level of dAmPurMP in DNA is expected in all three backgrounds, but a much larger reduction is expected in the case of L141 antimutator.

We find no evidence that the pools of the four common dNTPs undergo significant distortion during AmPur mutagenesis. Thus, rather than causing mutations in an indirect manner by creating an imbalance in the concentration of one nucleotide *versus* another, it seems likely that AmPur is involved directly in the formation of aberrant base pairs AmPur·T and AmPur·C in causing bidirectional A·T ⇄ G·C transition mutations.

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SUPPLEMENTARY MATERIAL TO

RIBONUCLEOSIDE AND DEOXYRIBONUCLEOSIDE TRIPHOSPHATE POOLS DURING 2-AMINOPURINE MUTAGENESIS IN T4 MUTATOR, WILD TYPE, AND ANTIMUTATOR INFECTED ESCHERICHIA COLI

Randi L. Hopkins and Myron F. Goodman

This supplement provides EXPERIMENTAL PROCEDURES and RESULTS.

EXPERIMENTAL PROCEDURES

Growth Conditions

2-Aminopurine mutagenesis was carried out on T4 ocUV199 (rIIB), UV199-tsL56(43), and UV199-tsL141(43) bacteriophage in an E. coli CR63 host as described in (Ref. 28). Control extracts were prepared from cultures identical except for the omission of 2-aminopurine, adenine, and guanine from the medium.

Extraction and Sample Preparation

Cells were harvested 20 minutes postinfection by vacuum filtration on a 15 cm diameter 0.45u type HA filter (Millipore, Maine). This process usually took between 30 and 60 seconds for a 200 ml culture (equivalent to ~ 10^{11} infected cells). The filters were inverted and placed in a 15 ml bath of 0.4M perchloric acid containing [^{3}H]- or [^{32}P]-labelled deoxyribonucleoside triphosphate markers kept in a Pyrex dinner plate on ice. Cells were scraped from the filter and placed in a chilled centrifuge tube. The filter was washed with an additional 5 ml aliquot of $HClO_4$. The pooled extracts were centrifuged for 10 seconds at 7000 RPM. Meanwhile, a third aliquot (5 ml) of $HClO_4$ was used to wash the filter,and subsequently to resuspend the pellet from the first centrifugation. After withdrawing the first supernatant, the resuspended pellet was centrifuged again. The supernatants were brought to pH 8 to 9.5 with 1M KOH and pooled. $KClO_4$ precipitate was removed by filtering through a 25 mm 0.8u type HA Millipore filter. Free bases were removed from the extract by passing it over a 1 cm × 60 cm column of LRP (Whatman) which had been wet with methanol and equilibrated with water at 4°. The extracts, whose volumes at this point were ca. 80 ml, were fractionated on a disposable 500 mg aminopropylsilane cartridge (Analytichem International, Harbor City, CA). Extracts were loaded at 1 ml/min on ice. A step gradient of $NH_4H_2PO_4$/NH_4OH at pH 9.5 was employed to fractionate ribo- and deoxyribonucleoside triphosphates by level of phosphorylation. 0.001M was applied until A_{260} reached a baseline. 0.01M eluted a small peak representing most of the diphosphates in ca. 2 ml. Triphosphate compounds were eluted in ca. 10 ml 0.2 M. The triphosphate and diphosphate peaks were each pooled, divided into 2 or 3 aliquots, shell-frozen and lyophyllized.

HPLC

Analysis was performed on a Waters μBondapak C18 8 mm id × 10 cm radial compression cartridge using two different mobile phase systems. Reverse phase system I with a mobile phase of 0.2 $NH_4H_2PO_4$/NH_4OH pH 5.5 at a flow rate of 0.5 or 1 ml per minute was used for the quantitation of ribo- and deoxyribonucleoside triphosphates. Reverse phase system II with a mobile phase of 2.8M $NH_4H_2PO_4$/H_3PO_4, pH 3.1 was used to to confirm identity and sometimes quantity of the pyrimidine compounds and rAmPurTP. A flow rate of 1 ml/min was used.

Data Acquisition and Analysis

UV absorption data were collected simultaneously at 313nm and 254 nm and later processed using Appligration software (Dynamic Solutions, Pasadena) in conjunction with an Apple II+ computer. These peak detection and integration algorithms gave linear results of molar quantity vs. peak height or peak area even when peaks were under 0.001 A tall. This contrasts to the findings of Garrett and Santi (29).

Nucleotides

10^6 cpm of [^{32}P]-dNTP (New England Nuclear) at a specific activity of ca. 10 Ci per mmol was sufficient to allow quantitation of recovery from subsequent steps without contributing significantly to the molar quantity of any compound. Tritium markers were much more difficult to trace, due to an enormous and variable quench in these large extracts.

RESULTS

A small aliquot (ca. 10 μl) of prepared sample was injected into reverse phase system I (Fig. 1). From this injection we quantitated normal nucleotide concentrations (CTP, UTP, HMdCTP, GTP, TTP, ATP and dATP). A shoulder often seen on the tail of GTP, quite possibly GDP, was not further analyzed. dGTP fractions from system I were reinjected into the same system to confirm quantification. A quantity of standard sufficient to double peak height for each of the normal ribo- and deoxyribonucleoside triphosphates was mixed with an identical small sample, and injected into reverse phase system I (not shown). In this manner we simultaneously confirmed each compound's retention time and molar extinction coefficient in units of height and area under the exact conditions of the chromatographic assay. Errors due to peak distortion were thus avoided.

A large aliquot of sample was injected into the same reverse phase system I (Fig. 2). Minor constituents (dAmPurTP and rAMPurTP) were quantitated from this run. The packing material was overloaded with ATP and GTP. Peak distortion rendered dGTP unresolved from ATP. Resolution of early peaks was diminished. Late peaks, dATP and dAmPurTP, remain relatively free of distortion. In this region of the chromatogram, computer processed detector response in area (and sometimes in height) remained linearly related to molar quantity for peaks whose heights were as small as 0.2 milliabsorbance units (mA) at 313 nm. 0.25 ml fractions were collected during chromatography of the large aliquot. Marker label recovery was determined by counting 50 to 100 μl aliquots in Liquiscint. Quench was determined by adding 2 μl containing at least 100-fold more lable to the sample vials and recounting. Overall recoveries were 40 to 60%. The identities of HMdCTP and rAmPurTP were confirmed by injecting fractions from reverse phase system I into reverse phase system II. (See standard chromatograms, Fig. 3). The retention time and ratio of absorbance 254 nm/absorbance 313 nm for HMdCTP in system II were compared to standard.

Calculations of molar quantity from the areas of HMdCTP, TTP (254 nm only) and dATP (313 nm and 254 nm) peaks from large aliquot-system I were used to cross check small aliquot-system I results. dAmPurTP was quantitated by area from large aliquot injections into system I. Tracings at 313 nm and 254 nm yield the same result.

We believe that rAmPurTP appeared as a peak at 313 nm (Fig. 2) corresponding to the leading edge of dTTP at about 10 ml in the 254 nm channel. Because no standard rAmPurTP was available to us, a tentative identification and estimate of molar quantity were derived in two ways. The first approach exploited the unusual spectral properties of AmPur compounds. Standard dTTP and non-mutagenized extracts run in reverse phase system I yielded a peak at about 10 ml whose height at 313 nm was about 3.5% of that at 254 nm. The peak maximum was the same time point for both wavelengths. Standard AmPur compounds, in contrast, had slightly greater (ca. 1.5 times) height at 313 nm than at 254 nm. Our AmPur mutagenized extracts yielded a peak at 254 nm which corresponded in time to dTTP, but whose maximum in 313 nm tracings is significantly earlier (about 0.5 ml). The ratio of absorbance at 313 nm/absorbance 254 nm was sometimes 10-fold greater than for dTTP. Computer acquired data points from the appropriate 254 nm and 313 nm channels were converted to absorbance units. 3.5% of the A_{254} for each datum or time point was subtracted from the corresponding A_{313} datum. The resultant "difference chromatogram" was processed using the molar extinction in area for dAmPurTP to estimate rAmPurTP concentration.

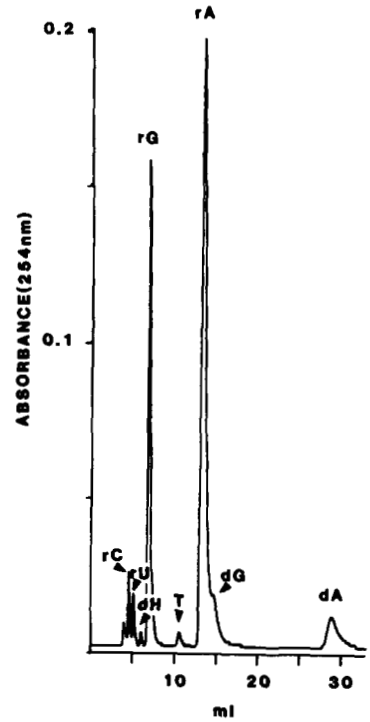

Fig. 1. Representative chromatogram of AmPur mutagenized T4 rUV199-tsL56-infected E. coli. 20 μl equivalent to about 2 × 10^9 cells is injected into reverse phase system I at a flow rate of 1 ml/min. Ribo- and deoxyribonucleoside triphosphates are represented as follows: rC, CTP; rU, UTP; dH, HmdCTP; rG, GTP; T, dTTP; rA, ATP; dG, dGTP; dA, dATP.

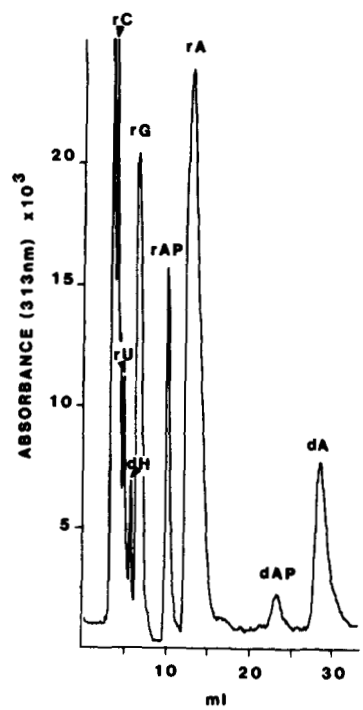

Fig. 2. A 200 μl aliquot of samples from (Fig. 1) representing 2 × 10^{10} cells is injected into reverse phase system I at a flow rate of 1 ml/min. Note absorbance is monitored at 313 nm. Ribo- and deoxyribonucleoside triphosphates are labelled as in (Fig. 1) with the addition of rAP, rAmPurTP; and dAP, dAmPurTP.

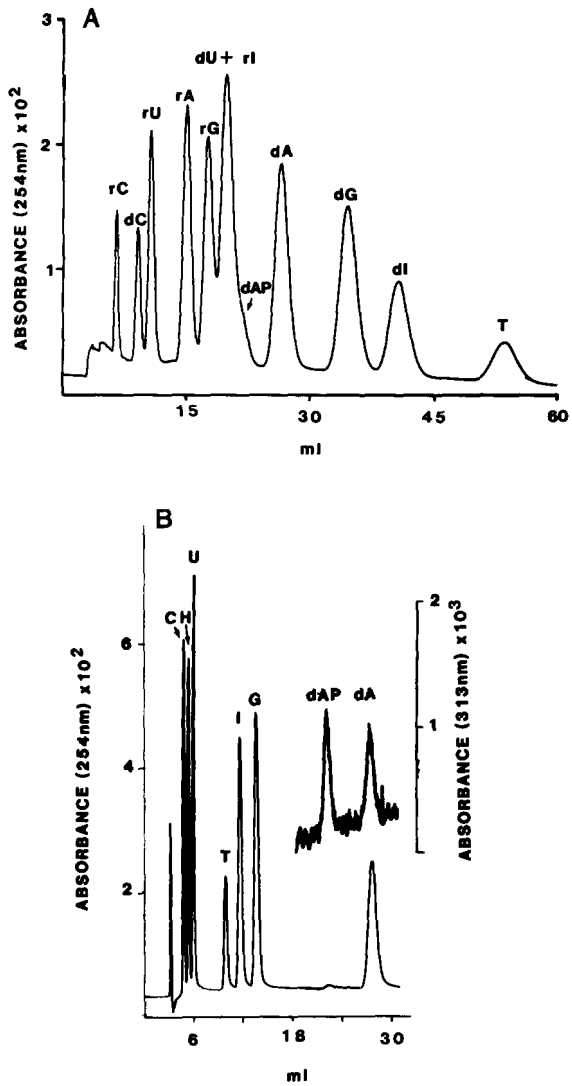

Fig. 3. Ribo- and deoxyribonucleoside triphosphate standards. A. 0.2 nmol dAmPurTP and 2 nmol of the other compounds are injected at a flow rate of 3 ml/min into reverse phase system II. B. The same quantity of each compound is injected at a flow rate of 0.5 ml/min into reverse phase system I. The insert indicates a portion of the 313 nm tracing taken from this same chromatogram. Ribo- and deoxyribonucleoside triphosphates are labelled as in Fig. 2 with the addition of dC, dCTP; dU, dUTP; rI, ITP; and dI, dITP; which are compounds not found in our extracts.

A second approach exploited the difference in elution order or retention times as a function of base moiety observed in comparison of reverse phase system I vs. system II (Fig. 3A vs. Fig. 3B). The putative rAmPurTP had a retention time relative to ATP and GTP equal to the retention time of dAmPurTP relative to dATP and dGTP in both systems. In reverse phase system I, the order was GTP, rAmPurTP, ATP, dGTP, dAmPurTP, dATP. dTTP eluted very near rAmPurTP. In system II the elution order of ribonucleotides was rAmPurTP, ATP, GTP and of deoxyribonucleotides is dAmPurTP, dATP, dGTP. dTTP was the last compound to elute. Thus we eliminated dTTP in the fractions collected from system I while comparing relative retention times in system II. Molar quantity is deduced again using the area extinction at 313 nm for dAmPurTP. From both methods, we estimate rAmPurTP pools were roughly equal to the dAmPurTP pool in each extract (0.1 to 0.5 $\times 10^5$ molecules per cell).

Intracellular concentrations of DNA and RNA precursors in AmPur-mutagenized and nonmutagenized T4-infected E. coli CR63 are shown in Tables I and II. The pools of ATP and GTP expand during AmPur mutagenesis in all three genotypes (Table I). The pools of CTP and UTP may contract slightly during mutagenesis. While the purine deoxyribonucleoside triphosphates may expand and pyrimidine dNTPs contract during mutagenesis, data are equivocal. Moreover, the gene 43 alleles and the rII lesion appear to be more important than AmPur mutagenesis in perturbing normal DNA precursor pools (compare (Ref. 30) to Table II). The dAmPurTP/dATP ratio for UV199-L56 is slightly higher (5 to 8%) than that of UV199-43$^+$ (1.7 to 5%) and UV199-L141 (1.3 to 4.6%) (Table I). Mutation rate data appear in Table III.

Table I. Nucleotide pools during AmPur mutagenesis of T4rUV199 bacteriophage, allelic for the DNA polymerase gene (43). Acid extracts were passed through C_{18} to remove free bases and fractionated on an NH_2 Bond-elute and analyzed on reverse phase systems I and II as described in EXPERIMENTAL PROCEDURES.

	UV199-L56 (3 trials)		UV199-43$^+$ (2 trials)		UV199-L141 (2 trials)	
	average	range	average	range	average	range
CTP	18	16-20	6.6	5.8-7.9	14.4	8.8-20
UTP	14	10-16	4.25	3.5-5.0	14.6	5.6-24
GTP	19	17-20	23.5	19-28	17	12-22
ATP	92	77-100	95	89-101	81.5	70-93
HMdCTP	1.6	1.3-2	5.5	1.9-9.0	8.2	2.3-14
TTP	2.5	1.2-4	2.4	2.3-2.4	13.5	11-16
dGTP	5.9	0.8-11	6.6	6.6,N.D.	4.2	1-7.3
dATP	2.2	0.73-4	8.0	4.0-12	9.5	7-12
dAmPurTP	0.12	0.06-0.2	0.2	0.2-0.2	0.24	0.16-0.32

Mathews has reported on nucleotide pools in DNA-defective amber mutants of T4 using an assay involving ^{32}P-radiolabel (30). We compared our analytical technique to that of Mathews by extracting 200 ml of T4D-infected E. coli B grown in his "media A." Extracts were either treated with $NaIO_4$ (30) or were passed through a borate-gel equilibrated with 1M triethyl-ammonium bicarbonate buffer pH 10 to remove ribo- compounds. Subsequent analysis of extracts on the SAX system used by Garret and Santi (29) yielded quantitation of dNTP pools equivalent to 1-4 $\times 10^5$ molecules per cell in agreement with Mathews (30). $NaIO_4$-treated extracts could not be analyzed on C18 due to interference from breakdown products. However, borate-treated extracts gave the same results when analyzed on the SAX system as when analyzed on reverse phase system I.

Table II. Nucleotide pools from control extracts of T4 rUV199 bacteriophage allelic for the DNA polymerase gene (43) prepared and analyzed as in Table I.

	UV199-L56	UV199-43$^+$	UV199-L141
	molecules per cell $\times 10^{-5}$		
CTP	33	5.5	20
UTP	23	7.1	30
GTP	12	3.6	12
ATP	33	9.4	38
HMdCTP	37	8.5	9
TTP	2.7	2	8
dGTP	6.4	2.7	3
dATP	1.5	1.4	5.8

Table III. rII UV199 reversion frequencies in gene 43 alleles expressed as titer on restrictive host, CR63(λ), divided by titer on permissive host, E. coli B.

	UV199-L56	UV199-43$^+$	UV199-L141
AmPur-induced	2×10^{-4}	4×10^{-5}	1×10^{-7}
Spontaneous	5×10^{-5}	5×10^{-7}	1×10^{-9}