On the Cofactor Specificity of Glycinamide Ribonucleotide and 5-Aminoimidazole-4-carboxamide Ribonucleotide Transformylase from Chicken Liver†

Gary K. Smith, W. Thomas Mueller, Patricia A. Benkovic, and Stephen J. Benkovic*

ABSTRACT: Tests of 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) and glycinamide ribonucleotide (GAR) transformylase cofactor specificity were conducted with 5- and/or 8-deazafolate analogues formylated at N-10. Several of these compounds were found to serve as cofactors for both the enzymes. The finding that 10-formyl-8-deazafolate can be used by AICAR transformylase eliminates those mechanisms requiring cyclization to a methenyl derivative prior to carbon unit transfer for this transformylase. Surprisingly, a similar analogue, 10-formyl-5,8-deazafolate, is very effective as a cofactor for GAR transformylase in the presence or absence of the trifunctional protein which is required for 5,10-methenyl-H_4-folate activity with this transformylase. This finding suggests that the trifunctional protein modulates GAR transformylase cofactor specificity by supplying the active cofactor as the N^{10}-formyl species, possibly through a transport process that avoids its dissociation into solution.

The AICAR and GAR TFases[1] carry out the two reduced folate cofactor requiring steps of de novo purine biosynthesis. The reactions are shown in Scheme I, where GAR TFase and AICAR TFase require 5,10-methenyl-H_4-folate, and 10-formyl-H_4-folate, respectively (Hartman & Buchanan, 1959).

We have previously shown that two enzymes, the trifunctional 10-formyl-, 5,10-methenyl-, 5,10-methylene-H_4-folate synthetase (combined) and serine transhydroxymethylase, copurify through several steps including affinity chromatography columns for either AICAR or GAR TFase (Smith et al., 1980). Further, we have found that protein interactions with the trifunctional protein are necessary for the GAR TFase activity but not necessary for AICAR TFase activity (Smith et al., 1980; Mueller & Benkovic, 1981).

In the present paper we demonstrate that both transformylases can use fully oxidized deazafolate cofactor analogues in their reactions; consequently the 5,10-methenyl structure per se surprisingly is not needed for GAR TFase activity, nor is its formation at either active site required for activity. Further, we show that interactions with the trifunctional protein are not necessary for GAR TFase activity if the cofactor analogues are used, permitting us to further define the mode for the trifunctional protein's activation of GAR TFase.

Experimental Procedures

Materials

5,8-Deazafolate and 10-formyl-5,8-deazafolate were gifts of Dr. John B. Hynes, Department of Pharmaceutical Chemistry, Medical University of South Carolina; 5-deazafolate was kindly furnished by Dr. E. C. Taylor, Department of Chemistry, Princeton University; and 8-deazafolate was available through the generosity of Dr. Roy Kisliuk, Department of Biochemistry, Tufts Medical College.

The 5-deazafolate sample was found to contain impurities which were removed by cellulose chromatography. A 12-mg sample of the 5-deazafolate was dissolved in 1 mL of 0.1 M NH_4HCO_3 and was applied to a 2.4 × 34 cm column of cellulose which had been equilibrated with the same buffer. The column was then eluted with 0.1 M NH_4HCO_3 and the absorbance at 295 nm was monitored. Peak fractions were checked by cellulose TLC with 0.1 M NH_4HCO_3 as the developing solvent and were homogeneous by these criteria.

AICAR TFase, GAR TFase, the trifunctional protein, and their substrates and cofactors were prepared as described previously (Smith et al., 1980; Mueller & Benkovic, 1981; Chettur & Benkovic, 1977). All other compounds were of the highest purity available.

Folic Acid Purification. Commercial folic acid (Sigma) has been found to contain three fluorescent impurities when analyzed on nonfluorescent cellulose TLC with 0.1 M K_2HPO_4 as developing solvent. Purification was affected by a modification of a reported technique (Sakami & Knowles, 1959). Commercial folic acid (350 mg) was dissolved in 10 mL of 0.1 M K_2HPO_4 and adjusted to pH 8 with 3 N KOH. The solution was then passed through a 0.7 × 25 cm column containing a mixture of 2:1 Whatman cellulose–Norite (neutral) which had been prewashed with 25 mL of 6 N HCl and 100 mL of 0.1 M K_2HPO_4, sequentially. The column was developed with 0.1 M K_2HPO_4 under mild suction, and the yellow folic acid fraction was collected. HCl (2 N) was added to precipitate the product at 4 °C. The collected precipitate was redissolved in K_2HPO_4 as before and passed through a 2.5 × 100 cm column of Whatman cellulose (medium fiber) which had been packed under mild suction and equilibrated with 0.1 M K_2HPO_4. The column was eluted as above and the folic acid collected and precipitated as previously. The product then was washed 2 times with cold 0.01 M HCl, lyophilized, and found to be homogeneous by the above TLC criterion; yield, 190 mg.

Methods

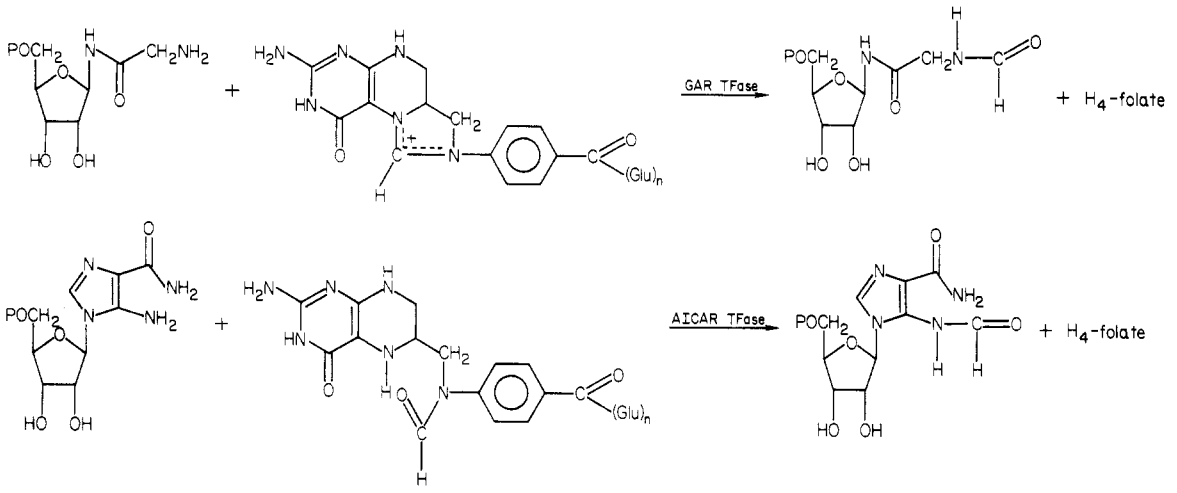
N^{10}-Formylation of Folate Analogues. The reaction was typically carried out on a 0.1–10-mg scale. In a modification

† From the Department of Chemistry, The Pennsylvania State University, University Park, Pennsylvania 16802. *Received August 28, 1980.* This investigation was supported by Grant GM 24129 from the National Institutes of Health.

[1] Abbreviations used: AICAR, 5-aminoimidazole-4-carboxamide ribonucleotide; GAR, glycinamide ribonucleotide; ATP, adenosine 5′-triphosphate; H_4-folate, tetrahydrofolate; TFase, transformylase; FAICAR, formamidoimidazolecarboxamide ribonucleotide; FGAR, formylglycinamide ribonucleotide; trifunctional protein, 5,10-methenyl-, 5,10-methylene-, and 10-formyl-H_4-folate synthetase (combined); 2-ME, 2-mercaptoethanol.

0006-2960/81/0420-1241$01.25/0

Scheme I[a]



[a] P = PO_3^{2-}.

of the technique of Hynes et al. (1977), the analogue was dissolved in a 200-fold molar excess of formic acid (95–97%), and the reaction was allowed to proceed for 1 h at 90 °C in a sealed tube. Formic acid was removed by lyophilization. The product was used directly as the lyophilized powder. Reprecipitation (vide infra) from aqueous NH_4OH after adjusting the pH to 4 with concentrated HCl gave material with no change in spectral or kinetic properties.

Structures were verified by (^{1}H) NMR (D_2O/NaOD) on a Bruker WP-200 Fourier transform NMR, and UV spectra on a Cary 118. The chemical shift for the phenyl protons of the (*p*-aminobenzoyl)glutamate moiety changed from approximately 6.6 and 7.5 ppm to 7.2 and 7.6 ppm upon formylation relative to Me_4Si. Chemical shift of the formyl proton was typically 8.4 ppm and integrated as 1 proton. UV spectra showed a characteristic blue shift of the 290–300-nm peak to 255–280 nm upon formylation (Table I). TLC carried out on Eastman cellulose plates with fluorescent indicator using 0.1 M potassium phosphate (pH 6.5) as the developing solvent for the deazafolates and folate showed the N^{10}-formyl products in all cases to contain only one minor fluorescent impurity, possibly resulting from side-chain cleavage.

^{14}C-Formylation of 5,8-Deazafolate. 5,8-Deazafolate (0.492 mg, 1.04 μmol) was dissolved in a solution of 20 μL (520 μmol) of carrier 95–97% formic acid plus (0.149 mg, 2.13 μmol) sodium [^{14}C]formate (sp act. 3.06×10^5 cpm/μmol). Formylation was carried out as described above. After lyophilization the product was redissolved in 40 μL of acetic acid and relyophilized. This was repeated until no more volatile radioactivity was found. The product was dissolved in 0.1 M potassium phosphate, pH 6.8, and its concentration was determined by A_{255} (Table I).

Synthesis of [p-(Benzylamino)benzoyl]-L-glutamate. (*p*-Aminobenzoyl)-L-glutamate (Sigma) (260 mg, 1.04 mmol) and freshly distilled benzaldehyde (130 mg, 1.23 mmol) were dissolved in 10 mL of dry methanol and stirred for 1 h at 25 °C. To the light yellow solution was added (90 mg, 1.43 mmol) $NaCNBH_3$, and the mixture was stirred for an additional 5 h. The reaction is complete when a stable absorbance at λ_{max} 290 nm is found for a reaction aliquot in aqueous solution at pH 6.8. Solvent was removed in vacuo, and the product was redissolved in H_2O. HCl was then added to precipitate the product (pH 3.5), which was collected by centrifugation. The product was resuspended in H_2O, and NH_4OH was added until it redissolved. Precipitation and dissolution were repeated 2 more times. The product was then lyophilized as the ammonium salt. The ^{1}H NMR spectrum (D_2O/NaOD) was consistent with the stated structure [δ 2.0 (m, $-CH_2CH_2-$, 4 H), 3.8 (s, $-CH_2N-$, 2 H), 4.1 (m, –CONCH, 1 H), 6.3 and 7.4 (2 d, C_6H_4, 4 H), and 6.9 (s, C_6H_5, 5 H)] and the UV spectrum showed λ_{max} 290 nm (ε 17000) (pH 6.8). TLC carried out on Eastman cellulose plates with fluorescent indicator run with 0.1 M dipotassium phosphate showed a single spot, R_f 0.84. The formylated compound shows small amounts of this spot as the only impurity.

Enzyme Assays. The transformylation of AICAR and GAR were followed spectrophotometrically at 290, 295, or 300 nm for the production of the free base folate analogue.

GAR TFase was assayed at pH 7.0 at 35 °C (pH 6.8 at 25 °C) in 50 mM potassium maleate buffer containing 0.234 mM (α,β)GAR, 0.1 mM EDTA, plus varied 10-formylfolate analogue concentrations. For inhibition studies of the unreactive analogues, (±)-L-5,10-methenyl-H_4-folate was included and the GAR TFase assay carried out as previously described (Smith et al., 1980).

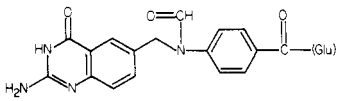
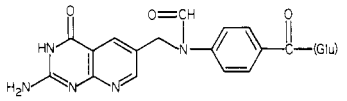
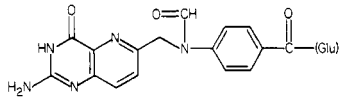
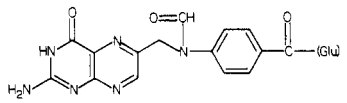
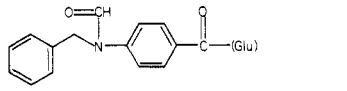
AICAR TFase was assayed at 25 °C in 32.5 mM Tris-HCl, pH 7.4 containing 5 mM 2-ME, 25 mM KCl, and 50 μM AICAR (Ba^{2+}) with varying 10-formylfolate analogue concentrations (Black et al., 1978). For inhibition studies of the unreactive analogues, (±)-L-10-formyl-H_4-folate was also included, and the change in absorbance at 298 nm for its conversion to H_4-folate was recorded.

The 10-formyl-H_4-folate synthetase assay with the natural cofactor was conducted as noted earlier (Smith et al., 1980). The assay with the cofactor analogues was performed by recording the appropriate change in absorbance (Table I) for the formylation of the analogue. Assay conditions were 100 μM analogue, 10 mM sodium formate, 2 mM ATP, 4 mM $MgCl_2$, and 100 mM NH_4Cl in 50 mM Tris-HCl buffer, pH 7.5.

Recombination Experiments. The protocol of Smith et al. (1980) was employed.

Products. The IMP produced by AICAR TFase (inosinicase is also present) was quantitated by anion exchange HPLC (Mueller & Benkovic, 1981). The FGAR produced by GAR TFase was measured by employing 10-[^{14}C]-formyl-5,8-deazafolate and determining the quantity of [^{14}C]FGAR. Separation was effected by passage through a QAE-Sephadex column equilibrated with 0.01 M Na_2CO_3, eluting with 0.02 M Na_2CO_3, pH 9.9, to purify the FGAR made in the reaction. Elution volume of FGAR was determined with authentic [^{14}C]FGAR (Caperelli et al., 1980).

Table I

compd	structure	λ_{max}, nm (ϵ, mM^{-1})[a] free base	λ_{max}, nm (ϵ, mM^{-1})[a] N^{10}-formylated	$\Delta\epsilon$, mM^{-1} (λ, nm)
1		295 (22.2)	255 (23.5), 310 (3.8)	18.9 (295)
2		277 (22.4)	265 (20.7), 320 (5.2)	17.8 (295)
3		277 (23.0)	260 (21.0), 312 (4.1)	18.3 (295)
4		245 (20.0), 269 (20.9), 348 (5.7)[b]	282 (27.6), 346 (7.2)[b]	17.0 (300)[b]
5		290 (17.0)	255 (16.4)	14.8 (290)

[a] Spectra determined at 25 °C, pH 6.8 (0.1 M potassium phosphate), or pH 7.4 (0.1 M Tris-HCl). Spectra were identical at either pH. [b] From Blakley (1969).

Table II: Activity of AICAR TFase with Cofactor Analogues

compd	V_{rel}	K_m^{app} (μM)[a]	K_i (μM)[a]	V_{rel}/K_m^{app}
10-formyl-H_4-folate[b]	1	67.9		0.015
1	(7.4 ± 0.4) × 10^{-4}	28.6 ± 3.5		2 × 10^{-5}
2			38.2 ± 2.3[c]	
3	0.47 ± 0.08	102 ± 22		4.6 × 10^{-3}
4	(1.7 ± 0.2) × 10^{-3}	86 ± 18		2.1 × 10^{-5}
5			>200	

[a] 50 μM AICAR. [b] V = 2.07 μmol min^{-1} mg^{-1} (25 °C) (Mueller & Benkovic, 1981). [c] Inhibition competitive with 10-formyl-H_4-folate.

Table III: Activity of GAR TFase with Cofactor Analogues

compd	V_{rel}	K_m^{app} (μM)[a]	K_i (μM)[a]	V_{rel}/K_m^{app}
5,10-methenyl-H_4-folate	1[b]	8.9[c]		0.11
1	0.77 ± 0.046	1.90 ± 0.77		0.41
2	0.234 ± 0.008	29.5 ± 2.2		7.8 × 10^{-3}
3	0.0017 ± 0.0002		4 ± 3[d]	4 × 10^{-4}
4	0.0033 ± 0.0002	29 ± 7		1.1 × 10^{-4}
5	0		>275	

[a] 0.234 μM (α,β)GAR. [b] 0.54 μmol min^{-1} mg^{-1} (35 °C). [c] K from Caperelli et al. (1980). [d] Relative to either 5,10-methenyl-H_4-folate or **2**.

Kinetics. All kinetic constants were calculated with the hyperbolic computer program of Cleland (1967).

Results

Table I shows the formylated folate analogues used in this study and $\Delta\epsilon$ for each. Tables II and III show the results of assays of AICAR and GAR TFases with these analogues.

AICAR TFase. Only compounds **1**, **3**, and **4** were accepted as cofactors by AICAR TFase, and only one of these (**3**) was reasonably active, 47% V_{rel}. However, this compound also has the highest value for K_m of the first four analogues. Compound **5**, besides not serving as a cofactor, also gave only slight inhibition of the enzyme at concentrations up to 0.2 mM.

The IMP produced from AICAR with 10-formyl-8-deazafolate in the presence of AICAR TFase was quantitated by using high performance liquid chromatography. At 16% reaction (as determined by ΔA_{295}) 0.0121 μmol of 10-formyl-8-deazafolate was consumed and 0.0119 μmol of IMP was formed. Thus 0.984 IMP molecule was produced per 10-formyl-8-deazafolate used, so that a 1:1 stoichiometry is indicated. Although similar product isolation was not carried out with the other analogues, no reaction was observed in the absence of either enzyme or AICAR.

GAR TFase. Analogues **1**–**4** are all accepted by GAR TFase at varying rates. Most noteably, the 10-formyl-5,8-deazafolate cofactor (**1**) is used at a rate which is 77% V_{rel}. This result is quite surprising in light of the fact that (±)-10-formyl-H_4-folate does not serve as a substrate (Hartman & Buchanan, 1959). Further, the intact pyrimidine ring appears essential for binding since compound **5** shows no activity or inhibition up to 275 μM.

At 89% reaction (as determined by ΔA_{295}) 0.0556 μmol of

10-formyl-5,8-deazafolate was consumed, and on the basis of a specific activity of 0.306×10^6 cpm/μmol for the 10-[^{14}C]formyl-5,8-deazafolate starting material, 0.0561 μmol of FGAR was produced. Thus, 1.01 FGAR molecules was produced per 10-formyl-5,8-deazafolate used, and a 1:1 stoichiometry is indicated. As noted earlier for AICAR TFase, no reaction was observed with the 10-formyl analogues in the absence of either GAR TFase or GAR.

GAR TFase Activation. A protein fraction has been obtained by NADP–Sepharose affinity chromatography which is 93% GAR TFase. Utilizing this enzyme sample, we had previously found that GAR TFase is inactive in the absence of the trifunctional protein and that upon recombination with the latter the activity of the former is restored, suggesting a necessary role for the trifunctional protein in the GAR TFase reaction (Smith et al., 1980).

In the present study we have performed a similar set of recombination experiments using the folate cofactor analogues in place of 5,10-methenyl-H_4-folate. It was found that the activity of GAR TFase toward the analogues was identical in the absence or presence of the trifunctional protein. Thus, trifunctional protein does not activate GAR TFase with the cofactor analogues.

Activity of 10-Formyl-H_4-folate Synthetase with the Cofactor Analogues. This enzyme carries out the formylation of H_4-folate according to

$$\text{formate} + H_4\text{-folate} + \text{ATP} \xrightarrow{NH_4Cl} \text{10-formyl-}H_4\text{-folate} + \text{ADP} + P_i$$

In light of the fact that the folate analogues serve as cofactors in both AICAR and GAR TFase reactions we tested their free base forms in the 10-formyl-H_4-folate synthetase reaction. No reaction was observed in any case with enzyme levels at least 500 times higher than is necessary to observe activity with H_4-folate.

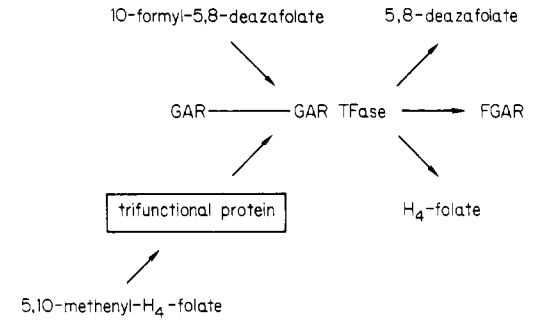
Discussion

Since three of the analogues tested (**1**, **3**, and **4**) serve as cofactors for AICAR TFase, any enzymatic mechanism involving an obligatory 5,10-methenyl-H_4-folate intermediate is ruled out on structural grounds. A pyrimidine ring is an absolute requirement for cofactor utilization as indicated by compound **5**'s insignificant binding to the active site of this enzyme.

The substitution of aromatic structures (benzene, pyrimidine, or pyrazine) for the tetrahydropyrazine of the reduced folate cofactor circumvents the problem of assymetry introduced by C-6 and the absolute preference of AICAR TFase for one isomer. Collectively the results in Table II suggest the enzyme "reads" this portion of the cofactor despite the fact that the transfer of the formyl group occurs from the N^{10} position, two atoms removed. Maximal activity in the analogues is found with the structure possessing a 5-nitrogen (**1**) with the activity falling by ca. 600- and 1250-fold when the analogue contains both 5- and 8-nitrogens (folate) or none (the 5,8-deazafolate). The failure of K_m^{app} values to reflect this change in activity cautions against their use as dissociation constants. However the low value of K_i for **2**, achieved without a polyglutamate side chain points to its possible use as a specific inhibitor of AICAR TFase, since **2** is relatively active with GAR TFase.

The results in Table III show that GAR TFase does not require the 5,10-methenyl-H_4-folate structure for activity when the tetrahydropyrazine ring of the cofactor is replaced by an aromatic ring. Indeed, 10-formyl-5,8-deazafolate and 10-formyl-5-deazafolate work 77% and 23% as well as the natural cofactor. Furthermore, although the trifunctional protein is absolutely required for activity with 5,10-methenyl-H_4-folate, the present results show that it is not needed for activity with the cofactor analogues. These results show that the cofactor analogues can bind directly to GAR TFase to produce an active enzyme–cofactor complex so that a protein–protein interaction with the trifunctional enzyme is not an absolute requirement for GAR TFase activity. Thus the trifunctional protein may activate GAR TFase by chemically modifying the 5,10-methenyl-H_4-folate, perhaps by conversion to 10-formyl-H_4-folate as implied by the results with **1** and implied in Scheme II.

Scheme II



The model in Scheme II makes an important prediction that will be tested, namely, that an inhibitor which binds to the analogue binding site should inhibit GAR TFase activity with either a cofactor analogue or 5,10-methenyl-H_4-folate as the cofactor. Conversely, an inhibitor which binds to the 5,10-methenyl-H_4-folate site on the trifunctional protein should not inhibit the GAR TFase activity with the cofactor analogues.

It is apparent from Table III that the major criterion for analogue reactivity with GAR TFase is the removal of both the 5- and 8-nitrogens from the pyrazine ring as in **1**. However, in contrast to AICAR TFase, the 5-nitrogen's presence blocks GAR TFase activity (**1** vs. **3**). This is essentially a V_{max} effect since $K_M^{app}(\mathbf{1}) \simeq K_i(\mathbf{3})$. Thus **3** can be used as a specific inhibitor of GAR TFase, since **3** is relatively active with AICAR TFase. On the other hand, as noted above, the presence of the 8-nitrogen (**2**) only marginally reduces V_{rel} for GAR TFase but is not active with AICAR TFase. As before, the presence of the pyrimidine ring is essential for binding (**5**).

In conclusion, the cofactor requirements of the GAR TFase and AICAR TFase may be satisfied with various analogues. Most striking is the finding that N^{10}-formyl derivatives function in formyl transfer catalyzed by GAR TFase and that the trifunctional protein requirement for GAR TFase activity may be bypassed. In addition, the sharp differences in analogue selectivity between the two transformylases permit the design of experiments to selectively inhibit one while retaining the activity of the other when both are present within a multifunctional protein complex. Finally the results with the analogues offer a possible rationale for the 0.8% V_{rel} observed by Caperelli et al. (1980) for the unnatural stereoisomer of 5,10-methenyl-H_4-folate with GAR TFase, inferring that a 10-formyl-7,8-dihydrofolate species formed by air oxidation during the course of the experiment may function as the formyl donor.

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Unambiguous Determination of the Stereochemistry of Nucleotidyl Transfer Catalyzed by DNA Polymerase I from *Escherichia coli*†

Richard S. Brody‡ and Perry A. Frey*

ABSTRACT: Nucleotidyl transfer catalyzed by DNA polymerase I from *Escherichia coli* proceeds with greater than 97% inversion of configuration at P_α of the α-phosphorothioate analogue of dATP. This is shown by experiments in which dAMPS,$^{18}O_2$ is stereospecifically phosphorylated to (*Sp*)-dATPαS,$\alpha^{18}O_2$, which is then copolymerized with dTTTP by DNA polymerase. The product of the polymerization is degraded to dAMPS,^{18}O by methods that do not affect the configuration of the phosphorothioate. After the dAMPS,^{18}O is stereospecifically phosphorylated, the resulting (*Sp*)-dATPαS,$\alpha^{18}O$ is copolymerized as before with dTTP. The ^{18}O is found in the displaced pyrophosphate by mass spectral analysis and so must have been in the pyrophosphate bridge of (*Sp*)-dATPαS,$\alpha^{18}O$. Since this ^{18}O was originally nonbridging in (*Sp*)-dATPαS,$\alpha^{18}O_2$, the phosphorothioate configuration must have been inverted in the polymerization reaction. This confirms the determination of P. M. J. Burgers & F. Eckstein [(1979) *J. Biol. Chem. 254*, 6889–6893], who used kinetic correlations based on the stereoselectivity of snake venom phosphodiesterase to deduce the stereochemistry of this reaction.

DNA polymerase I from *Escherichia coli* catalyzes the polymerization of deoxynucleotide triphosphates in the presence of primer template to produce DNA-like polymers (Kornberg & Kornberg, 1974). The incorporation of a deoxynucleotide into the growing polymer involves either a single bond cleavage at the α-phosphorus of the nucleotide triphosphate as the 3′-hydroxyl of the growing chain displaces pyrophosphate or two bond cleavages if a covalent nucleotidyl enzyme is involved. The use of chiral phosphorothioate nucleotides, already extensively employed as stereochemical probes of phospho- and nucleotidyltransferases (Pliura et al., 1980; Gerlt et al., 1980; Webb & Trentham, 1980; Burgers & Eckstein, 1979a–c; Sheu et al., 1979; and references found therein), can distinguish between these two reaction possibilities. As the accumulated evidence indicates that all cleavages of phosphate bonds in enzymatic reactions occur with inversion of configuration, a single bond cleavage will result in inversion and two bond cleavages will yield retention of the configuration about the phosphorothioate [see Westheimer (1980) and Knowles (1980) for discussions of the stereochemistry of nonenzymatic and enzymatic reactions of phosphate esters].

The polymerization of dATPαS[1] catalyzed by DNA polymerase I has recently been investigated by Burgers & Eckstein (1979c). They found that the *Sp* diastereomer of dATPαS is polymerized in the presence of a poly(dT) template to poly(deoxyadenylic acid) containing phosphorothioate internucleotide linkages. The kinetic constants for the polymerization of (*Sp*)-dATPαS are similar to those for dATP, while the *Rp* diastereomer is not a substrate for the enzyme. The chirality of the poly[d(A_s-A)] produced in the polymerization reaction was assigned by using the stereoselectivity of the exonuclease activity of snake venom phosphodiesterase for phosphorothioate diesters having the *Rp* configuration (Burgers & Eckstein, 1978). In a dinucleotide model system, Burgers & Eckstein (1979b) found that 3′-uridyl 5′-adenosyl *Rp* diastereomer hydrolyzed by the diesterase 110 times faster than the *Rp* diastereomer of 3′-*O*-uridyl-5′-*O*-adenosyl phos-

† From the Department of Chemistry, The Ohio State University, Columbus, Ohio 43210. *Received August 14, 1980.* Supported by Grants PF-1534 from the American Cancer Society and GM-24390 from the National Institute of General Medical Sciences.
‡ American Cancer Society Postdoctoral Fellow.

[1] Abbreviations used: dAMPS, 2′-deoxyadenosine 5′-*O*-phosphorothioate; dAMPS,^{18}O, 2′-deoxyadenosine 5′-*O*-[^{18}O]phosphorothioate; dATPαS, 2′-deoxyadenosine 5′-*O*-(1-thiotriphosphate); dATPαS,$\alpha^{18}O$, 2′-deoxyadenosine 5′-*O*-(1-thio[1-^{18}O]triphosphate); pdTp_sdA, 3′-*O*-(5′-phospho-2′-deoxythymidyl)-5′-*O*-(2′-deoxyadenosyl) phosphorothioate; poly[d(T_s-A)], alternating copolymer of 2′-deoxyadenosine 5′-*O*-phosphorothioate and 2′-deoxythymidine 5′-phosphate; poly[d(A-T)], alternating copolymer of deoxyadenylate and deoxythymidylate; poly[d-(T)], poly(deoxythymidylic acid); poly[d(A)], poly(deoxyadenylic acid); poly(rA), poly(adenylic acid); poly[r(A_s-A)], poly(adenylic acid) containing phosphorothioate internucleotide linkages; *Rp*, the *R* configuration of the chiral phosphorus in a nucleotide; *Sp*, the *S* configuration of the chiral phosphorus in a nucleotide; A_{260} units, absorbance at 260 nm if entire sample were in 1 mL; Me_2SO, dimethyl sulfoxide; EDTA, ethylenediaminetetraacetic acid; GCMS, gas chromatograph interfaced with a mass spectrometer; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Tris, tris(hydroxymethyl)aminomethane; TLC, thin-layer chromatography.

0006-2960/81/0420-1245$01.25/0