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Active-Site Modification of Mammalian DNA Polymerase β with Pyridoxal 5′-Phosphate: Mechanism of Inhibition and Identification of Lysine 71 in the Deoxynucleoside Triphosphate Binding Pocket†

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ABSTRACT: Pyridoxal 5′-phosphate is a potent inhibitor of the DNA polymerase activity of recombinant rat DNA polymerase β. Kinetic studies indicate that the mechanism of PLP inhibition is complex. In a lower range of PLP concentration, inhibition is competitive with respect to substrate dNTP, whereas at higher levels of PLP several forms of enzyme combine with PLP and are involved in the overall inhibition, and a possible model for these interactions during the catalytic process is suggested. Reduction of the PLP-treated enzyme with sodium [^{3}H]borohydride results in covalent incorporation of about 4 mol of PLP/mol of enzyme, and the modified enzyme is not capable of DNA polymerase activity. The presence of dNTP during the modification reaction blocks incorporation of 1 mol of PLP/mol of enzyme, and the enzyme so modified is almost fully active. This protective effect is not observed in the absence of template-primer. Tryptic peptide mapping of the PLP-modified enzyme reveals four major sites of modification. Of these four sites, only one is protected by dNTP from pyridoxylation. Sequence analysis of the tryptic peptide corresponding to the protected site reveals that it spans residues 68–80 in the amino acid sequence of the enzyme, with Lys 71 as the site of pyridoxylation. These results indicate that Lys 71 is at or near the binding pocket for the dNTP substrate.

All known DNA polymerases have a common catalytic mechanism and exhibit an absolute dependence on the template (with the exception of terminal deoxynucleotidyltransferase) for DNA synthesis (Kornberg, 1980). While template-dependent substrate selection is a crucial step in error-free DNA replication or repair processes, the mechanism of base selection and the structural components of the enzyme protein involved in this process have not been clarified. To gain insight into structural components involved in the recognition and binding of individual reaction components of the polymerase reaction, we have used site-specific reagents that are capable of producing covalent linkage at the site of their reaction on the enzyme protein. Definition of that site, in turn, reveals the active-site domain, as well as an important amino acid residue(s) in that domain which is essential for catalysis. Thus, we found that pyridoxal 5′-phosphate (PLP), under appropriate conditions, is a reagent with specificity for the substrate deoxynucleoside triphosphate (dNTP) binding site in many DNA polymerases (Modak, 1976; Modak & Dumaswala, 1981). For example, lysine residues invoved in the substrate binding function of *Escherichia coli* DNA polymerase I (Basu & Modak, 1987), murine leukemia virus reverse transcriptase (Basu et al., 1988), and human immunodeficiency virus reverse transcriptase (Basu et al., 1989) have been identified via their covalent modification with PLP followed by peptide mapping and amino acid sequencing. We have now extended this analysis to mammalian DNA polymerase β as a representative of mammalian DNA polymerases, since the primary amino acid sequence of this enzyme has been deduced from the nucleotide sequence of a cDNA (Zmudzka et al., 1986). The successful subcloning of the coding sequence in an expression vector has been accomplished (Abbotts et al., 1988a), and this has made available sufficient quantities of enzyme protein for detailed structural studies. Furthermore, the relatively small size (M_r = 40 000) and simple structure in the form of a single polypeptide chain have made this enzyme most attractive for structure function studies. In this paper, we describe the mechanism of PLP-mediated inactivation of β-polymerase and report the identification of a PLP-reactive lysine residue that appears to be in the substrate binding pocket of the enzyme.

Materials and Methods

[^{3}H]dTTP was from New England Nuclear. Tritiated $NaBH_4$ was from ICN. To prepare the template-primer complex, poly(dA) and $d(T)_{14}$ were mixed in a weight ratio of 2:1 in 10 mM KCl. Poly(rA)·$(dT)_{12-18}$ was obtained from P-L Biochemicals. The template-primers were heated in boiling water for 3 min and were then allowed to cool to room

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temperature spontaneously. The annealed template-primer could be stored at −20 °C. Sources of other materials have been described (Detera et al., 1981). Pyridoxal, pyridoxamine, pyridoxal 5′-phosphate, and trifluoroacetic acid were from Sigma Chemical Co. Acetonitrile and HPLC grade water were products of Fisher Scientific Co.

Homogenous recombinant rat β-polymerase was purified from *E. coli* essentially as described by Abbotts et al. (1988a). A rat polymerase expression vector and near-complete amino acid sequencing of the purified protein will be described elsewhere (Wilson, et al., unpublished results). The first residue in the open reading frame of the enzyme, Met 1, is not present in the purified enzyme. Rather, the NH_2-terminal sequence is Ser-Lys-Arg-Lys-. Ser is therefore considered residue 1 of the purified 334 amino acid recombinant protein.

SKRKAPQETLNGGITDMLVELANFEKNVSQAIHKYNAYRKAASVIAKYPHKIKSGAEAK
KLPGVGTKIAEKIDEFLATGKLRKLEKIRQDDTSSSINFLTRVTGIGPSAARKLVDEGIK
TLEDLRKNEDKLNHHQRIGLKYFEDFEKRIPREEMLQMQDIVLNEVKKLDPEYIATVCGS
FRRGAESSGDMDVLLTHPNFTSESSKQPKLLHRVVEQLQKVRFITDTLSKGETKFMGVCQ
LPSENDENEYPHRRIDIRLIPKDQYYCGVLYFTGSDIFNKNMRAHALEKGFTINEYTIRP
LGVTGVAGEPLPVDSEQDIFDYIQWRYREPKDRSE

To introduce the purified enzyme into reaction mixtures, an aliquot of the stored enzyme solution at 0.51 mg/mL protein was diluted 100-fold in the reaction mixture buffer containing 500 mM NaCl and 50% glycerol, and 2 μL of this solution was added last to individual reaction mixture.

Pol Assay. Standard reaction mixtures in a final volume of 40 μL containing 20 mM Tris-HCl, pH 8.2, 10 mM NaCl, 0.2 mM $MnCl_2$, and 6 nM enzyme, plus template-primer and dNTP were incubated for 10 min at 37 °C. [^{3}H]dTTP was 11 μM, poly(dA) was 32 μg/mL, and $d(T)_{14}$ was 16 μg/mL. Poly(rA)·$(dT)_{12-18}$, when used, was present at 1 μg per assay. Reactions were terminated by the addition of a solution containing 10% TCA, and [^{3}H]DNA products were collected on nitrocellulose filters and washed with 10% TCA; radioactivity was measured as described (Schrier & Wilson, 1976). The specific activity of [^{3}H]dNTP was 2500 dpm/pmol. Strict linearity of dNMP incorporation with time of incubation was observed, and the rate of incorporation was measured in a range of proportionality with the amount of enzyme. Data points shown are the average from two or more determinations. The velocity of polymerization is expresssed as picomoles of dNMP incorporated per reaction.

Inactivation of β-Pol with Pyridoxal Phosphate and Stoichiometry of Binding. β-Polymerase (adjusted to 200 μg/mL) was incubated in the dark in a reaction mixture containing 50 mM Hepes, pH 7.8, 20% glycerol (v/v), 1 mM $MnCl_2$, 10 mM NaCl, and the desired amounts of PLP. After 20 min at 37 °C, the reaction mixture was immediately cooled to 0 °C, and a freshly prepared chilled solution of either unlabeled $NaBH_4$ or tritiated $NaBH_4$ (sp act. = 2 Ci/mmol) in 5 mM NaOH was added to give a final concentration of 10 mM. After all pyridoxal phosphate was neutralized, as judged by the disappearance of the yellow color, the reaction mixture was incubated for an additional 15 min on ice. To determine DNA polymerase activity, a small aliquot of the PLP-treated enzyme was diluted in a solution containing 50% glycerol and used for polymerase assay. For protection experiments, desired amounts of substrate dNTPs or template-primer were added to the PLP-containing reaction mixture. For protection with substrate dNTPs in the presence of appropriate template-primer, the enzyme was preincubated with PLP and template-primer for 10 min followed by the addition of the desired dNTP. After an additional 2 min at 37 °C, the reactions were stopped by the addition of sodium borohydride. The extent of PLP incorporation into enzyme protein was determined by reducing the pyridoxylated enzyme by NaB^3H_4 as described above. A small aliquot of the labeled enzyme was removed to determine enzyme activity, and the remainder of the protein then was precipitated by the addition of 10% TCA along with 100 μg of BSA as a carrier protein. The protein was collected in a microfuge tube and washed extensively with 5% TCA and water and finally dried with ether. The precipitate was dissolved in a small volume of 8 M urea solution and counted in a liquid scintillation counter.

Protein Chemistry of PLP-Modified β-Pol. For peptide analyses, 2 nmol of the enzyme protein was modified with PLP and $NaBH_4$ as indicated above. The protein was precipitated with 10% TCA and collected by centrifugation at 10 000 rpm for 10 min. The precipitate was washed extensively with 5% cold TCA and finally with ether to remove TCA. It was then suspended in 200 μL of 50 mM ammonium bicarbonate, pH 8.0, and trypsin (TPCK treated) was added at a protein:trypsin ratio of 50:1. The mixture was incubated at 37 °C. After 2 h, another aliquot of trypsin (50:1 protein:trypsin ratio) was added, and incubation was allowed to proceed overnight. After tryptic digestion, peptides were either injected indirectly onto a Vydac C-18 reverse-phase column or stored frozen after acidification (pH 2) with trifluoroacetic acid (TFA). The peptides were resolved on a Vydac C-18 column (0.45 cm × 25 cm, 5-μm particulate size, 300-Å pore size) that had been preequilibrated with 0.1% TFA (solvent A). Elution of peptides was effected by linearly increasing the concentration of 70% acetonitrile containing 0.1% TFA (solution B) with the following time schedule: 0–40% solution B (0–60 min) and 40–70% solution B (60–90 min). Flow rate was maintained at 0.7 mL/min. All the HPLC analyses were carried out on a Varian Vista 5500 HPLC system equipped with a Polychrome 9060 diode array detector system. Peptides were monitored simultaneously at 215 and 280 nm. The peptides of interest were dried under vacuum, redissolved in 100 μL of 0.1% TFA, and further purified on a Vydac C-18 reverse-phase column equilibrated in buffer A.

Amino Acid and Peptide Sequence Analyses. Purified peptide was hydrolyzed in 6 N HCl containing 0.2% phenol for 16 h at 115 °C in a Waters picotag work station. The resulting amino acids were converted to their PTC derivative and analyzed on Waters Nova-Pak columns (two 0.45 × 15 cm columns attached in series) by the method described by Stone and Williams (1986). Amino acid sequencing of the purified peptide was carried out on an Applied Biosystems 470A gas-phase sequenator at the protein chemistry facility at Yale University, under the supervision of Dr. K. R. Williams.

Results

PLP Inhibition of DNA Synthesis. PLP is an inhibitor of the initial rate of DNA polymerization by purified β-polymerase. Inhibition is not observed with two PLP analogues lacking the phosphate group, pyridoxal and pyridoxamine at 0.3 mM, or with phosphate alone at 1 mM (data not shown). Inhibition was also observed with an alternate template-primer system, poly(rA)·oligo(dT). We found that the inhibition by PLP was not due to simple chelation of Mn^{2+} by PLP, as revealed through study of Mn^{2+} concentration curves between 0 and 2 mM (data not shown).

Mechanism of PLP Inhibition. The PLP inhibition curve obtained with the polyd(A)·oligo(dT) template-primer system revealed that the enzyme activity could be inhibited completely, but the shape of the curve indicated that the mechanism of inhibition is complex. Analysis of the data by a Hill plot method (Hazra et al., 1984) revealed that the inhibition has two distinct linear components with slopes of about 1 and 3,

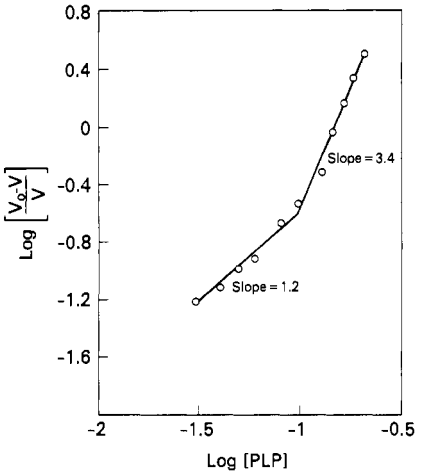


FIGURE 1: Hill plot analysis of PLP inhibition of β-polymerase activity. Experiments were conducted as described under Materials and Methods. Data are plotted according to the equation [see p 2076 of Hazra et al. (1984)].

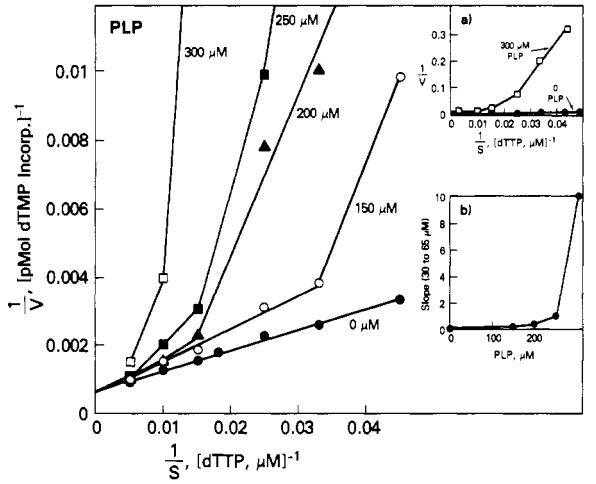


FIGURE 2: Steady-state kinetic analysis of PLP inhibition. dTTP was used as variable substrate in the presence of fixed levels of PLP, as indicated. Insert a shows results with 300 μM PLP on a much larger ordinate; insert b shows a replot of slopes as indicated versus PLP concentration.

respectively (Figure 1). Thus, inhibition with less than 80 μM PLP corresponds to a molecular order of 1 and K_i of 63 μM, whereas inhibition with more than 100 μM PLP corresponds to a molecular order of 3 and K_i (overall) of 10^{-3} M. This change of slope with increasing PLP concentration indicates that PLP combines with two or more forms of enzyme in the ordered reaction scheme (Tanabe et al., 1979); in the case of slope equal to 3, several reversibly connected forms of the enzyme combine with PLP [for discussion, see Abbotts et al. (1988b)].

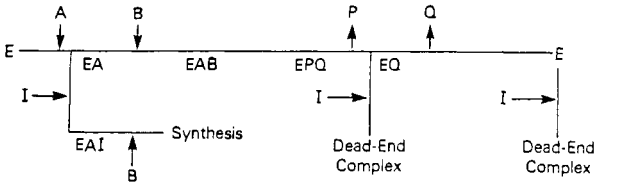
Kinetics of PLP Inhibition. The mechanism of PLP inhibition was examined further by using steady-state kinetic methods with dTTP as variable substrate (Figure 2). The double-reciprocal plot in the absence of PLP was perfectly linear, in accord with earlier results with β-pol purified from mouse tissue (Tanabe et al., 1979). In the presence of PLP, however, the plots were parabolic. These results indicate that PLP alters the normal reaction scheme, such that dTTP now combines with two or more "reversibly connected" forms of the enzyme. This parabolic effect is clearly illustrated by the curves at 250 and 300 μM PLP (Figure 2, insert a). At each concentration of PLP, the inhibition is overcome by dTTP, and at the lowest PLP level, 150 μM, most of the data points from a pattern indicative of competitive inhibition.

Table I: Effects of Substrates and Template-Primer on Inactivation of β-Polymerase by Pyridoxal 5′-Phosphate

addition[a]	pmol of TMP incorporation/15 min[b]	% activity
control (no PLP)	17.5	100
PLP (0.5 mM)	1.4	8
PLP + TTP (1 mM)	1.6	9
PLP + $(rA)_n$·$(dT)_{12-18}$ (5 μg)	2.5	14
PLP + $(rA)_n$·$(dT)_{12-18}$ (5 μg) + TTP (1 mM)	13.2	75
PLP + $(rA)_n$·$(dT)_{12-18}$ (5 μg) + dCTP or dGTP (1 mM)[b]	3.5	20

[a] Five micrograms of β-pol was incubated for 10 min at 37 °C in 25 μL of a solution containing 50 mM Hepes–KOH, pH 7.8, 10 mM NaCl, 1 mM $MnCl_2$, 1 mM DTT, 20% (v/v) glycerol, and 0.5 mM PLP. Other components were added to this reaction mixture at the indicated concentrations. [b] dNTPs were added after a 10-min preincubation with PLP followed by an additional 2-min incubation at 37 °C. All the reactions were stopped by the addition of 5 mM sodium borohydride. For the determination of DNA polymerase activity, modified enzyme was diluted in 0.1% BSA solution, and aliquots representing 20 ng of enzyme were used in the standard DNA polymerase assay (see Materials and Methods).

A replot of slopes versus PLP concentration (Figure 2, insert b) shows that the inhibition is parabolic with respect to PLP also, as expected from the Hill plot analysis in Figure 1. This curve deviates upward sharply at the highest level of PLP, 300 μM. The results, taken together, are consistent with the kinetic scheme shown here where PLP is I, template-primer is A, and dTTP is B. Products are pyrophosphate (P) and primer + I (Q), respectively. I combines with EA (competitively vs B) and with EQ, and with E·B combines with both EA (competitively vs I) and with EAI.



Covalent Modification of β-Pol and DNA Synthesis. Inactivation of *E. coli* DNA polymerase I by PLP has been shown to result from covalent modification of selective lysine residues, one of which is in the vicinity of a substrate dNTP binding site and another is in the template binding site (Basu & Modak, 1987; Basu et al., 1988). We have found that mammalian β-pol is inhibited by PLP and that some properties of the PLP inhibition are similar to those for PLP inhibition of pol I (Modak, 1976). PLP treatment of β-polymerase followed by sodium borohydride reduction, to covalently incorporate PLP into the enzyme, results in the irreversible inactivation of β-pol activity. Increasing inactivation of β-pol occurs with increasing concentration of PLP, and approximately 50% inactivation is effected by 0.2 mM PLP. To examine the specificity of PLP action, we determined the protective effect of various reaction substrates on the inactivation. As shown in Table I, addition of substrate dNTP (dTTP) provides significant protection from PLP inactivation of enzyme. However, this protective effect requires the presence of template-primer. Addition of either template-primer alone or a noncomplementary dNTP [e.g., dGTP or dCTP for template-primer poly(rA)·oligo(dT)] with template-primer failed to produce a protective effect. These results indicate that the substrate dNTP binding site is one of the targets of the PLP action.

Stoichiometry of PLP Incorporation and Protection by dNTP. Reduction of PLP-treated enzyme protein with tri-

Table II: Stoichiometry of PLP Incorporation in β-Polymerase[a]

addition	polymerase activity (%)	mol of PLP/mol of enzyme[b]
control	100	
PLP	8	4.2 ± 0.2
PLP + $(rA)_n \cdot (dT)_{12-18}$ (200 μg/mL)	12	4.0 ± 0.3
PLP + $(rA)_n \cdot (dT)_{12-18}$ (200 μg/mL) + dTTP (1 mM)	75	2.9 ± 0.2
PLP + $(rA)_n \cdot (dT)_{12-18}$ (200 μg/mL) + dGTP (1 mM)	22	3.9 ± 0.4

[a] Two nanomoles of β-pol was treated with 0.5 mM PLP as described under Materials and Methods. The enzyme–PLP complex was reduced with tritiated $NaBH_4$ (sp act. 2 Ci/mmol), and the incorporation was quantitated by acid precipitation of the complex. Enzyme activity was measured as described in the legend to Table I. One hundred percent activity represents incorporation of about 18 pmol of dTMP/15 min into $(rA)_n \cdot (dT)_{12-18}$. [b] Values represent average of three experiments.

tiated sodium borohydride results in the covalent incorporation of 4 mol of PLP/mol of enzyme with concomitant loss of nearly all enzyme activity (Table II). Inclusion of any one of the four dNTPs or addition of template-primer in the absence of dNTPs in the PLP modification mixture had little effect on the amount of PLP incorporation. However, when a combination of template-primer and complementary dNTP was added to the reaction mixture, approximately 3 mol of PLP/mol of enzyme was found to be incorporated. Thus, the presence of dNTP appeared to block the modification of a single Lys residue.

Comparative Tryptic Peptide Mapping of PLP-Labeled Enzyme. To locate PLP reactive sites and to assess the modification of an individual site in the presence of various reaction components, tryptic digests were prepared from enzyme treated with PLP in the presence of substrate dNTP, template-primer, template-primer with noncomplementary dNTP, and template-primer with complementary dNTP. Various peptides were resolved on a reverse-phase column and monitored for PLP-specific absorbance and the presence of radioactivity (resulting from tritiated borohydride reduction of PLP-treated protein prior to trypsin digestion). Among the many well-resolved peptides (Figure 3), only four peptide peaks exhibited radioactivity as well as absorbance maxima at 325 nm, characteristic of reduced Schiff base containing PLP modified Lys (Basu & Modak, 1987). Only one of these four peptides, peptide 3 (Figure 3), was found to be protected from labeling when both template-primer and complementary dNTP were present in the PLP modification mixture. The results clearly indicate that peptide peak 3 contains the PLP-reactive residue that is protected by substrate binding.

Amino Acid Composition and Sequencing of Peptide 3. The peptide fraction eluting at 59 min from the reverse-phase C-18 column (Figure 3) was further purified by rechromatography on a C-18 column, and the pyridoxylated peptide was located as described before (Basu & Modak, 1987). A portion of the peptide was hydrolyzed and subjected to amino acid composition analysis while the remainder was processed for sequence determination (see Materials and Methods). Results of composition and sequence analyses (data not shown) clearly show that peptide 3 corresponds to amino acid residues 68–80 in the primary amino acid sequence of rat β-pol (see Materials and Methods). This peptide actually represents two consecutive tryptic peptides since lysine 71 joining the two peptides could not be cleaved as a result of pyridoxylation. Absence of this lysine residue at the expected cycle (cycle 4) during amino acid sequencing indicates that lysine 71 is the target of pyridoxylation.

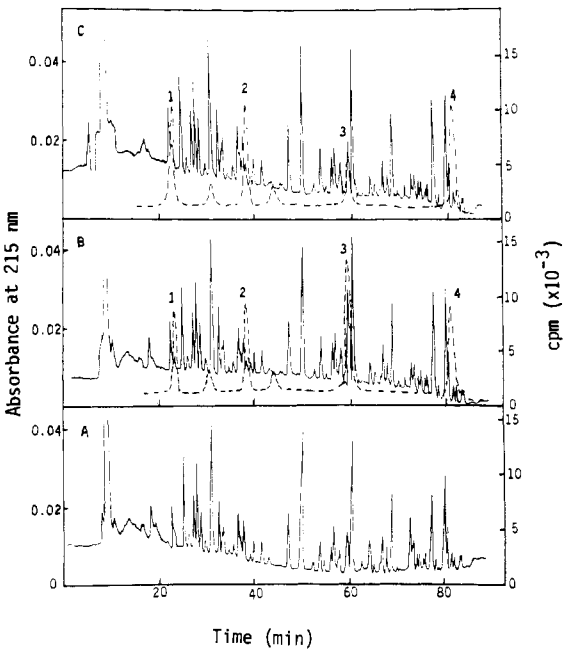


FIGURE 3: Separation of tryptic peptides of DNA polymerase β by reverse-phase HPLC. One nanomole (~40 μg) of DNA polymerase β was modified by PLP and labeled by reducing with tritiated sodium borohydride. The tryptic peptides were resolved on a Vydac C-18 reverse-phase column, equilibrated with 0.1% TFA. Peptides were eluted by increasing the concentration of 70% acetonitrile (solution B) as follows: 0–40% solution B (0–60 min); 40–70% solution B (60–90 min) at a flow rate of 0.7 mL/min. A small aliquot from each fraction was counted to determine the presence of radioactivity. The maps shown here corresponds to (A) control enzyme, (B) enzyme modified with PLP in the presence of $(rA)_n \cdot (dT)_{12-18}$, and (C) enzyme modified with PLP in the presence of $(rA)_n \cdot (dT)_{12-18}$ and TTP. Note that the tryptic peptide maps of the enzyme modified by PLP in the presence of TTP alone or in the absence of $(rA)_n \cdot (dT)_{12-18}$ were similar to that shown in (B).

Discussion

Mammalian DNA polymerase β exhibits strong sensitivity to PLP with its inhibition properties similar to those of other DNA polymerases (Modak, 1976). Our results of binding stoichiometry showed that 3–4 mol of PLP reacted per mole of enzyme (Table II). The protection from PLP-mediated modification required the presence of template-primer and complementary substrate dNTP (Table I). Thus, template-primer alone or with noncomplementary dNTP was not an effective blocker of PLP-mediated inactivation. These results together with the kinetic studies, particularly those showing a competitive mode of PLP inhibition with respect to substrate dNTP, indicated that the substrate dNTP binding site is one of the targets of PLP action.

Hill plot analysis of enzyme activity inhibition data and substrate kinetic studies indicate a complex mechanism in which multiple forms of enzyme react with PLP, yet, at all levels of PLP tested, the inhibition appeared to be fully overcome by TTP. Thus, it is likely that PLP interaction at the dNTP binding site, rather than at other PLP reactive sites, contributes to most of the overall inhibition of DNA synthesis. This interpretation is in line with the results in Table II, where enzyme labeled in the presence of dTTP and template-primer retains almost full activity, but contains 3 mol of PLP/mol of enzyme. In *E. coli* DNA polymerase I (Basu et al., 1988) as well as eukaryotic DNA polymerase α (Diffley, 1988), PLP reactivity occurs with at least two lysine residues, one involved in substrate dNTP binding and the other required in the processive mode of DNA synthesis. At present, the role of each of the four PLP-reactive sites in DNA polymerase β has

not been clarified. Yet, insight into the participation of one of the reactive sites in the inhibitory process and in dNTP binding has been indicated by the studies reported here.

The protection from PLP inactivation in the presence of template-primer and substrate dNTP was found to correlate with the protection of a single PLP-reactive site in β-pol by stoichiometric studies. The presence of template-primer or substrate dNTP alone neither protected the enzyme from PLP inactivation nor reduced the amount of PLP incorporated into β-pol. Peptide maps prepared from β-pol treated with PLP under different conditions appear to confirm the results of stoichiometry measurements. In the PLP-treated enzyme, four peptides were found to contain the sites reactive with PLP (Figure 3). The only site that could be protected by dNTP was present in peptide 3; this protection required the simultaneous presence of both template-primer and substrate dNTP. It could also be argued that the PLP-sensitive residue may be involved in the processive mode of DNA synthesis, similar to that found in *E. coli* DNA pol I (Basu et al., 1988), since in the presence of template-primer and appropriate substrate dNTP, processive synthesis may be expected to begin. However, since DNA polymerase β is known to catalyze DNA synthesis via a distributive mode (Abbotts et al., 1988a; Detera et al., 1981), i.e., it dissociates from template-primer after addition of a single nucleotide, this possibility is unlikely. Earlier studies on the mechanism of catalysis by β-polymerase have clearly indicated that kinetically significant dNTP substrate binding to the enzyme occurs only after the enzyme has formed a complex template-primer (Tanabe et al., 1979). The competitive mode of inhibition with respect to substrate dNTP in β-pol is also indicative of interference of PLP in the process of substrate binding. It therefore appears that the pyridoxylated peptide 3 in the tryptic digest of PLP-treated β-pol contains a portion of the substrate binding pocket. Amino acid composition and sequence analyses of this peptide revealed the identity of this region in the primary amino acid sequence of β-pol; this region is encompassed by residues 68–80, and lysine 71 was found to be the actual site of pyridoxylation.

Finally, it appears that cross-linking with PLP under appropriate conditions can be a valid approach for identifying amino acid residues that are in close contact with bound dNTP in the active site. Previously, by use of a large fragment of *E. coli* DNA polymerase I, where both the dNTP-specific site of PLP cross-linking and the three-dimensional structure of the protein are known, the site of PLP cross-linking has been shown to be in or near the dNTP binding pocket of the enzyme (Basu & Modak, 1987). The sequence containing the dNTP-specific PLP–Lys residue is in an α-helix that is a part of the proposed polymerase active center; the sequence of this region is shown here with Lys 758 as the PLP target, Tyr 766 as the 8-azido-dATP photoaffinity labeled residue, and Ile–Tyr 765–766 as the probable residues implicated by NMR studies (Basu & Modak, 1987; Ollis et al., 1985; Ferrin & Mildvan, 1986; Joyce et al., 1985).

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          PLP          8-azido dATP
           |               |
754 R R S A K A I N F G L I Y G M 768
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We have found that a portion of the substrate binding pocket in β-pol appears to be relatively close to the N terminus of the polypeptide chain, in a region that shows some homology to terminal deoxynucleotidyltransferase (Anderson et al., 1987; Matsukage et al., 1987). It is important to point out here that the substrate binding domain of terminal deoxynucleotidyltransferase, revealed by affinity labeling with dTTP and ATP (Pandey & Modak, 1988a, 1989), lies in the same general area. Lysine 71 of β-pol and the domain containing this residue represent at least one portion of the dNTP binding pocket. In *E. coli* DNA polymerase I, at least three distinct amino acid residues that recognize three distinct structural features of dNTP, namely, sugar, triphosphate, and base moieties, have been inferred from active-site-labeling studies (Basu & Modak, 1987; Pandey et al., 1987; Pandey & Modak, 1988b). It seems likely that DNA β-polymerase may utilize a similar mechanism for recognition of substrate and that these can be revealed through further structural and chemical modification studies.

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