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Photoaffinity Labeling of the Thymidine Triphosphate Binding Domain in *Escherichia coli* DNA Polymerase I: Identification of Histidine-881 as the Site of Cross-Linking†

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Received May 18, 1987; Revised Manuscript Received July 14, 1987

ABSTRACT: Using the technique of ultraviolet-mediated cross-linking of substrate deoxynucleoside triphosphates (dNTPs) to their acceptor site [Abraham, K. I., & Modak, M. J. (1984) *Biochemistry 23*, 1176–1182], we have labeled the Klenow fragment of *Escherichia coli* DNA polymerase I (Pol I) with [α-^{32}P]dTTP. Covalent cross-linking of [α-^{32}P]dTTP to the Klenow fragment is shown to be at the substrate binding site by the following criteria: (a) the cross-linking reaction requires dTTP in its metal chelate form; (b) dTTP is readily competed out by other dNTPs as well as by substrate binding site directed reagents; (c) labeling with dTTP occurs at a single site as judged by peptide mapping. Under optimal conditions, a modification of approximately 20% of the enzyme was achieved. Following tryptic digestion of the [α-^{32}P]dTTP-labeled Klenow fragment, reverse-phase high-performance liquid chromatography demonstrated that 80% of the radioactivity was contained within a single peptide. The amino acid composition and sequence of this peptide identified it as the peptide spanning amino acid residues 876–890 in the primary sequence of *E. coli* Pol I. Chymotrypsin and *Staphylococcus aureus* V8 protease digestion of the labeled tryptic peptide in each case yielded a single smaller fragment that was radioactive. Amino acid analysis and sequencing of these smaller peptides further narrowed the dTTP cross-linking site to within the region spanning residues 876–883. We concluded that histidine-881 is the primary attachment site for dTTP in *E. coli* DNA Pol I, since during amino acid sequencing analysis of all three radioactive peptides loss of the histidine residue at the expected cycle is observed.

The discovery, purification, and extensive characterization of *Escherichia coli* DNA polymerase I (*E. coli* Pol I)[1] by Kornberg and colleagues (Kornberg, 1969, 1982) led to the early groundwork which adequately describes the general nature of a typical DNA polymerase reaction in all life forms which contain DNA. The second important advance relates to the successful cloning of the gene for *E. coli* Pol I (Kelley et al., 1977; Murray & Kelly, 1979; Kelley & Stump, 1979) and its enzymatically active fragment (Klenow fragment) (Joyce & Grindley, 1982) which permitted the elucidation of the nucleotide sequence followed by the prediction and actual demonstration of the primary amino acid sequence (Joyce et al., 1982; Brown et al., 1982). The Pol I overproducer strain of *E. coli* essentially eliminated severe constraint on the availability of enzyme protein. The abundance of enzyme protein in turn culminated in the third important advance, namely, the elucidation of the crystal structure of this enzyme (Ollis et al., 1985). The solving of the Pol I crystal structure has opened a molecular window through which molecular aspects of polymerase and associated reactions catalyzed by this enzyme can be visualized.

The site-specific chemical modification of the enzyme followed by the identification of that site together with its functional attributes has been one of our approaches to relate the structure and function of DNA polymerase I. Thus, we have determined that a pyridoxal phosphate sensitive lysine (residue 758) is essential for substrate binding (Basu & Modak, 1987). Similarly, methionine-512 (Basu et al., 1987) and arginine-841 (Mohan et al., 1987) have been found to be important residues required in the template-primer binding function of this enzyme. Simultaneously, we also developed protocols for the affinity labeling of DNA polymerases, first using azido analogues of nucleotides (Abraham et al., 1983) and subsequently using the unsubstituted substrate dNTPs themselves as affinity labeling reagents (Modak & Giller-

† Supported by grants from the USPHS–National Institute of General Medical Sciences (NIH-NIGMS-36307 and NIH GM 31539).
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[1] Abbreviations: Pol I, *Escherichia coli* DNA polymerase I; dNTP, deoxynucleoside triphosphate; EDTA, ethylenediaminetetraacetic acid; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; HPLC, high-performance liquid chromatography; TPCK, tosylphenylalanine chloromethyl ketone; PTH, phenylthiohydantoin; DMPTU, *N,N*-dimethyl-*N*-phenylthiourea; DPTH, *N,N'*-diphenylthiourea; SDS, sodium dodecyl sulfate.

0006-2960/87/0426-7744$01.50/0

man-Cox, 1982; Abraham & Modak, 1984). We demonstrated that even though the final extent of labeling was relatively low (Abraham & Modak, 1984), all the characteristics of labeling were consistent with enzyme–substrate complex formation. Therefore, the affinity labeling approach in which the binding site is covalently blocked seemed quite attractive. In this paper, we describe the identification of a domain in *E. coli* Pol I that contains the dTTP binding site Histidine-881 has been found to be the amino acid residue in this domain that is involved in TTP binding.

Materials and Methods

α-^{32}P-Labeled deoxynucleoside triphosphates (dNTPs) were purchased from Amersham Corp. Nonradioactive dNTPs were the products of P-L Biochemicals. Chymotrypsin and TPCK–trypsin were obtained from Worthington Biochemical Corp., and *Staphylococcus aureus* V8 protease was purchased from Miles. DEAE-Sephadex A25 was from Pharmacia while polyacrylamide gel electrophoresis reagents were from Sigma Chemical Co. Klenow fragment of *E. coli* Pol I was purified from the extracts of an overproducer clone of *E. coli* (Joyce & Grindley, 1982) generously donated by Dr. Catherine Joyce of Yale University. The enzyme preparation used in this study was homogeneous (99% purity) as judged by SDS–polyacrylamide gel electrophoresis and had a specific activity of approximately 10^4 units/mg of protein with poly[d(A-T)] as template.

Affinity Labeling of E. coli Pol I with [α-^{32}P]dTTP. UV-mediated affinity labeling of the Klenow fragment of *E. coli* Pol I with [α-^{32}P]dTTP was carried out as previously described (Abraham & Modak, 1984) with slight modification. Briefly, [α-^{32}P]dTTP was converted to its metal chelate form by mixing equimolar concentrations of either Mg^{2+} or Mn^{2+} and dTTP prior to affinity labeling. The presence of excess Mg^{2+} or Mn^{2+} was found to reduce the extent of affinity labeling. For the preparative-scale cross-linking of [α-^{32}P]dTTP to the Klenow fragment, an enzyme to substrate ratio of 1:5 was used, and the irradiation mixture containing enzyme and substrate was preincubated for 6 h on ice prior to UV exposure. Usually 50 nmol of enzyme and 250 nmol of dTTP (specific activity 1 mCi/μmol) in a final volume of 1 mL received UV exposure (1300 μW/cm^2 at 15 cm), at a distance of 6 cm for 8 min. After irradiation, the solution was transferred to a dialysis bag and then electrodialyzed to remove free dTTP. An aliquot of labeled protein was subjected to SDS–polyacrylamide gel electrophoresis to determine the extent of labeling.

Tryptic Digestion of [α-^{32}P]dTTP-Labeled Pol I Protein. The lyophilized [^{32}P]dTTP-labeled Pol I protein was dissolved in 200 μL of 8 M urea in 50 mM Tris-HCl, pH 8.0, and then diluted to 2 M urea in the same buffer. Trypsin digestion was carried out at a protein:enzyme ratio of 50:1 (w/w), and the mixture was incubated at 37 °C. After 2 h, a second aliquot of trypsin (50:1 protein:enzyme ratio) was added, and the incubation was continued for 24 h. Digestion was stopped by quick freezing at −85 °C.

Chymotryptic and S. aureus V8 Protease Digestion of [α-^{32}P]dTTP-Labeled Tryptic Peptide. Further digestion of the purified tryptic peptide that was covalently attached to [α-^{32}P]dTTP was achieved with either *S. aureus* V8 protease or chymotrypsin. *S. aureus* V8 protease digestion was carried out in 0.5 M sodium phosphate/2 mM EDTA, pH 6.8, at 30 °C for 18 h. The ratio of peptide to enzyme was 30:1 (w/w). Under the conditions described here, we observed selective cleavage at the glutamyl peptide bonds. Digestion with chymotrypsin was carried out under conditions similar to those used in V8 protease except that EDTA was omitted. The digestion of *S. aureus* V8 protease or chymotrypsin was stopped by addition of guanidine hydrochloride to a final concentration of 4 M, and digests were loaded directly onto a C_4 reverse-phase column for purification of the resulting peptides.

HPLC Purification of Tryptic Peptides. Initial resolution of tryptic peptides was obtained by applying the tryptic digest to a Vydac C-18 reverse-phase column which was preequilibrated in 20 mM sodium phosphate buffer, pH 6.8. Peptides were eluted by increasing the acetonitrile concentration (see Figure 2), and 1-mL fractions were collected. Each fraction was monitored for absorbance at 215 nm as well as 280 nm. The radioactivity in each fraction was determined by Cerenkov counting. Fractions containing the radioactive peptides were pooled and further purified through DEAE-Sephadex and hydrophobic chromatography on C_4 matrix. We find that an intermediate purification step between the C-18 and C_4 columns, in the form of DEAE-Sephadex chromatography, consistently provides excellent purification of radioactive peptide. Essentially the radioactive fraction from the C-18 column is concentrated by adsorption onto a small column of DEAE-Sephadex A25, and the peptide containing the majority of the radioactivity is then eluted by increasing the salt concentration. After lyophilization, the desired fractions are dissolved in 20 mM sodium phosphate buffer containing 4 M guanidine hydrochloride and then directly loaded onto the C_4 column. It was then eluted with a linear acetonitrile gradient (see Figure 3). All the HPLC analyses were done with a Varian Vista 5500 liquid chromatography system equipped with a Polychrome 9060 diode array detector.

Amino Acid Composition and Sequence Analysis. Amino acid analysis was performed as previously described (Stone & Williams, 1986). Sequence analysis of peptides was performed, by using an Applied Biosystems 470 A gas-phase sequenator. The 03RPTH program supplied with this instrument was used without modification, and the resulting phenylthiohydantoin derivatives were analyzed by using an on-line Applied Biosystems Model 470A microbore HPLC.

Results

Comparison of UV-Mediated Cross-Linking of Various dNTPs to Klenow Fragment. With *E. coli* DNA polymerase I, we have shown that all four dNTPs could be cross-linked to the enzyme; however, the extent of cross-linking of individual dNTPs varied significantly (Abraham & Modak, 1984). The results shown in Figure 1, using Klenow fragment of Pol I, confirmed the earlier results which clearly indicate that among the four dNTPs, dTTP has the highest cross-linking efficiency. Cross-linking of dATP, dGTP, and dCTP amounted to only about 1–20% of that observed with dTTP. Similar types of cross-linking efficiencies were observed when laser energy was used for the cross-linking reactions (Hockensmith et al., 1986). The effect of addition of other dNTPs and pyridoxal phosphate on the extent of dTTP cross-linking to enzyme is presented in Table I. It is clear from the results that all four dNTPs, but not dNMPs, compete for binding to enzyme as judged by a similar degree of reduction in the incorporation of [^{32}P]dTTP into enzyme protein. Blockage of [^{32}P]dTTP cross-linking in the presence of PP_i and pyridoxal phosphate, a substrate binding site directed reagent (Modak, 1976), further confirms the fact that the cross-linking of nucleotide requires substrate binding at the appropriate site. We have, therefore, used [^{32}P]dTTP as a labeling reagent which is expected to represent binding of all four dNTPs.

Conditions for Optimal Cross-Linking of [^{32}P]dTTP to Klenow Fragment. In order to determine the conditions for

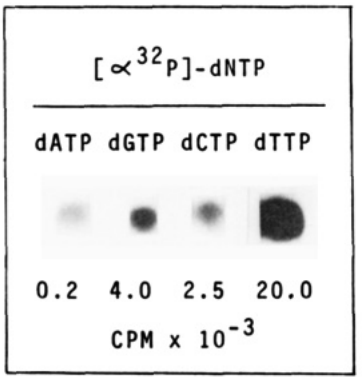

FIGURE 1: Extent of cross-linking of various [α-^{32}P]dNTPs to *E. coli* Pol I protein. A standard irradiation mixture in a final volume of 50 μL contained 20 μM [α-^{32}P]dNTP (specific activity ~1 Ci/mmol) and 2 μM *E. coli* Pol I (Klenow fragment). The mixture was subjected to UV irradiation as described under Materials and Methods, and the extent of covalent attachment of each [α-^{32}P]dNTP was assayed by SDS–polyacrylamide gel electrophoresis. Labeled protein was localized by autoradiography of the gel followed by excision of the labeled protein from the gel and determination of the radioactivity by Cerenkov counting (Abraham & Modak, 1984).

the maximal labeling of the Klenow fragment with dTTP, the extent of dTTP cross-linking to enzyme protein was determined as a function of irradiation time and substrate and divalent cation concentrations. The cross-linking of dTTP to enzyme increased linearly with time for about 12 min. Inactivation of enzyme during this time also increased linearly and amounted to about 25% (data not shown). The optimal dTTP concentration was found to be a 5 molar excess of that of input enzyme protein, and the optimal Mn^{2+} and Mg^{2+} concentrations were equimolar to that of dTTP. Under these conditions, approximately 20% of the enzyme can be modified (data not shown).

Preparative-Scale Cross-Linking of dTTP to Klenow Fragment and Tryptic Digestion. In order to determine the site of dTTP cross-linking to the enzyme, we resorted to HPLC tryptic peptide mapping. For this purpose, 50 nmol of enzyme and 250 nmol of dTTP (containing 250 μCi of ^{32}P) were cross-linked in an appropriate reaction mixture for 8 min. Since the covalent nucleotide–DNA polymerase complex is not stable under acid conditions, the cross-linked enzyme was not precipitated with acid. An extensive electrodialysis of cross-linked enzyme or SDS–polyacrylamide gel electrophoresis provides enzyme which is free from noncovalently bound dTTP. The cross-linked protein is fully digested (in the presence of 2 M urea) by trypsin as judged by the identical tryptic peptide patterns obtained on dTTP-cross-linked and control enzyme (data not shown). A typical tryptic peptide

Table I: Effect of Various dNMPs, dNTPs, and Pyridoxal Phosphate on the Cross-Linking of [α-^{32}P]dTTP to *E. coli* Pol I (Klenow Fragment)[a]

additions (40 μM)	pmol of [α-^{32}P]dTTP cross-linked	% inhibition
none	19.5	0
dATP	6.2	68
dGTP	6.4	68
dCTP	3.6	81
pyridoxal phosphate	9.4	51
PP_i	12.6	35
dTMP	20.0	0
dAMP	20.2	0
dGMP	20.2	0
dCMP	20.4	0

[a] 0.1 nmol of *E. coli* Pol I (Klenow fragment) was incubated with 0.5 nmol of [^{32}P]dTTP (specific activity 1 Ci/mmol) in the absence or presence of indicated dNTP, dNMP, PP_i, or pyridoxal phosphate in their Mg chelate form. The reaction mixture (50 μL) was UV irradiated, and the extent of cross-linking of [α-^{32}P]dTTP was determined as described under Materials and Methods.

pattern of 2 nmol of dTTP-cross-linked Klenow fragment is shown in Figure 2. Among the many well-resolved peptide peaks, a peak eluting at 79 min from the C_{18} column appears to contain the majority of radioactivity. Nearly 90% of the radioactivity applied to the C_{18} column was recovered, 80% of which was contained in the fractions near 79 min of the gradient. This peak was, therefore, considered to represent peptide(s) which contained a major site of dTTP cross-linking. Further purification of this peptide was followed by monitoring the coincidence of both optical density and radioactivity.

Purification of [^{32}P]dTTP-Cross-Linked Peptide. In order to purify ^{32}P-labeled peptide so that it would be suitable for amino acid analysis and sequencing, an anion-exchange step was found to be extremely useful. The fact that cross-linked nucleotide introduced additional negative charges in the peptide made that peptide bind unusually strongly to the DEAE-Sephadex. The ^{32}P-labeled peptide was retained on the column throughout the 0.2 M salt wash. When the DEAE-Sephadex-eluted peptide fraction was further resolved on a C_4 reverse-phase column, two distinct peaks were found that eluted at 79 and 81 min and which contained 80% and 10% of the total input radioactivity, respectively (Figure 3). The amino acid composition (composition data not shown) and sequence analysis of both radioactive peptides corresponded to residues 876–890 in the primary sequence of *E. coli* Pol I (see Figure 3 inset). The second radioactive peak may result from degradation of covalently attached dTTP to either dTDP or dTMP. Conspicuously missing in both analyses was histidine residue 881. On the basis of the specific activity of [α-^{32}P]-

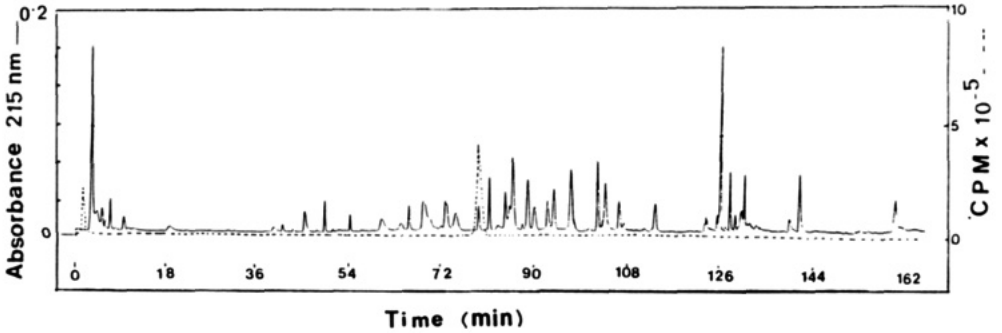

FIGURE 2: Resolution of tryptic peptides of *E. coli* Pol I (Klenow fragment) cross-linked to [α-^{32}P]dTTP. Tryptic peptides, derived from 2 nmol of [^{32}P]dTTP-labeled Pol I protein, were injected directly onto a Vydac C_{18} column (0.46 cm × 25 cm) that was equilibrated with 20 mM sodium phosphate buffer, pH 6.8 (solvent A), at a flow rate of 1 mL/min. The peptides were then eluted by increasing the concentration of solvent B (70% CH_3CN) as follows: 0–10 min (0% B), 10–115 min (35% B), 115–150 min (70% B), 150–160 min (100% B). One-milliliter fractions were collected and counted directly for radioactivity by Cerenkov counting.

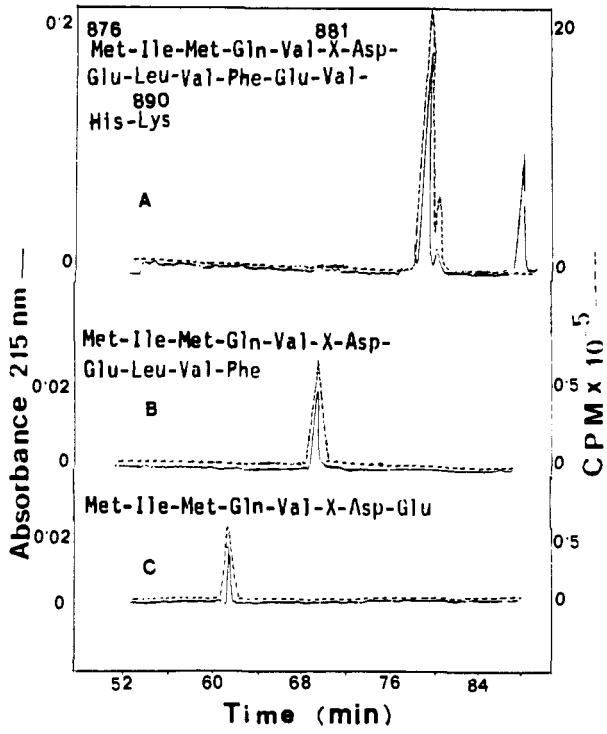

FIGURE 3: Purification of [^{32}P]dTTP-labeled tryptic peptide. Radioactive peptide material obtained from the preparative C_{18} HPLC column was concentrated on a small DEAE-Sephadex column. It was then purified to homogeneity using a Vydac C_4 reverse-phase column. The flow rate was 1 mL/min. The profile was developed with a linear gradient of solvent B (70% CH_3CN) into solvent A (20 mM sodium phosphate buffer, pH 6.8) as follows: 0–5 min (0% B), 5–15 min (5% B), 15–130 min (30% B). Fractions were collected every minute and counted directly for Cerenkov cpm. Each radioactive peptide peak was subjected to amino acid sequence analysis. The sequence is shown in the inset. (A) Purification of ^{32}P-labeled tryptic peptides. Note that the amino acid sequence of both radioactive peptides is identical. (B and C) Purification of secondary proteolytic digestion products of a portion of [α-^{32}P]dTTP-labeled tryptic peptide: chymotryptic digest (B); *S. aureus* V8 protease digest (C).

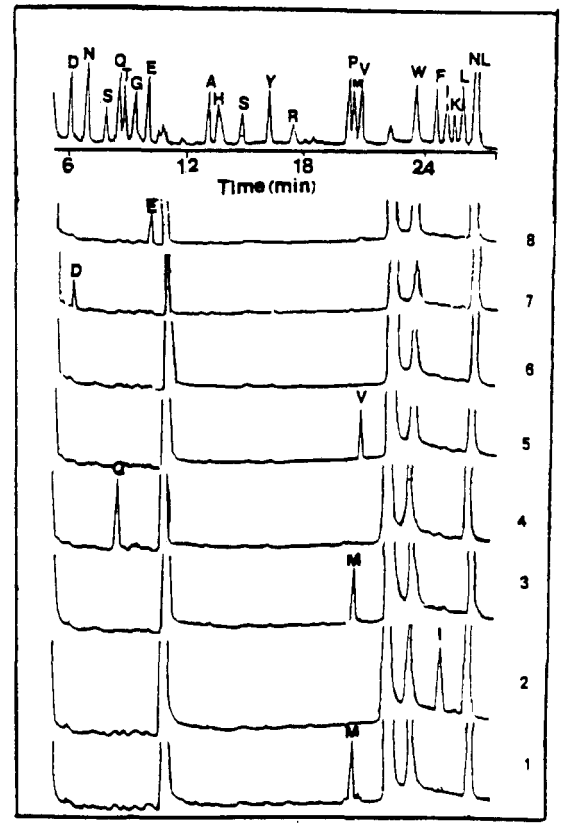

FIGURE 4: HPLC tracing of phenylthiohydantoin derivatives from cycles 1–8 of the gas-phase sequencing of the tryptic peptide fragment that remained after it had been digested with *S. aureus* V8 protease and then repurified. An internal standard of PTH–norleucine (100 pmol) was added to each sequenator cycle. The PTH derivatives produced at each cycle were automatically chromatographed on an Applied Biosystems Model 120 "on-line" HPLC. The tracing at the top represents 10 pmol of a standard mixture of PTH-amino acids. The sequence is indicated by the one-letter amino acid symbol adjacent to the corresponding peak. The peaks eluting at 10.6 and 22.6 min correspond to DMPTH and DPTH, respectively.

dTTP in the photolabeling reaction, and the recovery of peptide in both peaks, we estimate a value of 0.9 mol of dTTP cross-linked/mol of peptide.

Chymotrypsin and V8 Protease Digestion of ^{32}P-Labeled Tryptic Peptide and Amino Acid Sequence of Radioactive Peptide. In order to absolutely confirm that the ^{32}P radioactivity in dTTP indeed covalently linked to the tryptic peptide, we subjected the above tryptic peptide to secondary digestion with chymotrypsin and V8 protease. Individual digests were separated on a C_4 column (Figure 3B,C). The change in the elution pattern of the chymotryptic and V8-cleaved peptides from that of the original tryptic peptide, together with a corresponding change in the radioactivity peaks, confirmed that the new peaks were indeed derived from the original labeled tryptic peptide. Amino acid sequence analysis of both the chymotryptic and V8 peptides (Figure 4 and Figure 3B,C inset) confirmed the original sequence of the tryptic peptide and narrowed the site of cross-linking to a single histidine residue at position 881. The identification of histidine-881 as the site of cross-linking is based on the observation that this residue does not appear at its appropriate place (sequencing cycle) during the sequencing of tryptic, chymotryptic, and V8 protease generated peptides.

Discussion

Photoaffinity labeling with azido analogues of nucleotides as well as affinity labeling mediated via UV energy using unsubstituted nucleotides in DNA polymerases has been qualitatively shown to represent the process of substrate binding in DNA polymerases (Abraham & Modak, 1984). The application of these protocols in the identification and isolation of reactive sites on the enzyme has been difficult, mainly due to poor efficiency of labeling and instability of the labeled nucleotide–protein linkage to acid pH. By optimizing conditions for the cross-linking of [α-^{32}P]dTTP to Klenow fragment, we have been able to achieve modification of nearly 20% of enzyme molecules as judged by the incorporation of radioactivity into the enzyme protein. This fact, together with the changes in the protein chemistry protocols, which avoid use of acidic media, has allowed us to label the enzyme and identify the site of labeling. As detailed under Results, the site of dTTP cross-linking appears to be histidine-881 in the primary sequence of Pol I. This conclusion is derived from the observation that the majority of radioactivity remained associated with a single peptide, eluting at 79 min in the initial reverse-phase HPLC run on a C_{18} support. Adsorption of the labeled peptide on DEAE-Sephadex provided a decisive purification step since the DEAE-bound peptide yielded a homogeneous peak of radioactivity that was coincident with a peptide peak when resolved on a C_4 HPLC column (Figure 3). In fact, when a control enzyme digest was similarly processed, there was a total absence of peptide peak in that area (data not shown), indicating that the presence of cross-linked nucleotide was responsible for the adsorption onto the DEAE matrix. The association of radioactive [^{32}P]dTTP with peptide was further confirmed by treatment of this peptide with

chymotrypsin and V8 protease. The latter treatments resulted in a change in the elution profile of labeled tryptic peptide (Figure 3). Amino acid sequence analysis of the original tryptic peptide, as well as those derived from chymotryptic and V8 digestion of this peptide, revealed that the tryptic peptide containing radioactivity corresponded to amino acid residues 876–890. During the amino acid sequencing of these peptides, the absence of histidine-881 in the appropriate cycle was consistently noted. We therefore conclude that histidine is the site of dTTP cross-linking and that nucleotide-cross-linked histidine does not elute at its normal position.[2]

Histidine-881 is located at the lower part of B sheet number 12 in the structure of Klenow predicted by Steitz and associates (Ollis et al., 1985). This residue thus lies almost in the center of the active-site cleft proposed in the model. Earlier, our studies with pyridoxal phosphate modification have shown that Lys-758, which resides on helix O, is required for the binding of the metal chelate form of dNTP (Basu & Modak, 1987). The lysine residue most likely provides a binding site for the metal–triphosphate moiety of dNTPs. Using 8-azido-dATP as a labeling analogue, Joyce et al. (1985) find tyrosine-766 to be the reactive site on the same helix. In *E. coli* DNA polymerase I, all four dNTPs bind to a single site as determined by kinetic studies (Englund et al., 1969), pyridoxal phosphate sensitivity (Modak, 1976), and NMR measurements (Ferrin & Mildvan, 1985). Our affinity labeling studies also confirm this fact since all four dNTPs compete with each other for binding to enzyme (Abraham & Modak, 1984; Table I). Thus, our present studies using dTTP as a labeling reagent suggest that His-881 is possibly involved in the binding of all four dNTPs. However, similar studies with other dNTPs would be necessary to ascertain this conclusion. It appears that the pyrimidine ring moiety of the purine and pyrimidine nucleotides may bind to the enzyme at histidine-881 which may provide a common contact point. The suggestion of Tyr-766 involvement, observed when 8-azido-dATP is used as the cross-linking agent, may be explained by the reactivity of the 8-azido group which is clearly away from the pyrimidine moiety of dATP. Alternatively, the ring structure of nucleotide which is involved in the base paring of individual dNTPs may exhibit distinct contact points for their binding to enzyme, with lysine-758 being the common anchoring point for the metal–triphosphate moiety. Additional studies would be needed to clarify this point. Our present studies have been performed in the absence of template-primer, and it is not known what effect, if any, the template-primer would exert on dNTP cross-linking specificity. Nevertheless, the present studies together with identification of other important residues in this enzyme, e.g., Arg-841 in template-primer binding function (Mohan et al., 1987), are progressing toward clarification of the overall mechanism of template-dependent DNA synthesis.

[2] Although a dTTP–histidine complex has not been directly identified, amino acid sequencing of the cross-linked peptide strongly suggests that histidine-881 represents the site of cross-linking. No PTH derivative of histidine was observed at this position during amino acid sequencing either of the original cross-linked tryptic peptide or of its chrymotryptic or staphylococcal protease cleavage products. Since acid treatment of this peptide results in loss of radioactivity (data not shown), we presume that the trifluoroacetic acid cleavage step that occurs during amino acid sequencing breaks the covalent [α-^{32}P]dTTP–histidine linkage. Rather than generating free histidine, histidine derivative(s) must be produced whose PTH derivative no longer elutes from the reverse-phase support as PTH–histidine. Even if the [α-^{32}P]dTTP–histidine linkage were acid stable, it would be too polar to be extracted as anilinothiazolinone with chlorobutane used in the Applied Biosystems sequencer. Hence, a blank cycle would be expected.

Registry No. Pol I, 9012-90-2; $MgPP_i$, 20768-12-1; MgdTTP, 72781-90-9; MgdATP, 74386-14-4; MgdGTP, 89705-26-0; MgdCTP, 79295-52-6; L-His, 71-00-1; magnesium pyridoxal phosphate, 70061-30-2.

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