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Isolation of Multiple Forms of DNA Polymerase δ: Evidence of Proteolytic Modification during Isolation†

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ABSTRACT: The subunit structures of a number of human placenta DNA polymerase δ preparations were investigated by Western blotting with polyclonal antisera and by activity staining following polyacrylamide gel electrophoresis. When immunoblots and activity stains were performed on different enzyme preparations, putative catalytic subunits of (a) 170, (b) 120, or (c) 50–70 kilodaltons (kDa) were observed. It was also observed that the lower molecular weight forms could be generated upon storage of the preparations. Western blotting of human placental tissue extracts showed that the major immunoreactive polypeptide was 160–170 kDa. Treatment of the extracts with trypsin or *Staphylococcus aureus* V8 protease led to the generation of immunoreactive polypeptides of lower molecular weights. These studies suggest that the 120-kDa and lower forms of the enzyme are generated via uncontrolled proteolysis and provide a rationale for the observation of different apparent subunit structures previously reported for DNA polymerase δ. In addition, these findings suggest that DNA polymerase δ has a catalytic domain which resides in a protease-resistant domain.

DNA polymerase δ is a high molecular weight DNA polymerase which has been described in rabbit reticulocytes (Byrnes, 1984), calf thymus (Lee et al., 1981, 1984; Crute et al., 1986), and human placenta (Lee & Toomey, 1987). DNA polymerase δ is of particular interest since it differs from DNA polymerase α in that it is associated with a 3′ to 5′ exonuclease activity which is not removed by any conventional fractionation methods, and therefore appears to be intrinsic to the enzyme. We have reported studies of a highly purified DNA polymerase δ preparation from human placenta which possessed a 170-kilodalton (kDa) polypeptide, with smaller polypeptides in the 60–70-kDa range; in this study, murine antisera were shown

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to Western blot the 170-kDa polypeptide which was shown to be the catalytic polypeptide by activity staining (Lee & Toomey, 1987). In previous studies, various compositions have been reported for the subunit structure of DNA polymerase δ from different sources, with polypeptides ranging from 45 to 245 kDa (Byrnes et al., 1984; Lee et al., 1981, 1984; Crute et al., 1986). In this report, we describe further studies using murine antisera to DNA polymerase δ to examine the behavior of the enzyme in purified enzyme preparations and in tissue extracts by immunoblotting. Activity staining was also used in parallel with immunoblotting in order to monitor the subunit structure of several human placental DNA polymerase δ preparations. These studies show that the 170-kDa polypeptide of DNA polymerase δ is highly susceptible to proteolysis.

Materials and Methods

Materials. Prestained protein standards for sodium dodecyl sulfate–polyacrylamide gel electrophoresis were obtained from BRL. ^{14}C-Methylated protein standards for autoradiography following sodium dodecyl sulfate–polyacrylamide gel electrophoresis were obtained from New England Nuclear. Biotinylated sheep anti-mouse IgG and preformed streptavidin–biotinylated peroxidase complex were obtained from Amersham Co.

Purification of DNA Polymerase δ. DNA polymerase δ was purified by the procedure described by Lee and Toomey (1987) from human placental tissue. Several preparations were examined in the study described in this work.

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis. This was performed by the procedure of Laemmli (1970) in slab gels with a gradient of 5–15% acrylamide. Gels were stained by the silver staining procedure (Merril et al., 1981).

Activity Staining for DNA Polymerase after Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis and Renaturation. This was performed as described by Spanos et al. (1981). Samples were prepared as described by Blank et al. (1983) by heating for 3 min at 37 °C. These were then electrophoresed in a 5–15% sodium dodecyl sulfate–polyacrylamide gel containing 6 A_{260} OD U of poly(dA-dT) and 0.75 mg of fibrinogen per 15 mL of acrylamide. After electrophoresis, the gels were incubated in a series of renaturing buffers [100 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), 5 mM 2-mercaptoethanol, and 20% 2-propanol, pH 7.5 for 30 min; 50 mM Tris-HCl and 10 mM 2-mercaptoethanol, pH 7.5 for 30 min; and finally 50 mM Tris-HCl, 10 mM 2-mercaptoethanol, and 1 mM ethylenediaminetetraacetic acid (EDTA), pH 7.5 for 16–18 h]. The gels were then incubated at 37 °C for 18 h in an assay mixture for polymerase activity (40 mM Tris-HCl, pH 7.4, 0.8 mM $MgCl_2$, 1 mM mercaptoethanol, and 20 μM [α-^{32}P]dTTP containing 180 μCi in a total volume of 45 mL). The gels were washed in 5% trichloroacetic acid containing 20 mM sodium pyrophosphate at 4 °C, with a change of wash liquid every 30 min for 4 h, and then with a final wash for 18 h. The gels were then stained for protein and dried. The dried gels were then autoradiographed at −70 °C using a Cronex "Lightning" screen and preflashed film (Kodak XAR-5). ^{14}C-Methylated proteins were used as molecular weight standards. For samples with weak activity, the gels were incubated in dimethyl sulfoxide for 30 min followed by 22% 2,5-diphenyloxazole in dimethyl sulfoxide before being dried and autoradiographed.

Preparation of Antibodies. Antisera to DNA polymerase δ were obtained by immunization of five female BALB/c mice with purified DNA polymerase δ (Lee & Toomey, 1987). Each mouse was injected intraperitoneally with ca. 200 units of enzyme emulsified with an equal volume of Freund's complete adjuvant. After 21 days, they were injected with the same amount of enzyme emulsified with Freund's incomplete adjuvant. The mice were bled from the tail veins. Positive antisera were obtained from three of the mice; these were partially purified by ammonium sulfate precipitation before use.

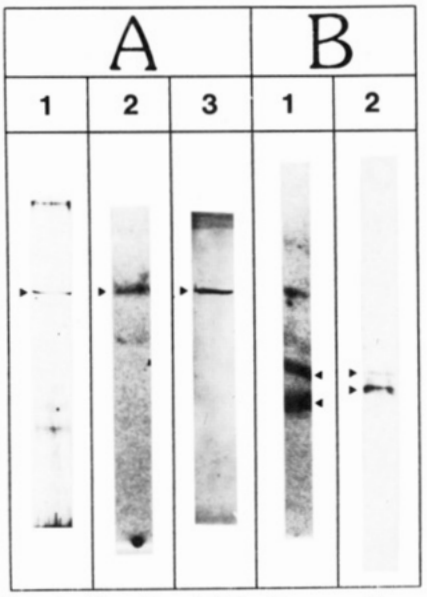

FIGURE 1: Examination of two DNA polymerase δ preparations by immunoblotting and activity staining. Two DNA polymerase δ preparations (panels A and B, respectively) were subjected to immunoblotting using mouse polyclonal antiserum and activity staining essentially as described under Materials and Methods. Gradient gels of 5–15% acrylamide were used. Prestained and ^{14}C-methylated proteins were used as molecular weight markers for the immunoblot and activity-stained gels, respectively. Panel A: lane 1, silver stain (5 units); lane 2, activity stain (8 units); lane 3, immunoblot (8 units). The arrowheads indicate an estimated molecular weight of 120K. Panel B: lane 1, activity stain (10 units; arrowheads indicate estimated molecular weights of 72K and 50K; lane 2, immunoblot (7 units, arrowheads indicate estimated molecular weights of 65K and 48K).

Immunoblotting. Immunoblotting was performed essentially as described by Towbin et al. (1979). Samples were electrophoresed in 5–15% polyacrylamide gradient gels and then transferred to nitrocellulose paper. Prestained proteins (BRL) were used as molecular weight markers and also used to provide confirmation of efficient transfer. The blots were incubated with 3% bovine serum albumin or nonfat dry milk in phosphate-buffered saline as a blocking agent. The blots were then incubated with a 1:1000 dilution of mouse antisera for 16 h and then washed with the blocking solution. The blots were visualized by incubation with biotinylated sheep anti-mouse IgG, followed by incubation with streptavidin–biotinylated peroxidase preformed complex (Yolken et al., 1983) obtained from Amersham. Color development was performed by incubation with 4-chloro-1-naphthol and hydrogen peroxide and terminated with sodium azide.

Results

In the course of the study of human placental DNA polymerase δ enzyme preparations, we obtained variable results following sodium dodecyl sulfate–polyacrylamide gel electrophoresis in regard to (a) the polypeptide composition as determined by protein staining, (b) the identity of the DNA polymerase catalytic polypeptide(s) by activity staining, and (c) the immunoreactive polypeptides which were blotted with a polyclonal antibody to a 170-kDa polymerase δ polypeptide. An example of our findings is given in Figure 1, in which two

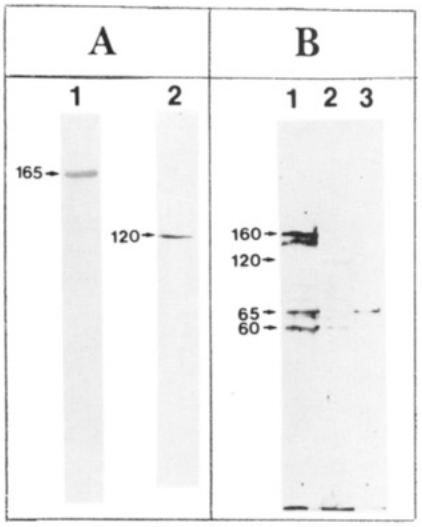

FIGURE 2: Immunoblots of several DNA polymerase δ preparations. Several DNA polymerase δ preparations were examined by immunoblotting with murine polyclonal antisera after electrophoresis on 5–15% gradient gels as described under Materials and Methods. Panel A: lane 1, immunoblot of a DNA polymerase δ preparation immediately after preparation (5 units of enzyme); lane 2, immunoblot of the same preparation (5 units) as in lane 1, after 2 weeks of storage at −70 °C. Panel B: lanes 1–3, immunoblots of three separate DNA polymerase δ preparations which had been stored for periods of several months at −70 °C (10, 4, and 3 units of enzyme, respectively).

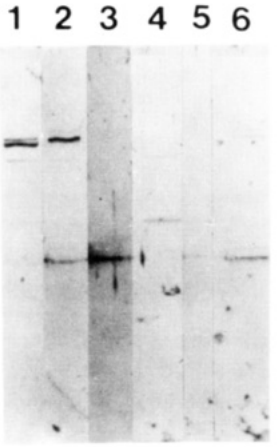

FIGURE 3: Effect of proteases on the immunoreactive polypeptides recognized by polyclonal antibodies to polymerase δ. A fresh placenta extract (20 μL of 1.2 mg of protein/mL solution) prepared as described under Materials and Methods was incubated at 22 °C for 1 h with the following additions or treatments: lane 1, boiled prior to incubation; lane 2, no additions; lane 3, subtilisin; lane 4, *S. aureus* V8 protease; lane 5, 5 mM $CaCl_2$; lane 6, trypsin. Protease additions were 10 μL of 0.1 mg of protein/mL solutions. The samples were then subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis in a 5–15% polyacrylamide gradient gel and immunoblotted as described under Materials and Methods.

human placental DNA polymerase δ preparations were examined. Panel A shows that the first preparation consists of a major polypeptide of 120 kDa on silver staining following sodium dodecyl sulfate–polyacrylamide gel electrophoresis (lane 1). Activity staining of the same preparation showed that the 120-kDa species was catalytically active for DNA polymerase activity (panel A, lane 2). Immunoblotting with polyclonal antisera to DNA polymerase δ also revealed a 120-kDa immunoreactive polypeptide (panel A, lane 3). Thus, this preparation appears to be an enzyme which consists of a 120-kDa polypeptide which possesses the catalytic activity, and which is also immunoreactive to DNA polymerase δ antisera. A second preparation which was studied is shown in panel B. This preparation when examined by activity staining was found to consist of a doublet of low molecular mass polypeptides, of 50 and 72 kDa by activity staining (lane 1) and of 48 and 65 kDa by Western blotting (lane 2).

These findings are in contrast to our evidence for a 170-kDa catalytic polypeptide for DNA polymerase δ (Lee & Toomey, 1987) and suggest the 120-kDa and smaller species may be derived by proteolysis of the 170-kDa polypeptide. We have examined this issue using the immunoblot technique. Examination of several other DNA polymerase δ enzyme preparations is shown in Figure 2. Figure 2A, lane 1, shows the immunoblot of a DNA polymerase δ preparation immediately after the final step of purification, which reveals of 165-kDa polypeptide as reported previously (Lee & Toomey, 1987). After the enzyme had been stored for 2 weeks at −70 °C, and subjected to several freeze–thaw treatments, it was now observed that immunoblotting revealed a 120-kDa polypeptide (Figure 2A, lane 2). This demonstrates that the enzyme is converted from a ca. 170-kDa to a 120-kDa immunoreactive species. In Figure 2B are shown three other preparations, which were stored for various periods over several months at −70 °C, and which had been thawed several times. The first preparation shows a 160-kDa large species, as well as prominent bands at 60 and 65 kDa (Figure 2B, lane 1). The second preparation shows major bands at 120 and 60 kDa (Figure 2B, lane 2), while in the third preparation only the 65-kDa species is present (Figure 2B, lane 3). These results again show that isolated polymerase δ presents variable polypeptide compositions, which can be attributed to proteolytic modification.

These findings bring into question the native size of the DNA polymerase δ catalytic subunit and the relationship of the various polypeptide species found in purified enzyme preparations. It is noted that these purifications were all performed in the presence of protease inhibitors. Our findings suggest that despite these precautions, sufficient protease activity of an unknown nature remains in the preparations to be a serious problem. Experiments were performed to examine the immunoreactive species recognized by the antibody in crude placental extracts and the effects of protease treatments (Figure 3). As we previously have reported, the antibody recognizes a major 165–170-kDa polypeptide in freshly prepared placental extracts in the presence of protease inhibitors (lane 1). After incubation at room temperature (lane 2), some breakdown of the polypeptide is evident; complete conversion to a ca. 58-kDa species is observed in the presence of 5 mM calcium ion (lane 5), suggesting that even in the presence of protease inhibitors not all endogenous protease activity is nullified and that endogenous calcium-dependent proteases may represent a significant source of the problem. In the presence of added subtilisin A (lane 3), a complete conversion to the 58-kDa form was observed. In the case of *Staphylococcus aureus* V8 protease (lane 4), a complete disappearance of the 170-kDa species and the appearance of ca. 120- and 80-kDa species were observed. With trypsin (lane 6), most of the 165-kDa species was converted to the 58-kDa form. In this experiment, a large excess of protease (33 μg of protein/mL) was added, as the original homogenate contained protease inhibitors; essentially the same patterns were observed when the amount of protease was increased to 500 μg/mL (not shown), except in the case of *S. aureus* V8 protease, when both the 120- and 80-kDa species were reduced to the 58-kDa species as for trypsin and subtilisin A.

The time course of the effects of added trypsin and *S. aureus* V8 protease on the pattern of immunoreactive polypeptides observed by immunoblotting was examined. These were compared to that of control homogenates which were treated

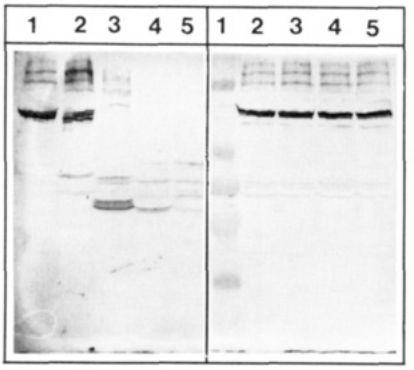

FIGURE 4: Immunoblotting of human placental homogenates before and after trypsin treatment. A freshly prepared human placental homogenate (1.2 mg of protein/mL) was incubated at 22 °C with trypsin (40 μg/mL); aliquots were taken at different times and inactivated in equal volumes (20 μL) of hot (100 °C) sodium dodecy sulfate–polyacrylamide gel electrophoresis sample buffer. The samples (40 μL) were then subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis in a 5–15% polyacrylamide gradient gel and immunoblotted as described under Materials and Methods. Left panel: lane 1, control, fresh placental homogenate boiled in sodium dodecyl sulfate–polyacrylamide gel electrophoresis sample buffer; lanes 2, 3, 4, and 5, placental homogenate incubated with trypsin for 2, 5, 10, and 30 min, respectively. Right panel: lane 1, BRL "prestained" protein standards—myosin (200 kDa), phosphorylase *b* (93 kDa), bovine serum albumin (68 kDa), ovalbumin (43 kDa), and α-chymotrypsinogen (26 kDa); lanes 2–5, control samples of placental homogenate incubated in the absence of trypsin for 2, 5, 10, and 30 min, respectively.

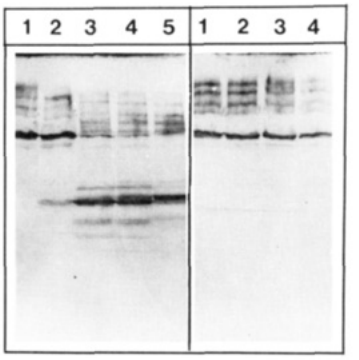

FIGURE 5: Immunoblotting of human placental homogenates before and after *S. aureus* V8 protease treatment. A human placental homogenate (1.3 mg of protein–mL) was prepared and incubated at 22 °C with *S. aureus* V8 protease (40 μg/mL); aliquots were taken at different times and inactivated in equal volumes (20 μL) of hot (100 °C) sodium dodecyl sulfate–polyacrylamide gel electrophoresis sample buffer. The samples (40 μL) were then subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis in 5–15% polyacrylamide gradient gels and immunoblotted as described under Materials and Methods. Left panel: lane 1, control fresh placental homogenate boiled in sodium dodecyl sulfate–polyacrylamide gel electrophoresis sample buffer; lanes 2, 3, 4, and 5, placental homogenate incubated with V8 protease for 5, 10, 20, and 30 min, respectively. Right panel: lanes 1–4, placental homogenate incubated in the absence of added V8 protease for 5, 10, 20, and 30 min, respectively.

in boiling sodium dodecyl sulfate buffer immediately after homogenization. In the case of trypsin (Figure 4), a major immunoreactive polypeptide of 165 kDa was found in the crude placental extract; this was followed by the appearance of a 82-kDa species after 2 min of trypsin treatment, and species of 62 and 58 kDa after 5 min of treatment. It should be noted that some staining of even larger species is apparent in the blots; this might suggest the presence of a larger precursor of polymerase δ. However, this is due to slight overstaining of the blots, as no larger bands were seen under normal staining conditions as can be seen for the blots shown in Figure 3. Similar results were obtained with *S. aureus* V8 protease; in this experiment, the major immunoreactive species in the crude placental extract was estimated as 160 kDa; this was converted to a 70-kDa species, with minor species of 80, 54, and 44 kDa (Figure 5). We have also examined the effect of a calcium-dependent protease activity partially purified from human placenta, using the procedure of Mellgren et al. (1979). Using this calcium-dependent protease, the 170-kDa species was shown to be degraded into immunoreactive species of ca. 74–45 kDa (not shown). It is noted parenthetically that DNA polymerase δ is usually purified in the presence of calcium chelators, except during hydroxylapatite chromatography, when it is conceivable that any calcium-dependent protease present could be activated. The fact that the major immunoreactive polypeptide in the whole tissue homogenate is the 170-kDa polypeptide confirms that this is the native form, and its breakdown by exogenous proteases supports the view that lower molecular weight species observed in the purified preparations can be explained by proteolysis during either preparation or storage.

Discussion

In this study, we have used activity stain and immunoblot analysis to probe the subunit structure of DNA polymerase δ. Our data support the view that the 170-kDa polypeptide represents the native form of the catalytic subunit. However, in our experience, human placental DNA polymerase δ can be isolated in forms containing polypeptides of 170 or 120 kDa or a mixture of 72–48-kDa polypeptides. The most commonly observed form was that containing the 120-kDa polypeptide. Taken together with the effects of proteolysis on the major 170-kDa immunoreactive polypeptide in placental extracts, there is therefore strong evidence that the smaller species are generated by breakdown of the 170-kDa species. Our findings also demonstrate the sensitivity of this polypeptide to proteolysis, despite the presence of a cocktail of protease inhibitors including phenylmethanesulfonyl fluoride, benzamidine, leupeptin, pepstatin, EDTA, and ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) during the preparations, and demonstrate that some endogenous protease activity, including an active calcium-dependent protease, survives this treatment. On the other hand, it is also of interest that human placental DNA polymerase δ is highly resistant to complete degradation by exogenous proteases, as demonstrated by both immunoblotting and activity staining, despite a significant size reduction of the 170-kDa polypeptide to as low as 50 kDa. These findings that DNA polymerase δ has a highly resistant active "core" on protease treatment are significant, as they suggest that the polymerase active site may reside in a specific domain of the protein which is highly resistant to proteolysis and independently capable of renaturation. Thus, it may be speculated DNA polymerase δ may have discrete functional domains including a well-defined catalytic domain which encompassses only a fraction of the total linear amino acid sequence.

Our findings also provide a rationalization of the variability of the subunit compositions reported for DNA polymerase δ enzymes. The rabbit reticulocyte enzyme has been reported to consist of a single 120-kDa polypeptide (Byrnes, 1984), while the calf thymus enzyme was observed to contain polypeptides of 60 and 49 kDa (Lee et al., 1981) and 125 and 48 kDa (Lee et al., 1984). In a recent study of the calf thymus polymerase δ, two forms were obtained, both exhibiting multiple polypeptide bands ranging from 45 to 245 kDa (Crute et al., 1986). However, it is likely that the latter preparations

may represent less purified preparations than those studied previously.

In our previous studies using the mouse antisera to polymerase δ (Lee & Toomey, 1987), it was observed that these antisera inhibited DNA polymerase δ activity and also immunoblotted a ca. 170-kDa species in the freshly prepared enzyme. Although this evidence alone gave a strong indication that the polyclonal antibody was directed toward the polymerase subunit, such evidence did not preclude the possibility, however remote, that the antibody recognized an adventitious protein of a similar size as polymerase δ. An important corollary of the present study, based on the correspondence between immunoreactivity and activity stains of the various polypeptide species encountered, is that this antibody is indeed directed toward the polymerase δ catalytic polypeptide. Nishida et al. (1988) have recently isolated a soluble factor from HeLa cells which restores DNA repair synthesis in permeabilized UV-irradiated cultured diploid human fibroblasts and identified the factor as DNA polymerase δ; their preparation consisted of a 220-kDa polypeptide. The question of whether the 170-kDa polypeptide we have identified is a product of an yet larger polypeptide is therefore raised and will require further investigation. At the present time, it can be said only that we have not observed such a polypeptide as a major immunoreactive component in human placental extracts.

The behavior of DNA polymerase δ shows a parallel with that of DNA polymerase α, where there have been major problems in the existence of multiple enzyme forms, as well as disparate reports of the sizes and compositions of the subunits [for reviews, see Fry (1982), DePamphilis and Wasserman (1980), and Holmes et al. (1983)]. In general, polypeptides of polymerase α preparations have fallen in the ranges of 140–180, 120, and 30–80 kDa. Determination of the subunit composition of this enzyme has been complicated by the possibilities that these polypeptides may represent associated replication proteins (Hübscher, 1983; Pritchard & DePamphilis, 1983; Pritchard et al., 1983; Baril et al., 1983; Wang et al., 1984; Plevani et al., 1984; Grosse & Krauss, 1985; Vishwanatha et al., 1986). Current evidence strongly supports a core polymerase α subunit of ca. 180 kDa for enzymes from a number of eucaryotic sources (Sauer & Lehman, 1982; Wahl et al., 1984; Kaguni et al., 1982; Wang et al., 1984; Karawaya et al., 1984; Yamaguchi et al., 1985; Plevani et al., 1985; Holmes et al., 1986). There is evidence that the 180-kDa polymerase α core polypeptide is also highly susceptible to proteolysis (Holmes et al., 1986; Wong et al., 1986), and activity staining of a number of mammalian DNA polymerase α preparations has revealed active polypeptides of 190, 110–125, and 47–68 kDa (Karawaya et al., 1984; Detera-Wadleigh et al., 1984; Wahl et al., 1984; Albert et al., 1982; Karawaya & Wilson, 1982; Hübscher, 1983). Thus, the behavior of DNA polymerase δ in terms of the active polypeptide species that have been observed has a parallel in the data reported for DNA polymerase α. It should be noted that we have found that our antisera to DNA polymerase δ do not cross-react with the 180-kDa polypeptide of purified DNA polymerase α from human placenta, as determined by immunoblotting, immunoprecipitation experiments, and enzyme inhibition studies.

In conclusion, our findings illustrate that the use of immunological methods may be of strong utility in the examination of the subunit compositions of mammalian DNA polymerases δ. We are currently using the immunoblotting technique as a tool for monitoring the integrity of our enzyme preparations, as well as attempts to develop isolation procedures that will abate the problems of endogenous proteolytic activity. In addition, our findings also suggest that proteolytic modification may be a useful tool in studying the nature of the functional domains of DNA polymerase δ.

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Probing the Surface of Z-DNA with Anti-Nucleoside Antibodies†

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ABSTRACT: Antibodies specific for cytidine (C) and guanosine (G) were used to probe the surface of two Z-DNA conformers. When tested by ELISA, anti-G reacted with poly(dG-dC)·poly(dG-dC) treated with bromine water [Br-poly(dG-dC)·poly(dG-dC)] but anti-C did not. A weak reaction with anti-C was detected by dot immunobinding. In contrast, anti-C reacted strongly with poly(dG-dC)·poly(dG-dC) treated with *N*-acetoxy-2-(acetylamino)fluorene [AAF-poly(dG-dC)·poly(dG-dC)]; anti-G reacted weakly, despite the fact that most G residues had not been substituted with AAF. Neither antinucleoside bound to the B conformation of poly(dG-dC)·poly(dG-dC). In competition experiments, GMP was the most efficient competitor of the reaction of anti-G with Br-poly(dG-dC)·poly(dG-dC); AMP and TMP were 100-fold less efficient, and CMP did not compete to a significant extent. In contrast, the reaction of anti-Z with Br-poly(dG-dC)·poly(dG-dC) was not inhibited by nucleotides. Of five possible sites recognized on guanosine by anti-G antibodies (N1, C6, O6, N7, and C8), AMP and TMP share three or their equivalent and CMP only one. The binding of anti-C to AAF-poly(dG-dC)·poly(dG-dC) was inhibited best by CMP; AMP was 8 times less efficient; GMP and TMP were about 35-fold less efficient than CMP. Thus, although the amino group on the C4 position of CMP appears to be immunodominant, the capacity of GMP and TMP to inhibit the reaction indicates that other sites are also recognized in AAF-poly(dG-dC)·poly(dG-dC), e.g., the exposed C5 position. Recognition of cytidine in denatured DNA by anti-C is more specific: CMP is the best competitor; AMP did not compete, and GMP and TMP were 53-fold and 115-fold, respectively, less efficient. Therefore, different antigenic determinants are recognized in the two systems although parts may overlap. The data show that purine and pyrimidine residues in a Z-type double helix are accessible to nucleotide-specific proteins, in this case, specific antibodies. In addition, the accessibility of nucleotides depends upon the chemical nature of the Z duplex, illustrating that Z-DNA is polymorphic. Our findings also show that proteins with purine or pyrimidine specificity, but not necessarily specific for the Z conformation, can also bind to Z-DNA.

The possible existence of a novel form of double-stranded helical DNA was first suggested (Pohl & Jovin, 1972) when it was observed that the CD spectrum of poly(dG-dC)·poly(dG-dC) underwent an inversion when the concentration of salt in the solution was increased to 4 M NaCl. Subsequent proof that it was a left-handed or Z-DNA came from crystallographic studies, from fiber diffraction data, and from NMR and laser Raman studies [reviewed in Rich et al. (1984)].

The structure of left-handed Z-DNA is unlike that of right-handed DNA, e.g., B-DNA, in many respects (Rich et al., 1984). Z-DNA does not have a major and minor groove but, instead, has one deep helical groove analogous to the minor groove of B-DNA. In place of the major groove is a convex surface in which regions of the paired purines and pyrimidines in poly(dG-dC)·poly(dG-dC) bases are displayed. This is in marked contrast to B-DNA, in which the paired bases are internally located, i.e., "buried", and not accessible at the surface of the molecule.

The presence of purine and pyrimidine residues on the surface of Z-DNA has made possible the development of specific chemical probes that can detect Z regions in DNA that is predominantly right-handed (Herr, 1985; Johnson & Rich, 1985). These probes, e.g., diethyl pyrophosphate, are of low molecular weight. In the context of a possible role of Z-DNA in gene regulation (Rich et al., 1984), it would be of interest to determine whether the purine and pyrimidine bases are also accessible for binding to base-specific proteins.

Antibodies can be made that are specific for nucleosides and nucleotides sequences [Erlanger & Beiser, 1964; Wallace et al., 1971; reviewed in Stollar (1980)]. These antibodies have been shown to be highly specific for the base (or bases) in the immunogen and capable of reacting with the specific base in DNA only in regions where there is no base pairing, i.e., in denatured regions of DNA. In this regard, antinucleoside antibodies were recently used to detect local denaturation in

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