Specific Interaction of DNA Polymerase β and DNA Ligase I in a Multiprotein Base Excision Repair Complex from Bovine Testis

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Base excision repair (BER)1 is a cellular defense mechanism repairing modified bases in DNA. Recently, a G:U repair reaction has been reconstituted with several purified enzymes from Escherichia coli (Dianov, G., and Lindahl, T. (1994) Curr. Biol. 4, 1069-1076). Using bovine testis crude nuclear extract, we have shown that G:U is repaired efficiently in vitro, and DNA polymerase β (β-pol) is responsible for the single nucleotide gap-filling synthesis (Singhal, R. K., Prasad, R., and Wilson, S. H. (1995) J. Biol. Chem. 270, 949-957). To investigate potential interaction of β-pol with other BER protein(s), we developed affinity chromatography matrices by cross-linking purified rat β-pol or antibody against β-pol to solid supports. Crude nuclear extract from bovine testis was applied to these affinity columns, which were then extensively washed. Proteins that bound specifically to the affinity columns were co-eluted in a complex with β-pol. This complex had a molecular mass of approximately 180 kDa and was able to conduct the complete uracil-initiated BER reaction. The BER complex contained both β-pol and DNA ligase I. An antibody to β-pol was able to shift the complex in sucrose gradients to a much larger molecular mass (>300 kDa) that again contained both β-pol and DNA ligase I. Furthermore, DNA ligase I and β-pol were co-immunoprecipitated from the testis nuclear extract with anti-β-pol IgG. Thus, we conclude that β-pol and DNA ligase I are components of a multiprotein complex that performs BER.

Base excision repair (BER)1 is initiated by enzymatic removal of a damaged or inappropriate base residue in DNA by a DNA glycosylase. This class of enzymes recognizes and repairs a variety of single base lesions in DNA (1). Uracil-DNA glycosylase (UDG), which catalyzes the removal of uracil from DNA at the 5′-deoxyribose phosphate (5′-dRpase) or by a 5′→3′ exonuclease, generating a one-nucleotide gap with 3′-OH and 5′-phosphate termini (8–11). The single nucleotide gap is then filled by DNA polymerase I in E. coli or by DNA polymerase β (β-pol) in mammalian cells (12–14). Finally, the nick is sealed by DNA ligase. Dianov and Lindahl (12) reconstituted the uracil-initiated BER reaction with five purified enzymes from E. coli. Recently, Singhal et al. (13) have demonstrated β-pol-dependent BER reaction in mammalian cells with partially purified bovine proteins and T4 DNA ligase. Since mammalian cells contain multiple species of DNA ligase (15), one or more of these enzymes may complete the BER reaction (16). Studies with mutant mammalian cell lines have implicated both DNA ligase I and DNA ligase III in repair of single-strand breaks generated by BER enzymes (16, 17).

β-Pol is a 39-kDa monomeric enzyme that lacks intrinsic nuclease activity (18). However, it has recently been reported to possess 5′-deoxyribose phosphodiesterase activity (19). In recent years, β-pol has been implicated in the repair of several lesions that are repaired by the BER pathway, among them G:T and G:U mismatches (13, 14, 20), abasic sites (21–23), adducts introduced by monofunctional alkylating agents (14, 24), and in vitro bypass of a dGpG-cisplatin adduct (25). β-Pol also appears to function in some specialized cases of DNA replication (26–30).

Mammalian β-pol have been cloned and overexpressed in E. coli (31–34). The recombinant proteins are fully active in DNA synthesis and have been exploited for structure-function studies (35–38). We recently generated monoclonal antibodies and several neutralizing polyclonal antibodies specific for mammalian β-pol (13, 39). Using these reagents, we have investigated potential interactions among mammalian BER proteins. Our results indicate that BER may be mediated, at least in part, by a multiprotein complex that contains β-pol and DNA ligase I.

EXPERIMENTAL PROCEDURES

Materials—[γ-32P]JATP and [α-32P]CTP were purchased from ICN Radiochemicals. High-performance liquid chromatography-purified synthetic oligodeoxynucleotides primers were obtained from Gensys Biotechnologies Inc. Epoxy-activated Sepharose 6B and protein A-Sepharose 4B were from Pharmacia Biotech, Inc. Rat β-pol was purified as described (33, 34). Recombinant human DNA ligase I was purified from baculovirus-infected insect cells (40). The rabbit polyclonal antisera raised against bovine DNA ligase I and against a conserved peptide sequence that is present in all eucaryotic DNA ligases I have been described (33, 34). Recombinant human DNA ligase I was purified from baculovirus-infected insect cells (40). The rabbit polyclonal antisera raised against bovine DNA ligase I and against a conserved peptide sequence that is present in all eucaryotic DNA ligases I have been described (33, 34).

1 The abbreviations used are: BER, base excision repair; UDG, uracil-DNA glycosylase; AP, apurinic/apyrimidinic; APE, AP endonucleases; dRpase, deoxyribose phosphodiesterase; β-pol, DNA polymerase β; SDG, sucrose density gradient; PAGE, polyacrylamide gel electrophoresis; bp, base pair; BSA, bovine serum albumin; IOD, integrated optical density; DTT, dithiothreitol; PCR, polymerase chain reaction.
tion for 20 min at 15,000
and dialyzed against the same buffer for 14 h. Nuclear extract was then
phenylmethylsulfonyl fluoride, 0.5 mM EDTA, 1 mM DTT, 10 mM sodium metabisulfite. Buffer B was the same as buffer A, except it contained 1 M NaCl. Buffer C was composed of 25 mM Tris-HCl, pH 7.5, 1 mM EDTA, 10 mM sodium metabisulfite, 1 mM phenylmethylsulfonyl fluoride, 0.5 mM pepstatin A, and 20% glycerol. Buffer D is the same as buffer C, except it contains 100 mM NaCl. Buffer E contains 25 mM HEPES, pH 7.5, 100 mM NaCl, 1 mM EDTA, 1 mM DTT, and 10% glycerol.

Nuclear Extract Preparation—Nuclear extract was prepared from bovine testis as described (44). Briefly, 500 g of bovine testis was minced in 3 volumes of buffer A and homogenized with a blender. The homogenate was pelleted by centrifugation at 10,000 ¥ g for 30 min. The supernatant was centrifuged at 100,000 ¥ g for 1 h. The clear super-
natant was used as the crude nuclear extract.

Protein Affinity Column Chromatography—Purified rat b-pol (14 mg) or bovine serum albumin (BSA) (14 mg) was coupled to approximately 10 ml of epoxy-activated Sepharose 6B according to the manufacturer’s instructions (Pharmacia). After equilibration with buffer D, bovine testis nuclear extract (5 ml containing approximately 50 mg of protein) was loaded onto each column repeatedly. The columns were then washed extensively (30 column volumes) with buffer D. The bound proteins were eluted from these columns by stepwise increasing NaCl concentrations. Column fractions were dialyzed against buffer D, concentrated to 1 ml with Centriprep-10 (Amicon), and stored at –80°C until use.

Immunofluorescence Column Chromatography—Affinity purified anti-b-pol IgG (2 mg) and purified preimmune IgG (2 mg) from the same rabbit were covalently cross-linked to epoxy-activated Sepharose 6B according to the manufacturer’s suggested protocol. Equal volumes (5 ml) of bovine testis nuclear extract were applied to each column. After extensive washing with buffer D (30 column volumes), bound proteins were eluted from the columns as described above.

Sucrose Density Gradient Sedimentation—The nuclear extract preparations were dialyzed against buffer C without glycerol but containing 50 mM NaCl and then layered on a 15–40% sucrose gradient. Centrifugation at 100,000 ¥ g for 20 min. This clear super-

beration at 90°C for 3 min, followed by slow cooling to 25°C. 32P-Labeled duplex oligonucleotide was separated from unincorporated 32P-labeled ATP using a Nensorb-20 column according to the manufacturer’s suggested protocol. The radiolabeled oligonucleotide was resuspended in 70 ml of HEPES, pH 8.0, 1 mM EDTA, and 1 mM DTT. The DNA (50 ng) solution was treated with 1 unit of E. coli uracil-DNA glycosylase (Boehringer Mannheim) for 45 min at 37°C to create double-stranded DNA containing a site-specific AP site. AP endonuclease reaction mixture (10 ml) contained 50 ml HEPES, pH 7.9, 3 ml MgCl2, 2 ml DTT, and 135 mM 32P-labeled double-stranded oligonucleotide containing an abasic site at position 21. The reaction was initiated by adding 1 ml of the sucrase gradient fractions. Incubation was at 37°C for 5 min. The reaction was termi-
nated by adding 5 ml of gel loading buffer (95% formamide, 20 ml EDTA, 0.02% bromophenol blue, and 0.02% xylene cyanol). After incubation at 95°C for 2 min, the reaction products were separated by electrophoresis in a 15% polyacrylamide gel containing 7 M urea in 89 ml Tris-HCl, 89 M bovic acid, and 2 ml EDTA, pH 8.8, and visualized by autoradiography. The amount of the AP endonuclease product was measured in terms of ID of the radioactive bands and determined as above.

Base Excision Repair Assay—In vitro BER was performed essentially as described (13). Briefly, the standard reaction mixture (50 ml) con-
tained 100 ml Tris-HCl, pH 7.5, 5 ml MgCl2, 1 ml DTT, 0.1 mM EDTA, 2 ml ATP, 0.5 mM NAD, 5 ml dThiophospho
creatine, 10 units of creatine phosphokinase, 40 ml 51-bp duplex oligodeoxynucleotide substrate containing a uracil residue at position 22, and 0.3 mM [32P]dCTP (specific activity 6 ¥ 108 dpm/pmole). The BER reaction was initiated by addition of the reaction mixture. After incubation at 95°C for 2 min, the reaction products were separated by electrophoresis in a 15% polyacrylamide gel containing 7 M urea in 89 ml Tris-HCl, 89 M bovic acid, and 2 ml EDTA, pH 8.8. Gels were fixed, dried, and autoradiographed to visualize the reaction products.

RESULTS

A complete BER reaction with E. coli proteins has been reconstituted using uracil-DNA glycosylase, AP endonuclease IV, RecA protein which excises 5’-terminal deoxyribose phos-
phate, DNA polymerase I, and DNA ligase (12). We have recently demonstrated that a bovine testis crude nuclear extract conducts G:U-initiated base excision repair in vitro inserting dCMP opposite G (13). The DNA synthesis stage of this in vitro repair reaction is blocked by antibody to β-pol (panel a). In a parallel control experiment, 50 μg each of β-pol (39 kDa), bovine serum albumin (70 kDa), aldolase (158 kDa), catalase (220 kDa), and ferritin (440 kDa) were mixed and sedimented under similar conditions as the crude nuclear extract. SDG fractions of the control experiment were also analyzed for β-pol by immunoblotting to assess the position of β-pol in the gradients (panel b). An equal volume (1 μl) of the SDG fraction numbers 6 and 7 (panel a) was subjected to G:U-initiated BER assay. The BER products were separated by 12% PAGE containing 7 M urea and followed by autoradiography. The photograph of an autoradiogram is shown (panel c). The positions of the 51- and 22-mer BER products are indicated by arrows. The relative positions of the 39- and 160-kDa polypeptides in the gradients are shown at the bottom of panels a and b. The position of the β-pol is shown on the left side of the blots.

Fig. 1. Detection of BER complex in bovine testis nuclear extract by SDG sedimentation. Bovine testis crude nuclear extract was sedimented on 15–40% sucrose gradients as described under “Experimental Procedures.” SDG fractions were examined for β-pol by immunoblotting (panel a) and G:U-initiated BER activity (panel c). An equal volume (20 μl) of each SDG fraction was separated by SDS-PAGE, transferred to a nitrocellulose membrane, and probed with anti-β-pol (panel a). In a parallel control experiment, 50 μg each of β-pol (39 kDa), bovine serum albumin (70 kDa), aldolase (158 kDa), catalase (220 kDa), and ferritin (440 kDa) were mixed and sedimented under similar conditions as the crude nuclear extract. SDG fractions of the control experiment were also analyzed for β-pol by immunoblotting to assess the position of β-pol in the gradients (panel b). An equal volume (1 μl) of the SDG fraction numbers 6 and 7 (panel a) was subjected to G:U-initiated BER assay. The BER products were separated by 12% PAGE containing 7 M urea and followed by autoradiography. The photograph of an autoradiogram is shown (panel c). The positions of the 51- and 22-mer BER products are indicated by arrows. The relative positions of the 39- and 160-kDa polypeptides in the gradients are shown at the bottom of panels a and b. The position of the β-pol is shown on the left side of the blots.
The crude nuclear extract was applied to a Q-Sepharose column in a buffer containing 50 mM NaCl. Under these conditions, β-pol and other BER proteins including UDG, APE, and DNA ligases were not retained by the column, whereas contaminating nucleic acids were adsorbed. The flow-through fraction was subjected to SDG centrifugation and yielded the same size distribution of β-pol as that seen with the crude nuclear extract (data not shown). Taken together, these results initially suggested a complex with a molecular mass of ~180 kDa containing β-pol and other enzymatic activities required for BER. The slower sedimenting β-pol in fractions 11–13 can be attributed to the monomeric form of the enzyme (Fig. 1a).

Interaction of Base Excision Repair Proteins with Affinity Columns—To study interactions between β-pol and other BER proteins in the bovine testis crude nuclear extract, we constructed two types of affinity chromatography columns using, on the one hand, purified β-pol and, on the other hand, purified anti-β-pol IgG. Bovine testis nuclear extract, capable of BER in vitro, was applied to the affinity columns. For the β-pol column affinity chromatography, the crude extract was applied to the column and then washed extensively (>20 column volumes) with buffer containing 100 mM NaCl. Bound proteins were eluted with stepwise additions of buffer containing increasing [NaCl]. A column with bovine serum albumin was used as a negative control. Column fractions were assayed for G:U-initiated BER activity. The results (Fig. 2a) indicated that the proteins bound to the β-pol column and eluted with NaCl were able to confer full BER activity, whereas the proteins bound to the BSA column could not. These results also indicated that the various enzymes required for BER were immobilized on the β-pol affinity column in the presence of 100 mM NaCl but were eluted with 200 mM NaCl. The putative complex(es) isolated by this column chromatography was, therefore, sensitive to release from β-pol by a relatively low NaCl concentration. However, it is noteworthy that the proteins eluted from the β-pol affinity column confer full BER activity indicating that the complex also included β-pol (see “Discussion”).

To further explore complex(es) of β-pol and other BER proteins in the crude nuclear extract, we made use of immunoadfinity column chromatography with a β-pol antibody. We had shown earlier that our polyclonal antibody to β-pol is specific and can completely inhibit both purified β-pol and the nuclear extract-based BER activity in vitro (13). Therefore, we used IgG from this antiserum to conduct immunoadfinity chromatography to probe for a β-pol-containing protein complex. Purified anti-β-pol IgG or IgG isolated from preimmune serum of the same rabbit were covalently cross-linked to epoxy-activated Sepharose 6B as described under “Experimental Procedures.” An equal volume (5 ml) of bovine testis nuclear extract was applied to each column and then washed extensively (>20 column volumes) with buffer D. Bound proteins were eluted with buffer D containing increasing concentrations of NaCl. Protein fractions eluted with 200 mM NaCl were dialyzed and concentrated to equal volumes (1 ml), and then 2 μl of each protein fraction was examined for BER activity using a 51-mer duplex oligodeoxynucleotide containing G:U bp at position 22 as a substrate. Under standard base excision repair assay conditions, the incorporation of [32P]dCMP at position 22 results in either a fully repaired 51-mer product or an intermediate 22-mer product. Autoradiograms (panels a and b) were developed after separating the BER products by 12% PAGE containing 7 M urea. Panel c depicts the immunoblot analysis of the protein fractions isolated from the immunoaffinity columns (panel b). An equal volume (25 μl) of the concentrated protein fractions from the preimmune IgG (lane 2), anti-β-pol IgG (lane 3), or 40 μg of crude nuclear extract (lane 1) was separated by 12% SDS-PAGE and transferred onto a nitrocellulose membrane. The blots were developed with either anti-β-pol (panel c, I), anti-DNA ligase I (panel c, II), or anti-AP endonuclease (panel c, III). The relative positions of β-pol, DNA ligase I, and AP endonuclease are indicated.

**Fig. 2.** Isolation of BER complex using affinity column chromatography. Fourteen milligrams of purified rat β-pol or BSA (panel a) and 2 mg of affinity purified anti-β-pol IgG or preimmune IgG (panel b) were covalently cross-linked to epoxy-activated Sepharose 6B as described under “Experimental Procedures.” An equal volume (5 ml) of bovine testis nuclear extract was applied to each column and then washed extensively (>20 column volumes) with buffer D. Bound proteins were eluted with buffer D containing increasing concentrations of NaCl. Protein fractions eluted with 200 mM NaCl were dialyzed and concentrated to equal volumes (1 ml), and then 2 μl of each protein fraction was examined for BER activity using a 51-mer duplex oligodeoxynucleotide containing G:U bp at position 22 as a substrate. Under standard base excision repair assay conditions, the incorporation of [32P]dCMP at position 22 results in either a fully repaired 51-mer product or an intermediate 22-mer product. Autoradiograms (panels a and b) were developed after separating the BER products by 12% PAGE containing 7 M urea. Panel c depicts the immunoblot analysis of the protein fractions isolated from the immunoaffinity columns (panel b). An equal volume (25 μl) of the concentrated protein fractions from the preimmune IgG (lane 2), anti-β-pol IgG (lane 3), or 40 μg of crude nuclear extract (lane 1) was separated by 12% SDS-PAGE and transferred onto a nitrocellulose membrane. The blots were developed with either anti-β-pol (panel c, I), anti-DNA ligase I (panel c, II), or anti-AP endonuclease (panel c, III). The relative positions of β-pol, DNA ligase I, and AP endonuclease are indicated.
from the anti-β-pol column (Fig. 2c). As we will show below, the BER-proficient complex eluted from the anti-β-pol column also contains UDG activity. Thus, whereas a combination of individually purified proteins can perform the BER reaction in vitro (13), our results indicate an interaction between BER proteins and β-pol, suggesting that at least a portion of the BER enzymes in the testis nuclear extract exist as a complex.

Sedimentation of Bovine Testis BER Complex—To examine the physical properties of the BER complex obtained by immunoaffinity chromatography, the active fraction from the immunoaffinity column was sedimented through 15–40% sucrose gradients as described under “Experimental Procedures.” Fractions, −250 μl each, were collected from the bottom of the gradient and examined for BER activity. Two-microliter portion of the indicated SDG fractions was analyzed for BER (panel b) or UDG (panel c) activity as described under “Experimental Procedures.” The amounts of BER products (51- and 22-mer) were determined in terms of IOD and plotted against SDG fraction number (panel a). The relative positions of the protein markers in the gradient are indicated by arrows. The fraction numbers are indicated on each lane.

**Fig. 3. Sedimentation of BER complex isolated from the affinity column.** Protein fraction eluted from anti-β-pol IgG column was sedimented through 15–40% sucrose gradients as described under “Experimental Procedures.” Fractions, −250 μl each, were collected from the bottom of the gradient and examined for BER activity. Two-microliter portion of the indicated SDG fractions was analyzed for BER (panel b) or UDG (panel c) activity as described under “Experimental Procedures.” The amounts of BER products (51- and 22-mer) were determined in terms of IOD and plotted against SDG fraction number (panel a). The relative positions of the protein markers in the gradient are indicated by arrows. The fraction numbers are indicated on each lane.

Sedimentation of Bovine Testis BER Complex—To examine the physical properties of the BER complex obtained by immunoaffinity chromatography, the active fraction from the immunoaffinity column was sedimented through a 15–40% sucrose gradient, and the gradient fractions were assayed for BER activity (Fig. 3, a and b) and UDG activity (Fig. 3c). The results of these analyses revealed two peaks of BER and UDG activities, one centered in fractions 6–7 and the other at fraction 12 corresponding to molecular masses of approximately 180 and 40 kDa, respectively. The presence of the 22-mer intermediate product, which was not detected in assays of the immunoaffinity column eluate (Fig. 2b), suggested that some of the BER proteins that had been in a complex in the original sample had dissociated during the experimental protocol of dialysis, concentration, and/or centrifugation. The profile of the base excision repair activity of these SDG fractions also showed that fractions corresponding to a molecular mass of 100 kDa or less were limiting in DNA ligase activity (Fig. 3, fractions 10–14), since the 22-residue intermediate product molecule accumulated.

Properties of BER Complex—The immunoaffinity purified complex presumably must contain UDG, APE, dRpase, β-pol, and DNA ligase activities necessary to complete the BER reaction. The uracil-DNA glycosylase activity appears to be contributed by an enzyme that is present in the immunopurified
complex as UDG activity co-sedimented with the complex (Fig. 3c). β-pol presumably contributes both dRpase and DNA synthesis activities (19). To determine the identity of the DNA ligase in the BER complex, the crude nuclear extract was subjected to SDG centrifugation, and the gradient fractions were examined for DNA ligase polypeptides by immunoblotting (Fig. 4, b and c). As expected from experiments described above, β-pol was dispersed in fractions 6-14 (Fig. 4a), but it was quantitatively shifted by anti-β-pol IgG to fractions 2-4 (Fig. 4a) near the bottom of the gradient. When these fractions were tested by immunoblotting with DNA ligase I antibody, a similar shift of a portion of the DNA ligase I was observed, just as for β-pol (Fig. 4b). However, we did not observe any shift in DNA ligase II (Fig. 4c). In these experiments, DNA ligase III was detected at low levels in fractions containing DNA ligase II. These results suggest that β-pol was complexed with a portion of the DNA ligase I molecules in the nuclear extract but not with DNA ligase II.

3 P. R. Strauss and S. H. Wilson, unpublished observations.
DISCUSSION

Base excision repair is a sequential, multistep DNA repair pathway where a damaged or incorrect base is excised and replaced by a complementary nucleotide, through synthesis by a DNA polymerase. Singhal et al. (13) had demonstrated that uracil-initiated BER can be studied in vitro with purified and partially purified bovine proteins and that the DNA synthesis step is conducted by DNA polymerase β. From these results, it was clear that a robust BER system can be reconstituted in vitro with eucaryotic proteins and that at least four enzyme activities are required, UDG, APE, β-pol, and DNA ligase. Interactions between individual BER proteins may be important for the regulation of individual steps involved in the BER reaction.

In this paper, we provide evidence of protein-protein interactions among BER proteins, including direct association between β-pol and DNA ligase I. To demonstrate a multiprotein complex with bovine testis nuclear proteins, affinity chromatography was employed using β-pol or β-pol antibody as the immobilized ligand. A protein fraction purified by both types of affinity chromatography was capable of conducting complete uracil-initiated BER in vitro. Thus, these β-pol affinity columns were able to retain activities for UDG, APE, β-pol/dRpase, and DNA ligase. Biochemical and immunological characterization of the fractions eluted from the affinity columns demonstrated the presence of β-pol and DNA ligase. Furthermore, synthesis of the 51-mer product of the in vitro BER system indicated that these columns also retained UDGP and AP endonuclease activities, in addition to β-pol and DNA ligase. However, the accumulation of the 22-mer DNA ligase substrate, in some cases, indicated that our affinity purified fractions were limiting in DNA ligase activity. It is noted that the affinity purified fraction from the β-pol affinity columns contained β-pol itself. A similar phenomenon has been observed recently with XPA protein, where Park and Sancar (47) provided two possible explanations. First, XPA might dimerize at the concentrations provided on the column, or alternatively, the ERCC1/ERCC4 (XPF) complex, when applied to the affinity column, might alternately between complexes with free XPA and XPA on the column, resulting in retardation of free XPA protein and its subsequent co-elution with ERCC1/ERCC4 (XPF) complex. In any case, β-pol appears to behave in a similar fashion as XPA, in the affinity column chromatography.

Having established that the multiple activities required for BER bind to affinity columns, it was interesting to examine the hydrodynamic properties of the BER complex eluted from the affinity columns and compare it with a complex in the initial bovine testis crude nuclear extract. Using velocity sedimentation in linear sucrose gradients, we found that complexes capable of BER were heterodisperse and included species in the 180-kDa range (Figs. 1 and 3). The ~180-kDa complex also appeared to contain UDGP and AP endonuclease activities in addition to β-pol and DNA ligase I. The presence of these activities in a complex of ~180 kDa can generally be accommodated. Thus, stoichiometric levels of β-pol (39 kDa), UDGP (32 kDa), and possibly AP endonuclease (34 kDa) could be present in the 180-kDa complex, since sedimentation of DNA ligase I is consistent with a ~80-kDa molecule (41). In the in vitro BER system, synthesis of the 51-mer product molecule requires DNA ligase activity. Sucrose gradient analysis of the immunological properties of the BER complex indicate that DNA ligase I is a component of the ~180-kDa complex. Furthermore, biochemical and immunological properties of the BER complex indicate that DNA ligase I is a component of the ~180-kDa complex. The involvement of DNA ligase I in BER is consistent with the phenotype of the DNA ligase mutant cell line 46BR and the observed in vitro defect in BER in this cell line (16). We note that DNA ligase I also has been purified as a component of a 21 S DNA replication complex (48, 49).

The phenotype of the Chinese hamster ovary cell line EM9 suggests a defect in BER at a post-incision stage (17, 50). DNA ligase III activity is reduced in this cell line because of low levels of its interacting protein Xrcc1. It appears that there may be two pathways for repairing DNA single-strand breaks, one of which involves the BER complex described here with DNA ligase I and the other which is mediated by Xrcc1/DNA ligase III and probably poly(ADP-ribose) polymerase (51). Results in Fig. 4 are particularly noteworthy as they demonstrate a linkage between β-pol-mediated BER and DNA ligase I but not DNA ligase II. Recent studies indicate that DNA ligase II is encoded by the Lig3 gene and is probably derived from DNA ligase III by proteolysis (40, 32, 43). The BER complex described here may be mainly responsible for uracil-DNA glycosylase-initiated repair, whereas the Xrcc1/DNA ligase III/poly-(ADP-ribose) polymerase pathway may deal with DNA single-strand breaks. One compelling reason to have BER carried out by a multiprotein complex is to protect the DNA strand break intermediates from poly(ADP-ribose) polymerase, as this protein binds avidly to strand breaks; there is recent evidence that the cell uses this binding to activate checkpoints that may decide between cell cycle arrest and apoptosis (52). Therefore, it makes biological sense to have the BER reactions as concerted as possible, so that the mechanism of repair does not interfere with the surveillance mechanisms that monitor the genome for DNA damage.

In summary, if the BER enzymes noted here were in an equimolar macromolecular complex, the cumulative molecular mass is 196 kDa (i.e. UDGP, 32 kDa; β-pol/dRpase, 39 kDa; and DNA ligase I, 125 kDa). This is consistent with the molecular mass of the BER-proficient complex detected here by sucrose gradient centrifugation (~180 kDa), because DNA ligase I sediments as a ~125-kDa molecule (41). A significant portion of the ~180-kDa complex obtained by immunoadfinity chromatography appeared to dissociate in the elution and subsequent sucrose gradient centrifugation steps, pointing to the idea that proteins in this BER complex are only weakly associated with each other. The significance of such a BER complex in the cell may be that the lifetime of each DNA intermediate during the sequential BER pathway is reduced, thus enhancing the efficiency of the overall pathway.

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