DNA Helicase Associated with DNA Polymerase α: Isolation by a Modified Immunoaffinity Chromatography†

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ABSTRACT: We have developed a novel immunoaffinity method for isolating a DNA polymerase α-associated DNA helicase from the yeast *Saccharomyces cerevisiae*. Earlier we have reported the characterization of a DNA helicase activity associated with the multiprotein DNA polymerase α complex from yeast [Biswas, E. E., Ewing, C. M., & Biswas, S. B. (1993) *Biochemistry 32*, 3030–3027]. We report here the isolation of the DNA helicase from the DNA polymerase α (pol α) complex bound to an anti-pol α immunoaffinity matrix. The DNA helicase activity eluted at ~0.35 M NaCl concentration. The eluted ATPase/helicase peak was further purified by size-exclusion high-performance liquid chromatography (HPLC). At low ionic strength (50 mM NaCl), it remained associated with other proteins and eluted as a large polypeptide complex. At high ionic strength (500 mM NaCl), the helicase dissociated, and the eluted ATPase/helicase fraction contained 90-, 60-, and 50-kDa polypeptides. Photoaffinity cross-linking of helicase with ATP during the isolation process demonstrated a 90-kDa polypeptide to be the likely ATP binding component of the helicase protein. The DNA helicase has single-stranded DNA (ssDNA)-stimulated ATPase and dATPase activities. The ATPase activity was stimulated by yeast replication protein A (RPA). The DNA helicase activity was stimulated by *Escherichia coli* ssDNA binding protein and RPA. The DNA helicase migrated on a DNA template in the 5′→3′ direction which is also the overall direction of migration of pol α on the lagging strand of the replication fork. The procedure described here may prove equally useful in the isolation other DNA polymerase α-associated replication proteins, in addition to DNA helicase, from yeast and other eukaryotic cells.

Replication of chromosomal DNA requires a large number of proteins and enzymes with a variety of enzymatic activities (Kornberg & Baker, 1990). During DNA replication, neither DNA polymerases nor most other replication proteins are capable of unwinding the duplex DNA. Thus, a DNA unwinding enzyme or DNA helicase is required for unwinding the template DNA. A number of DNA helicases have been purified and characterized in prokaryotes and eukaryotes (Matson et al., 1983; Matson & Kaiser-Rogers, 1990). However, in *Escherichia coli*, only one of these helicases, the DnaB protein, has been shown to function as the sole helicase of the replisome in chromosomal and phage DNA replication (Fuller et al., 1981; Lebowitz & McMacken, 1986).

Studies on the *in vitro* replication of the mammalian virus SV40 genomic DNA have shown that the multifunctional virus-encoded T antigen functions as the helicase to unwind the viral DNA during replication and forms a complex with the large subunit of DNA polymerase α (Wold et al., 1987; Dornreiter et al., 1990, 1992). The helicase function of T antigen requires the presence of replication protein A (RPA)[1] in SV40 DNA replication. RPA has been shown to be a ssDNA binding protein that is involved in DNA replication and recombination (Wold & Kelly, 1988; Heyer & Kolodner, 1989; Heyer et al., 1990; Erdlie et al., 1991). The cellular homolog of the viral T antigen that functions in the unwinding of the chromosomal DNA in the replication fork remains unknown.

We have recently purified a multiprotein complex of DNA polymerase α from the yeast *Saccharomyces cerevisiae* which has both DNA-stimulated ATPase and RPA-dependent DNA helicase activities (Biswas et al., 1993a,b). Multiprotein complexes of mammalian pol α have been purified in several laboratories (Baril et al., 1988; Biswas & Biswas, 1988; Hübscher et al., 1982; Malkas et al., 1990; Pritchard & DePamphilis, 1983; Pritchard et al., 1983; Vishwanath et al., 1986). These complexes of pol α are presumably formed by complex protein–protein interactions of various polypeptide components with the core form of pol α, and as a result, these complexes appear to dissociate rather easily. Thus, purification and resolution of such complexes and analysis of their enzymatic components have remained a difficult challenge. We have employed a commonly used immunoaffinity chromatography of pol α and modified the procedure in order to isolate a fragile component of the multiprotein complex. In this paper, we describe the modified immunoaffinity procedure and discuss the utility of this strategy in the isolation of the rare and unstable components of the multiprotein complex.

MATERIALS AND METHODS

Yeast. Wild-type bakers' yeast (*Saccharomyces cerevisiae*) was obtained as a gift from the American Yeast Corp., Baltimore, MD. Yeast was removed from the fermenter at midlog phase, chilled to 4 °C, harvested by centrifugation,

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[1] Abbreviations: pol α, DNA polymerase α; Tris, tris(hydroxymethyl)-aminomethane; BSA, bovine serum albumin; EDTA, ethylenediaminetetraacetic acid; ATP, adenosine 5′-triphosphate; ATPase, adenosinetriphosphatase; ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; RPA, replication protein A; TPCK, *N*-tosyl-L-phenylalanine chloromethyl ketone; TLCK, N^{α}-*p*-tosyllysine chloromethyl ketone; PMSF, phenylmethanesulfonyl fluoride.

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and washed with chilled deionized water 3 times. The cell paste was suspended in 25 mM Tris-HCl (pH 7.5), 10% (v/v) glycerol, 0.01% (v/v) NP40, 1 mM EDTA, and 200 mM NaCl at OD_{600} = 400 and stored frozen at −90 °C until further use.

Nucleic Acids, Enzymes, and Other Reagents. Oligonucleotides were synthesized by Oligos Etc. (Portland, OR). Ultrapure deoxy- and ribonucleotides were obtained from Pharmacia (Piscataway, NJ) and were used without further purification. [α-^{32}P]ATP, [α-^{32}P]dATP, and [γ-^{32}P]ATP were obtained from Amersham (Arlington Heights, IL). RPA used in this study was obtained as described (Biswas et al., 1993a). *E. coli* ssDNA binding protein (SSB) and terminal deoxynucleotidyl transferase were purchased from United States Biochemical Corp. (Cleveland, OH). T4 polynucleotide kinase was obtained from New England Biolabs (Beverly, MA). The anti-pol α monoclonal antibody (MAb)–Sepharose column containing ~2 mg/mL antibody, prepared according to Chang et al. (1984) and Plevani et al. (1985), was received as a gift from Dr. Lucy Chang. All chemicals used to prepare buffers and solutions were reagent grade and were purchased from Fisher Chemical Co. (Pittsburgh, PA). Protease inhibitors were from Bachem (Los Angeles, CA). Poly(ethylenimine)–cellulose strips for thin-layer chromatography were from Fisher Chemical Co.

Buffers. Buffer I contained 25 mM Tris-HCl (pH 7.5), 2.5% (v/v) glycerol, 0.01% (v/v) NP40, and 1 mM EDTA. Buffer A contained 25 mM Tris-HCl (pH 7.5), 10% (v/v) glycerol, 0.1 mg/mL BSA, and 5 mM DTT. Buffer B contained 25 mM potassium phosphate (pH 7.5), 10% (v/v) glycerol, 0.01% NP40, and 5 mM DTT. All of the above buffers contained protease inhibitors as indicated; 1× TBE buffer was 89 mM Tris–borate/2.5 mM EDTA (pH 8.3).

Helicase Assays. The helicase assays were based on the methods described by Matson et al. (1983) and Biswas et al. (1993b).

(*A*) *Preparation of the 60-mer Primed Substrate.* A synthetic 60-mer oligonucleotide, complementary to a 50 bp sequence between nucleotides 6268 and 6317 of M13mp19 ssDNA, contained 5 nucleotide tails on both 5′ and 3′ termini. The oligonucleotide was labeled at its 3′ end using terminal deoxynucleotidyl transferase under conditions where on the average one nucleotide was added to the 3′ end. T4 polynucleotide kinase and [γ-^{32}P]ATP were used to label the 5′ end. The 60-mer was hybridized to M13mp19 as previously described (Biswas et al., 1993b). Excess unhybridized labeled 60-mer was removed by spin column (Promega Biotech, Madison, WI) purification. The purified substrate was diluted to 17 fmol/μL (10 000–20 000 cpm/μL) with 10 mM Tris-HCl (pH 7.5)/1 mM EDTA. The polarity substrate was prepared by digesting this substrate (where the 60-mer had been labeled at both the 5′ and 3′ ends) with *Sac*I enzyme, generating a linear substrate with duplex ends consisting of a 43-mer (with 38 bp duplex and 5 bp tail) at the 5′ terminus and a 17-mer (with 12 bp duplex and 5 bp tail) at the 3′ terminus.

Helicase activity was estimated as arbitrary units determined by densitometric scanning of the autoradiogram using a JAVA densitometric program of Jandel Scientific.

(*B*) *Assay Conditions.* Reaction mixtures were set up on ice as follows. A standard 20-μL reaction volume contained buffer A, 10 mM $MgCl_2$, 1 mM ATP, 17 fmol (10 000–20 000 cpm/μL) of substrate, and the indicated amount of DNA helicase. The mixtures were incubated at 30 °C for the times indicated, and the reactions were terminated by the addition of 4 μL of 1% SDS, 60 mM EDTA, and 1% bromophenol blue. A fraction (60%) of each reaction mixture was analyzed on 8% polyacrylamide gels in 1× TBE and 0.1% SDS. The electrophoresis was carried out in 1× TBE/0.1% SDS for 1 h at 160 V. Following electrophoresis, the gels were dried and autoradiographed at −80 °C for 12 h.

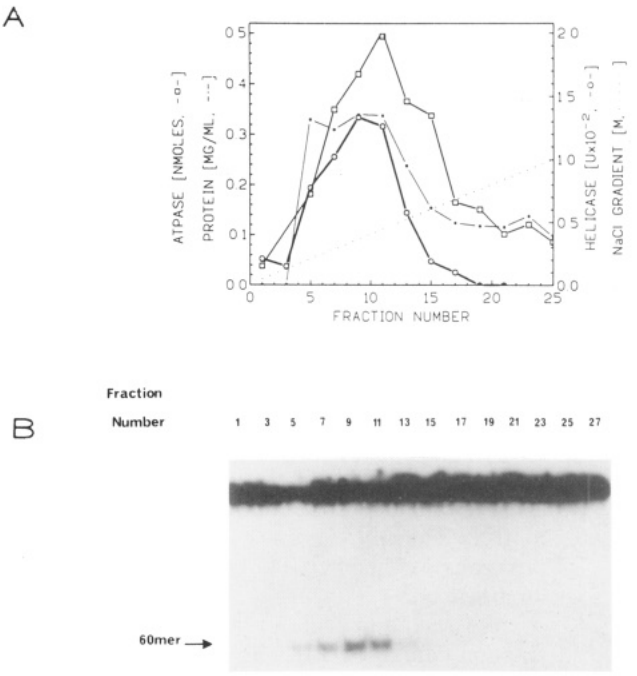

FIGURE 1: Chromatographic profile of the NaCl gradient elution of the anti–pol α MAb–Sepharose column. (A) The collected fractions were assayed for protein, ATPase, and helicase activities as described under Materials and Methods. In each case, 2.5 μL of the indicated fractions was assayed for helicase/ATPase. Helicase activities of the fractions were estimated by densitometric analysis of the autoradiogram shown in (B). (B) Autoradiogram of the helicase assay of the fractions as described above.

Photo-Cross-Linking of the ATP Binding Domain of the Helicase Protein. Photo-cross-linking was carried out essentially as described earlier (Biswas & Biswas, 1987; Biswas & Kornberg, 1984; Biswas et al., 1993b). This photo-cross-linking is highly specific as described in these publications.

Other Methods. Protein concentrations were estimated according to the method of Bradford (1976), using bovine serum albumin as a standard. The ATPase assays were carried out as previously described (Biswas et al., 1993b).

RESULTS

Isolation of the Pol α-Associated Protein Complex Containing Helicase and ATPase Activities. All steps in the isolation procedure were carried out at 4 °C unless otherwise indicated, and all buffers contained the following protease inhibitors: 1 μg/mL each of pepstatin A, leupeptin, antipain, and chymostatin; 0.1 mM each of benzamidine hydrochloride and $NaHSO_3$; and 2.5 μg/mL TPCK and TLCK unless otherwise indicated. The yeast extract (fraction I, Fr I) was prepared as previously described (Biswas et al., 1993a) using ~125 g of wild-type bakers' yeast in the presence of 0.2 M NaCl, 5 mM spermidine hydrochloride, 5 μg/mL soybean trypsin inhibitor (Worthington Biochemicals, Freehold, NJ), and 0.1 mM PMSF. The yeast extract was then dialyzed against buffer I for approximately 4 h until it had reached the conductivity of buffer I + 50 mM NaCl. The dialyzate was then centrifuged at 100000*g* for 1 h, and the supernatant was loaded slowly over a period of 15 h onto the 1-mL anti-pol α

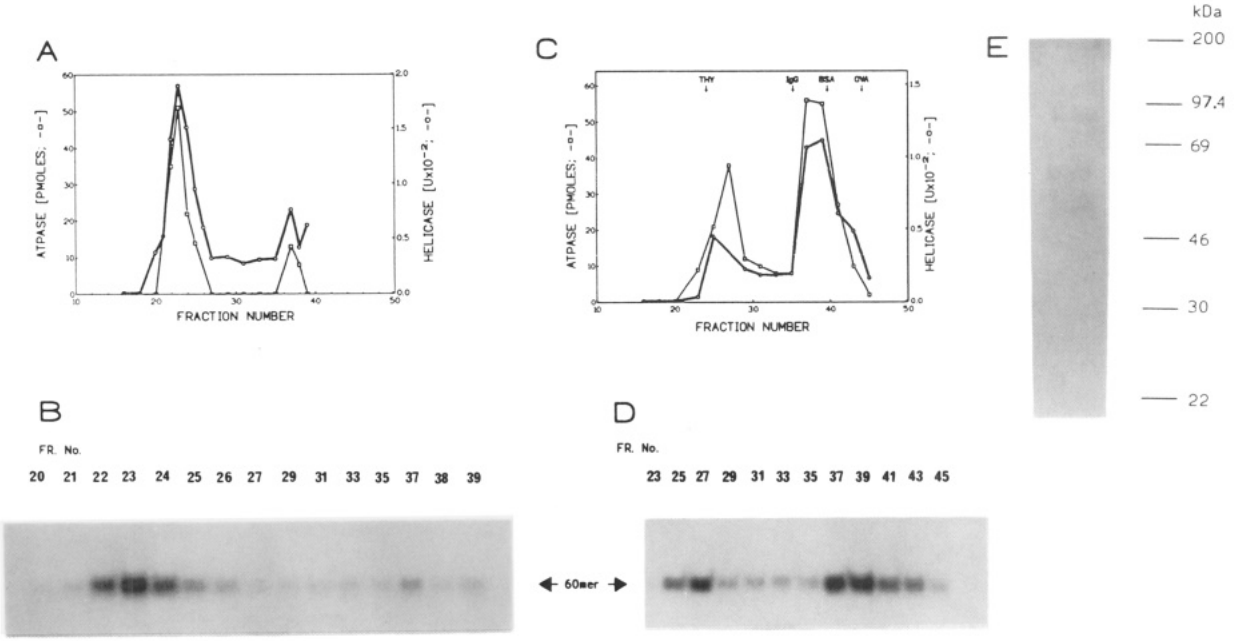

FIGURE 2: Fractionation of the pol α-associated ATPase/helicase by size-exclusion HPLC. (A and C) Pharmacia Superdex 200HR in buffer B + 50 mM NaCl (A) and TSK3000SW in buffer B + 500 mM NaCl (C) fractionation of the helicase as described under Results. The fractions in each of these chromatographic steps were assayed for protein, ATPase, and helicase activities as described under Materials and Methods. Five microliters of the indicated fractions was assayed for helicase/ATPase. The helicase activities of the fractions were estimated by densitometric analysis of the autoradiograms in panel B (for Superdex 200HR) and panel D (for TSK3000SW). Molecular weight markers were as follows: thyroglobulin (THY), immunoglobulin G (IgG), and bovine serum albumin (BSA). (B and D) Autoradiograms of helicase assays of HPLC fractions from Superdex 200HR chromatography (B) and for TSK3000SW chromatography (D). Please note that only the areas of the autoradiograms that represented the displaced 60-mer oligonucleotide are shown, and nuclease and phosphatase activities were negligible. (E) SDS–PAGE analysis of the pol α-associated helicase/ATPase, Fr IV (from the second SEHPLC purification, panel D). The active fractions (shown in panel D) corresponding to the major DNA helicase activity peak occurring at an elution volume of 11.4 mL were pooled and concentrated by ultrafiltration. Analysis of ~2 μg of Fr IV was carried out on a 10% SDS–polyacrylamide gel, stained with Coomassie Blue R250. Molecular weight standards were "Rainbow Marker" from Amersham.

MAb–Sepharose column. The column was then washed extensively with 1 L of buffer I + 50 mM NaCl; protein was not detectable in additional wash. The fractionation was then carried out using a linear gradient of NaCl from 50 to 1000 mM NaCl in buffer I (5.0 mL of each buffer). As DTT could not be added to buffers used on the immunoaffinity column, DTT was added to the fractions to a final concentration of 5 mM immediately following column elution. The gradient fractions were then assayed for protein, ATPase, and helicase activities. The helicase and ATPase eluted as a single broad peak in the gradient at a NaCl concentration of approximately 350 mM (Figure 1). The active fractions were dialyzed against buffer I and concentrated using Amicon YM30 membrane ultrafiltration. The total amount of protein recovered was 0.57 mg. In order to determine nonspecific protein binding, an analogous procedure was carried out where the immunoaffinity column was replaced with a 2-mL column of Sepharose CL-4B (Pharmacia Biotech). The fractions eluted in the NaCl gradient were assayed for protein and helicase activities. The total amount of protein eluted in the gradient was ~0.21 mg with no measurable helicase activities (data not shown).

Following elution of these pol α-associated proteins, pol α was eluted with 3.5 M $MgCl_2$ containing buffer essentially as described previously by Plevani et al. (1985). The 3.5 M $MgCl_2$ eluate contained the four-subunit pol α as well as the primase activity; however, no ATPase or helicase activities were detected in the pol α fractions.

To further resolve the proteins from the immunoaffinity chromatography, the active fractions of DNA helicase were pooled (Fr II), dialyzed against buffer I for 3 h, concentrated to 250 μL by ultrafiltration using an Amicon YM30 membrane, and subjected to size-exclusion HPLC (Pharmacia Superdex 200HR column, 1.0 × 30 cm) equilibrated with buffer B + 50 mM NaCl (Figure 2A,B). The majority of the ATPase and helicase activities appeared to comigrate as a large polypeptide complex with a major peak appearing at an elution volume of ~7.2 mL (peak I; >300 kDa as determined from a standard curve). A minor ATPase/helicase peak was observed at ~11.1 mL (peak II) (Figure 2A,B). It appeared that the removal of NaCl and size-exclusion chromatography at low ionic strength resulted in the reassociation of the polypeptides into a polypeptide complex. Consequently, the pool of ATPase/helicase activity was concentrated (Fr III) to 250 μL, and NaCl was added to 1 M concentration and fractionated on a TSK3000 column (0.8 × 30 cm glass column with a guard column) equilibrated with buffer B + 500 mM NaCl. The ATPase/helicase activities eluted as a peak of smaller size (120–150 kDa). The high-salt fractionation resolved the ATPase/helicase activities into two separate peaks: a minor peak at ~7.5-mL elution volume (peak I) and the major activity peak at ~11.4-mL elution volume (peak II) (Figure 2C,D). The two peaks differed with respect to the proportion of both activities present. It is important to note that the helicase activity is not influenced by NaCl concentration upto 250 mM (data not shown). SDS–PAGE analysis of the major helicase peak (concentrated by Amicon YM30) revealed the presence of three major polypeptides at 90, 60, and 48 kDa (Figure 2E). The 60-kDa polypeptide could be visualized on SDS–PAGE by Coomassie blue R250, but not silver staining. The yield of the helicase was ~5 μg from ~125 g of yeast (Table I).

RPA and SSB Stimulated the ATPase and Helicase Activities. In an earlier report (Biswas et al., 1993b), we described the association of a helicase activity with a high molecular weight, multiprotein complex of polymerase α

Table I: Purification Table of Pol α-Associated DNA Helicase

fraction	purification step	volume (mL)	protein (mg/mL)	total protein (mg)
I	extract	330	11.4	3762
II	immunoaffinity	0.25[a]	2.3	0.57
III	low-salt SEHPLC	0.3[a]	0.3	0.09
IV	high-salt SEHPLC	0.05[a]	0.11	0.0055

[a] Volume and protein concentration of the Amicon YM30 concentrated active pools are reported.

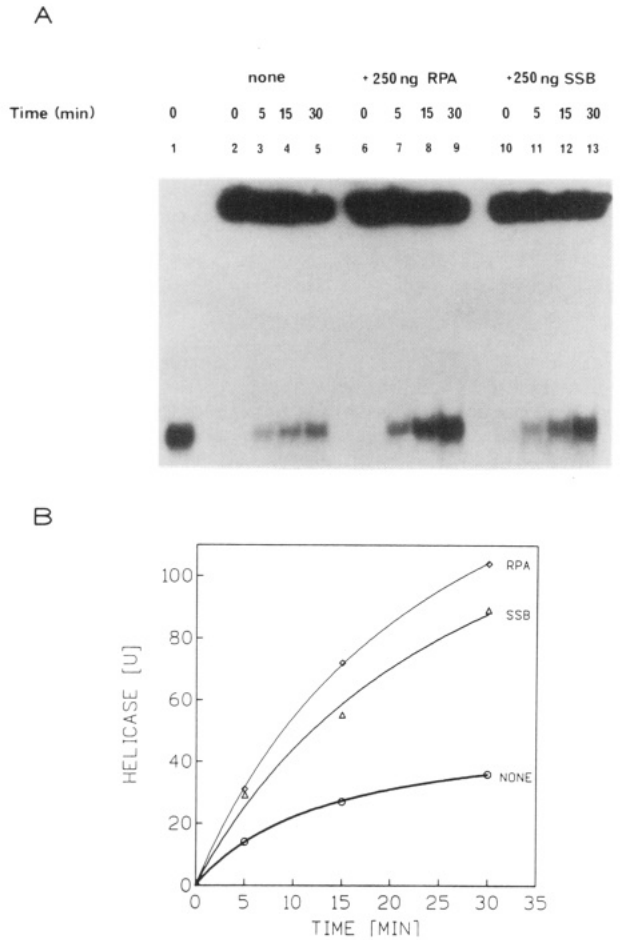

FIGURE 3: Effects of RPA and SSB on the helicase activity. (A) Kinetic analysis of 200 ng of helicase, Fr III, was carried out in the absence or presence of RPA or SSB as follows: lane 1, heat-denatured substrate; lanes 2–5, 0, 5, 15, and 30 min, respectively, without SSB or RPA; lanes 6–9, 0, 5, 15, and 30 min, respectively, in the presence of 250 ng of RPA; lanes 10–13, 0, 5, 15, and 30 min, respectively, in the presence of 250 ng of SSB. (B) Densitometric analysis of the autoradiogram shown in panel A and expressed as arbitrary scan units. The mean error is ±5 units.

(Biswas et al., 1993a,b). That activity was stimulated by RPA. Consequently, we have examined the effect of RPA on the ATPase/helicase as isolated by modified immunoaffinity chromatography. Results of the stimulation studies are presented in Figure 3A,B. It is evident from the autoradiogram in Figure 3A that the DNA helicase (200 ng, Fr III) alone can unwind the partial duplex DNA at a reasonably slow rate and appeared to saturate at about 15 min (Figure 3B). In the presence of 250 ng of RPA, the rate of unwinding was increased 3-fold and appeared to continue for a longer time period. A similar, although not as great, effect was observed when SSB was added to the reaction mixture. These results are in agreement with our previous studies with the DNA helicase activity associated with the multiprotein pol α complex (Biswas et al., 1993b).

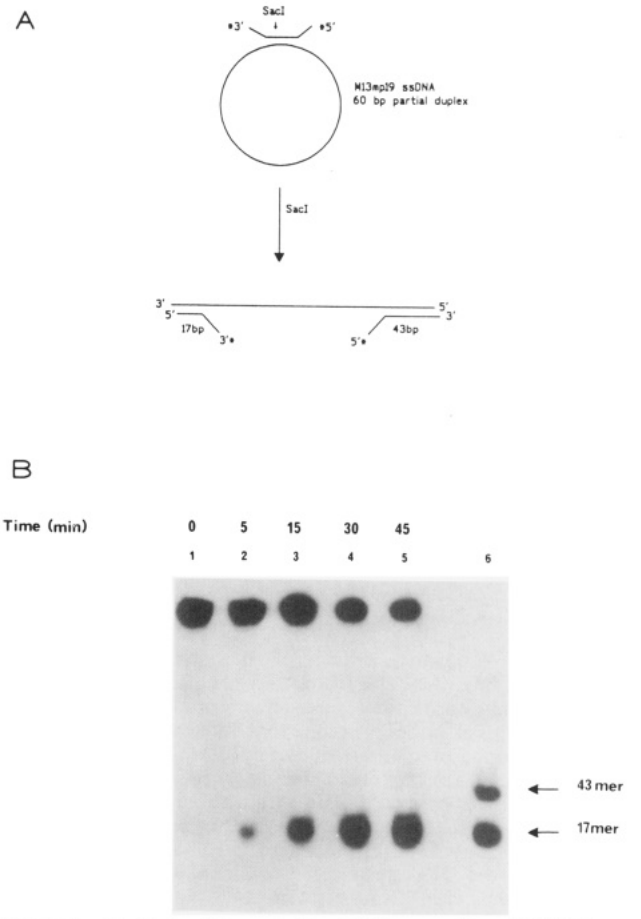

FIGURE 4: Analysis of the polarity of helicase action. (A) Scheme of the preparation of the linear polarity substrate used in this study. Preparation of the substrate has been described under Materials and Methods. (B) Kinetic analysis of the unwinding of the linear substrate shown in (A) with 200 ng of helicase, Fr III, and 250 ng of RPA. The reactions were allowed to proceed for the times indicated: lane 1, 0 min; lane 2, 5 min; lane 3, 15 min; lane 4, 30 min; lane 5, 45 min; lane 6, heat-denatured substrate. RPA alone (250 ng) did not have any helicase activity.

Polarity of the Helicase Action on the DNA Template. The polarity of the DNA helicase in a multiprotein pol α complex was ambiguous (Biswas et al., 1993b). The ambiguity was likely due to the presence of several other DNA binding factors in the complex that also contributed to the direction of migration. However, it was anticipated that separation of the DNA helicase from the other pol α-associated proteins would enable us to determine the polarity of the helicase unequivocally.

The polarity of the helicase was examined using the linear substrate with partial duplex ends depicted in Figure 4A. As described by Matson and Kaiser-Rogers (1990), we assumed that the DNA helicase would bind randomly to the RPA-coated M13 ssDNA template, and would migrate in a specific direction. The release of the 17-mer oligonucleotide would indicate a 5′→3′ direction of movement, while release of the 43-mer oligonucleotide would indicate a 3′→5′ direction of movement. Results of kinetic studies of unwinding using the linear polarity substrate and 200 ng of DNA helicase (Fr III) in the presence of 250 ng of RPA at time periods between 0 and 45 min are presented in Figure 4B. Clearly, the 17-mer oligonucleotide was the sole product, which indicated a 5′→3′ movement of the helicase along the substrate. The polarity of the helicase was not influenced by the RPA except that RPA enhanced the helicase activity (data not shown). In addition, the amounts of RPA used in the helicase activity

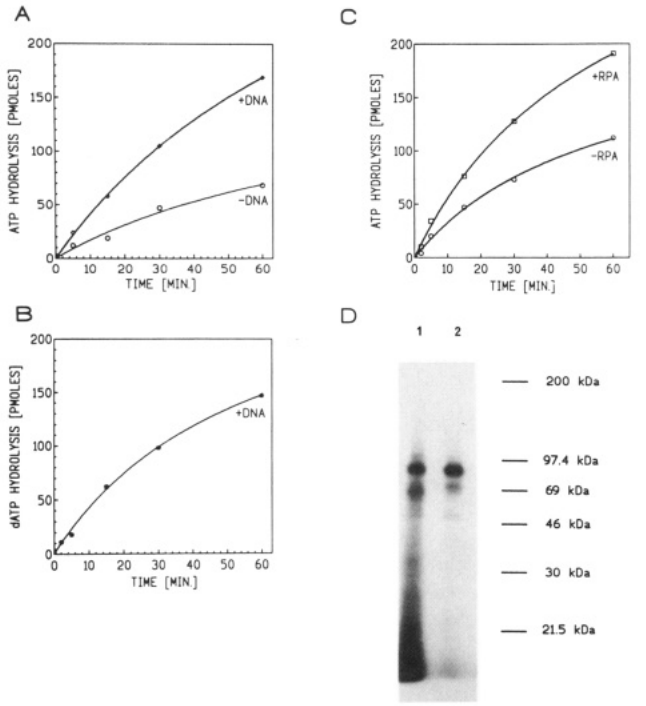

FIGURE 5: Analysis of the ATPase activity of the helicase. ATPase and dATPase assays were carried out using 150 ng of Fr III complex in the presence (or as indicated) of 200 pmol of M13mp18 ssDNA. (A) Kinetic analysis of the ATPase activity of the helicase in the presence and absence of M13 ssDNA. (B) Kinetic analysis of the dATPase activity of the helicase. (C) Influence of RPA on the ATPase activity of the helicase in the presence and absence of 250 ng of RPA. (D) Photo-cross-linking of [α-^{32}P]ATP to the helicase. The photo-cross-linking was carried out as described under Materials and Methods: lane 1, 1.0 μg of Fr III; lane 2, 1.0 μg of Fr IV.

assay did not unwind the partial duplex at either termini, similar to results reported earlier (Biswas et al., 1993b).

Analyses of the ATPase Activity and the ATP Binding Site of the DNA Helicase. Analysis of the ATPase activity showed a 2–3-fold stimulation in the presence of ssDNA (Figure 5A). The ATPase was also able to hydrolyze dATP with comparable efficiency (Figure 5B). Consequently, the isolated DNA helicase was a ssDNA-stimulated ATPase/dATPase. In the presence of M13mp19 ssDNA, the ATPase was stimulated approximately 80% by RPA (Figure 5C). RPA did not have any ATPase activities of its own under these reaction conditions (data not shown). The RPA stimulation, observed in the present study, was significantly higher than that observed with the ATPase activity of the multisubunit pol α. In order to identify the ATP binding polypeptide, photo-cross-linking with [α-^{32}P]ATP was carried out. It appeared that the 90-kDa polypeptide (p90) was the predominantly labeled polypeptide (Figure 5D). A polypeptide of similar size was found in our earlier studies to cross-link to [α-^{32}P]-ATP in the pol α multiprotein complex (Biswas et al., 1993a,b).

DISCUSSION

Concerted actions of multiple enzymes and proteins are often required for various nuclear events. Large multiprotein complexes that enhance the efficiency and coordinate the actions of a number of enzymes in a single event in the chromosome are involved in various processes such as DNA transcription and replication. As polymerases play central roles in many of these nuclear processes, they appear to be uniquely involved in the formation and organization of such complexes. DNA polymerase α has been shown, unequivocally, to execute a major function in the replication of the chromosomal DNA. Thus, pol α becomes a center for formation of a large multiprotein complex, bringing together and uniting a number of replication enzymes. Multiprotein DNA polymerase α complexes have been isolated and characterized from a number of organisms (Hübscher et al., 1982; Pritchard & DePamphilis, 1983; Pritchard et al., 1983; Vishwanath et al., 1986; Biswas & Biswas, 1988; Malkas et al., 1990; Biswas et al., 1993a,b). Therefore, it is becoming increasingly clear that a number of unique enzymatic activities, necessary for the efficient replication of the chromosomal DNA, are present in these complexes. However, because many of these enzymes appear to be of low copy number and the complexes fragile and prone to dissociation, it is difficult to identify and characterize these components. In the case of multisubunit DNA polymerase III holoenzyme of *E. coli*, the polypeptide assembly could be easily disrupted with high salt, and all of the accessory subunits could be dissociated from the core form of pol III by hydroxyapatite chromatography in 1 M NaCl (McHenry & Crow, 1979). In addition, Miles and Formosa (1992) have demonstrated by protein affinity chromatography that a number of yeast proteins specifically bind to the large subunit (180 kDa) of yeast pol α. The bound proteins were eluted from the protein affinity column using a linear NaCl gradient.

Immunoaffinity chromatographic techniques have been increasingly used in the purification of DNA polymerase α in recent years. Most of these procedures invariably result in stringent elimination of all but the core polypeptides from the antibody-bound pol α in the matrix. The standard method of procedure is treatment of the column with 1 M NaCl prior to elution of the antigen in order to completely remove all nonspecific proteins, and only antigen remains bound to the antibody in the matrix. However, during immunoaffinity purification of pol α, the high-salt elution should also efficiently remove any accessory proteins of pol α (except very tightly bound core proteins) in addition to the polypeptide impurities. Therefore, we have carried out the chromatography at low ionic strength (50 mM NaCl). The column was extensively washed, and bound pol α-associated proteins were eluted using a salt gradient. The results presented here indicated that the ATPase/helicase activities along with other pol α-associated polypeptides were eluted from the immunoaffinity matrix-bound pol α by a linear NaCl gradient from 50 to 1000 mM. Impurities, if any, and other accessory proteins were removed from the helicase by SEHPLC. Perhaps this is the simplest method that can be used for rapid isolation and identification of the rare enzymes that are involved in chromosomal DNA replication.

The purified DNA helicase appeared to contain three polypeptides of 90, 60, and 50 kDa. Photo-cross-linking studies clearly demonstrated that the 90-kDa polypeptide was the major polypeptide that bound ATP and thus the likely site of DNA helicase and ATPase activities, which is similar to that found with the multiprotein pol α complex (Biswas et al., 1993b). Its relationship with the other two polypeptides or the helicase activity that copurified remains unknown and will require further investigation. The ATPase and dATPase activities of the purified helicase were comparable and were similarly stimulated by ssDNA and RPA. A detailed analysis of various nucleic acid effectors on the ATPase activity has been published earlier (Biswas et al., 1993b). RPA-coated ssDNA appeared to be a better effector of the ATPase activity than DNA alone. The helicase activity was stimulated by both *E. coli* SSB and RPA. RPA appeared to be a better

stimulator of the helicase activity. DNA helicases have been shown to have unique polarity of action (Matson & Kaiser-Rogers, 1990). Our previous analysis of the polarity of the helicase in the pol α complex did not show a specific direction of movement, which was likely due to the presence of other subunits in the complex. In the polymerase-free state, the helicase displayed unequivocally a 5′→3′ direction of movement which is also the overall direction of migration of pol α in the lagging strand of the replication fork.

The modified immunoaffinity chromatography, described here, appeared to be a very rapid and efficient means of isolating the DNA helicase that forms a complex with pol α. We have not explored all of the various activities of the eluted polypeptides by this procedure; however, it appears that other pol α-associated proteins could be isolated and characterized from yeast and other eukaryotic organisms using this procedure.

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