Isolation and Characterization of a Polyol-Responsive Monoclonal Antibody Useful for Gentle Purification of *Escherichia coli* RNA Polymerase†

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ABSTRACT: A modified enzyme-linked immunosorbent assay (ELISA) was used to screen monoclonal antibodies (MAbs) that react with *Escherichia coli* RNA polymerase for the ability to release the RNA polymerase in the presence of a low molecular weight polyhydroxylated compound (polyol) and a nonchaotropic salt. This assay, termed the ELISA-elution assay, identified 19 presumptive "polyol-responsive" MAbs out of a total of 218 antigen-specific MAbs screened. One of these MAbs, designated NT73, was examined in detail for the ability to release the antigen in response to various combinations of polyol and salt. Using NT73 conjugated to Sepharose, highly active RNA polymerase could be prepared rapidly by a single immunoaffinity chromatography step, replacing two lengthy chromatographic steps in our conventional purification procedure. Because NT73 reacts with the β' subunit of RNA polymerase, a mixture of the core polymerase and holoenzyme was recovered from the immunoaffinity column. The holoenzyme ($E\sigma^{70}$) could be separated from the core polymerase by subsequent chromatography on a Mono Q column. This demonstrates that polyol-responsive MAbs can be easily identified and characterized by the ELISA-elution assay. The use of polyol-responsive MAbs provides a means of adapting immunoaffinity chromatography to the purification of labile proteins.

Transcription is a highly regulated sequence of biochemical reactions that results in an RNA product. In eubacteria, transcription of all classes of RNA is accomplished by one RNA polymerase. This enzyme is multimeric, consisting of five subunits designated β', β, α, σ, and ω with the α subunit present as a dimer Enzyme lacking the σ subunit ($\alpha_2\beta'\beta\omega$) is capable of synthesizing RNA in a nonspecific manner; this form of the enzyme is referred to as the core RNA polymerase. When the σ subunit is added to the enzyme ($\alpha_2\beta'\beta\omega\sigma$), RNA is synthesized that is specifically initiated at promoters; this form of the enzyme is referred to as the holoenzyme. Thus, the σ subunit is responsible for promoter recognition. Several σ factors have been identified that recognize different types of promoters and thus regulate certain classes of genes in response to specific stimuli [reviewed in Gross et al. (1992), Helmann and Chamberlin (1988), Kustu (1989), and Reznikoff et al. (1985)]. However, most genes in *Escherichia coli* are transcribed by RNA polymerase containing the major σ factor, σ^{70}. This enzyme is designated $E\sigma^{70}$. The β' subunit seems to be involved in interation with the DNA, and the β subunit seems to bind nucleotides. The α subunit seems to function in assembly of the enzyme. No function has been assigned to the ω subunit. Several reviews are available that address the structure of RNA polymerase and regulation of transcription in bacteria (Gross et al., 1992; Helmann & Chamberlin, 1988; Kustu, 1989; McClure, 1985; Reznikoff et al., 1985; von Hippel et al., 1984).

Examination of transcription in vitro requires the availability of highly purified RNA polymerase. Several procedures have been published that describe chromatographic separation of *E. coli* RNA polymerase from other proteins (Burgess & Jendrisak, 1975; Gonzalez et al., 1977; Lowe et al., 1979; Hager et al., 1990). Recently, eukaryotic RNA polymerase II was purified to near-homogeneity by an immunoaffinity chromatography procedure (Edwards et al., 1990; Thompson et al., 1990). This procedure took advantage of a monoclonal antibody (MAb) that releases the antigen in response to combinations of low molecular weight polyhydroxylated compounds (polyols) and nonchaotropic salts. This MAb (designated 8WG16) was described as "polyol-responsive." In addition, the response of MAb 8WG16 to elution by the salt/polyol combination could be predicted by a modified enzyme-linked immunosorbent assay (ELISA), designated ELISA-elution assay (Thompson et al., 1990).

In this study, we used the ELISA-elution assay to screen MAbs that react with *E. coli* RNA polymerase for identification of polyol-responsive MAbs. We then developed an immunoaffinity chromatography procedure for the purification of this enzyme. This purification procedure promises to be useful for rapid purification of *E. coli* RNA polymerase, for examining mutations in the enzyme, and for determining the competition of different σ factors for the core polymerase.

MATERIALS AND METHODS

Buffer and Reagents. All reagents were obtained from Sigma Chemical Co. (St. Louis, MO) unless otherwise indicated. A 10% solution of poly(ethylenimine) (Polymin P; BASF; Charlotte, NC) was prepared as described (Burgess & Jendrisak, 1975). TE buffer contained 50 mM Tris-HCl (pH 7.9) and 0.1 mM EDTA. Storage buffer (SB) contained 10 mM Tris-HCl (pH 7.9), 0.1 mM EDTA, 0.1 mM DTT, 100 mM NaCl, and 50% glycerol. TGED buffer contained 10 mM Tris-HCl (pH 7.9), 5% glycerol, 0.1 mM EDTA, and 0.1 mM DDT. Spectrophotometric-grade ethylene glycol, that was sealed to prevent extended exposure to oxygen, was obtained from Aldrich (Milwaukee, WI). Spectrophotometric-grade glycerol was obtained from Fisher (Fair Lawn, NJ). Poly(dA-dT) was obtained from Pharmacia (Piscataway, NJ). ^{3}H-Labeled UTP was obtained from Dupont (Wilmington, DE). Bacteriophage T7 DNA was a gift from Hans Liao at

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the University of Wisconsin Biotechnology Center. All pH values were determined at 23 °C.

Hybridomas and MAbs. Three female Balb/c ByJ mice (Jackson Laboratories, Bar Harbor, ME) were injected subcutaneously and intraperitoneally with 20 μg of core RNA polymerase that had been purified by the method of Burgess and Jendrisak (1975). Injections were administered 10 days apart. The first injection was contained in Freund's complete adjuvant. Subsequent injections were contained in Freund's incomplete adjuvant. Immune responses were monitored by ELISA assays performed on dilutions of serum collected by supraorbital bleeding. All three animals showed a titer of at least 1:6400 after the third injection. One animal was injected 30 days after the last injection with 70 μg of core RNA polymerase contained in PBS and administered intraperitoneally. Three days later, the animal was sacrificed, and the spleen cells were fused with either NS-1 or SP2/0 plasmacytoma cells by standard procedures. Fusions were screened for core-reactive MAbs by a standard ELISA; positive hybridomas were rescreened by the ELISA-elution assay (see below). Hybridomas of interest were cloned at least twice by limiting dilution. Hybridoma cells were injected into Pristane-primed Balb/c ByJ mice for the production of ascites fluid. Isotyping was performed by using an ELISA-based isotyping kit (Boehringer-Mannheim, Indianapolis, IN).

Antibody NT73 was purified from ascites fluid by precipitation with 45% saturated ammonium sulfate and subsequent chromatography on DE52 (Strickland et al., 1988). At pH 7.0, this MAb flows through the DE52 column. Antibody NT63 was purified from ascites fluid by adsorption onto protein A immobilized on cross-linked agarose (Repligen; Cambridge, MA) and elution with 0.75 M acetic acid. Antibody concentration was determined by the absorbance at 280 nm, using an extinction coefficient ($E^{1\%}_{1\,cm}$) of 13.8.

ELISA and ELISA-Elution Assay. ELISA and ELISA-elution assays were performed as described (Thompson et al., 1990), using 50 μL of core RNA polymerase (2 μg/mL) to coat each microtiter plate well.

Electrophoresis and Immunoblots. Proteins were separated by electrophoresis by the method of Laemmli (1970), using a separating gel containing a gradient of 4–20% polyacrylamide (Integrated Separation Systems, Hyde Park, MA) or continuous 7.5% polyacrylamide and 0.1% SDS. Electrophoresis was performed in a Mighty Small apparatus (Hoefer Scientific Instruments, San Francisco, CA). For immunoblots (Western blots), protein was transferred to nitrocellulose, blocked with 1% nonfat dried milk (Johnson et al., 1984), reacted with a 1:500 dilution of ascites fluid, and processed as described previously (Thompson et al., 1989).

Transcription Assays. Reaction volumes were 100 μL and contained diluted enzyme (0.3–2 μg per assay), 25 mM Tris-HCl (pH 7.9), 100 mM NaCl, 10 mM $MgCl_2$, 2 mM EDTA, 1 mM DTT, 0.2 mM each of CTP, GTP, and ATP, 0.05 mM UTP containing 1 μCi of [^{3}H]UTP, and 1 μg of either poly(dA-dT) or T7 DNA. Reactions were incubated at 37 °C for 10 min and then stopped by the addition of 10 μL of a solution containing 0.2 M sodium pyrophosphate and 0.2 M EDTA. A portion (75 μL) of each reaction was applied to DEAE-cellulose disks (McLeester Research Equipment, Inc., Madison, WI). The disks were air-dried and washed with sodium phosphate as described (Somers & Pearson, 1975). The disks were placed in scintillation vials containing 5 mL of BioSafe II (Research Products International) and counted.

Preparation of Immunoadsorbents. Purified antibody was conjugated to cyanogen bromide activated Sepharose 4B (Sigma) at a concentration of 2.5 mg of MAb/mL of swollen gel. Conjugation was performed in 0.1 M $NaHCO_3$ (pH 8.3) containing 0.5 M NaCl for 2 h at room temperature. Unreacted groups were blocked by reaction with 1 M ethanolamine at pH 8.3 for 2 h at room temperature. The antibody-conjugated Sepharose was washed alternatively with several changes of 0.1 M $NaHCO_3$ (pH 8.3) containing 0.5 M NaCl and 0.1 M sodium acetate (pH 4.0) containing 0.5 M NaCl. Antibody-conjugated Sepharose was stored in 0.1 M $NaHCO_3$ (pH 8.3) containing 0.5 M NaCl and 0.02% NaN_3 at 4 °C.

Purification of RNA Polymerase. Core RNA polymerase that was used for injections and ELISA was prepared by the method of Burgess and Jendrisak (1975). *E. coli* holoenzyme that was prepared by the conventional chromatography procedure was purified by the method of Hager et al. (1990).

E. coli cell extract that was to be subjected to the immunoaffinity chromatography procedure was prepared in the following way. Ten grams (wet weight) of *E. coli* K12 (MG1655) cells, that had been grown to mid-log phase in LB medium and frozen at −70 °C, was resuspended in 30 mL of grinding buffer containing 260 μg of lysozyme/mL. After incubation for 10 min at 10 °C, sodium deoxycholate was added to a final concentration of 0.2% (w/v). The extract was sonicated 3–5 times for 30 s each on ice. The volume was increased to 60 mL with TGED containing 0.2 M NaCl and centrifuged (15 min at 12000*g*). The supernatant was collected, and poly(ethylenimine) (Polymin P) was added to a final concentration of 0.6%. The precipitate was centrifuged (10 min at 12000*g*), and the pellet was washed twice with 30 mL each of TGED containing 0.5 M NaCl. RNA polymerase was extracted from the pellet with 30 mL of TGED containing 1 M NaCl. The extract was centrifuged to remove the residual precipitate, and the supernatant was collected. Ammonium sulfate (35 g/100 mL) was added to the supernatant, and after mixing for 30 min at 4 °C, the precipitate was collected by centrifugation. The ammonium sulfate pellet was washed with 15 mL of 50% saturated ammonium sulfate in TGED. The precipitate was dissolved by resuspending the washed pellet in TE buffer and diluting to a conductivity equivalent to that of 0.15 M NaCl (generally about 12 mL). RNA polymerase was adsorbed onto approximately 2 mL of NT73-conjugated Sepharose by gentle mixing for 3 h at 4 °C. The immunoadsorbent was collected by centrifugation, washed twice with 10 mL of TE containing 0.15 M NaCl at room temperature, and poured into an Omni column (Rainin, Woburn, MA). The RNA polymerase was eluted at room temperature with TE containing 30% ethylene glycol and 0.7 M NaCl using a flow rate of 0.5 mL/min. Fractions were analyzed by SDS–PAGE, and fractions that were enriched for the holoenzyme were pooled and dialyzed against TGED containing 0.25 M NaCl. This material was applied to a 1-mL Mono Q column (Pharmacia), washed with TGED containing 0.30 M NaCl for 10 min, and eluted with a linear gradient of TGED containing 0.35–0.45 M NaCl in 180 min as described (Hager et al., 1990). Fractions containing purified holoenzyme were pooled and dialyzed against SB.

Protein Determinations. Protein concentrations of purified RNA polymerase were determined by adsorbance readings at 280 nm corrected for light scattering, and using an extinction coefficient ($E^{1\%}_{1\,cm}$) of 6.2 (Burgess, 1976).

RESULTS

Identification of Polyol-Responsive MAbs. To identify polyol-responsive MAbs that react with *E. coli* RNA polymerase, hybridomas producing MAbs that reacted with the core

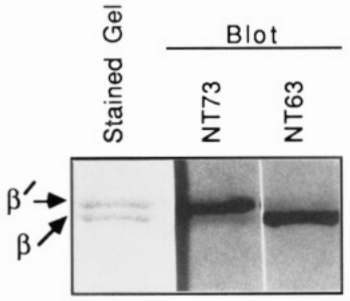

FIGURE 1: SDS–PAGE and immunoblots of the β' and β subunits of *E. coli* RNA polymerase. One-third of the 7.5% gel was stained with Coomassie Blue. The rest of the gel was transferred to nitrocellulose and reacted with a 1:500 dilution of ascites fluid containing either MAb NT73 or MAb NT63.

RNA polymerase in a standard ELISA were rescreened by the ELISA-elution assay. The immobilized core RNA polymerase was reacted with the MAb in duplicate wells. One well was treated with 100 μL of the TE buffer; the other well was treated with 100 μL of TE containing 1 M NaCl and 50% ethylene glycol as described previously (Thompson et al., 1990). After the eluting reagent was washed away, the enzyme-conjugated secondary antibody was allowed to bind. After being washed, substrate was added, and the reactions were read on a microtiter plate reader. A MAb was considered to be polyol-responsive if the optical density reading of the well treated with 1 M NaCl and 50% ethylene glycol was 50% or less than the optical density reading of the well treated with the TE buffer. A total of 218 hybridomas were rescreened by this method; 19 of these hybridomas appeared to produce polyol-responsive MAbs by the ELISA-elution method. Six of these presumptive polyol-responsive MAbs were cloned and examined in detail for the ability to purify RNA polymerase from *E. coli*. Two of the MAbs reacted with β' subunit, one antibody reacted with the α subunit, and three MAbs did not react with denatured protein on immunoblots. Preliminary experiments indicated that MAb NT73 was probably the superior MAb for use in immunoaffinity chromatography because of its higher affinity and its increased sensitivity to polyol and salt combinations. A control antibody was also selected. This antibody (NT63) did not show polyol-responsiveness in the ELISA elution assay.

The subunit specificity of the MAbs was determined by immunoblots. NT73 reacts with the β' subunit, and NT63 reacts with the β subunit (Figure 1).

When tested in an elongation reaction, NT73 did not inhibit transcription (data not shown).

NT73 is an IgG_1 antibody, and NT63 is an IgG_{2a} antibody.

Polyol-Responsiveness of NT73. Antibodies NT73 and NT63 were examined by the ELISA-elution assay for response to different combinations of polyols (40%) and salts (0.75 M). These levels of polyol and salt were selected because this was the maximum solubility of some of the salts in some of the polyols. Polyols tested were ethylene glycol, propylene glycol, 2,3-butanediol, and glycerol. The data in Table I show that NT73 responds to all polyols except glycerol when combined with NaCl, ammonium sulfate, sodium acetate, sodium pyruvate, and potassium glutamate. Antibody NT73 did not respond significantly to any of the salts in the absence of polyol; neither did it respond significantly to any polyol in the absence of salt. The control antibody, NT63, did not respond to any combination of polyol and salt (data not shown).

Effective Levels of Polyol and Salt on NT73. Because high levels (50%) of ethylene glycol can inactivate eukaryotic RNA polymerase II (Thompson et al., 1990), MAb NT73

Table I: Response[a] of NT73 to Different Combinations of Polyols and Salt[b] in the ELISA-Elution Assay

	polyol				
salt	no polyol	ethylene glycol	propylene glycol	2,3-butanediol	glycerol
no salt	1.86	1.85	1.82	1.72	1.78
NaCl	1.73	0.86	0.39	0.34	1.54
ammonium sulfate	1.79	0.51	0.35	0.32	1.56
sodium acetate	1.83	1.05	0.43	0.39	1.62
sodium pyruvate	1.77	0.77	0.40	0.32	1.58
potassium glutamate	1.80	1.04	0.44	0.38	1.68

[a] Average optical density readings of two trials. [b] All solutions were contained in TE buffer. All polyols were at 40% (v/v) and all salts were 0.75 M final concentration.

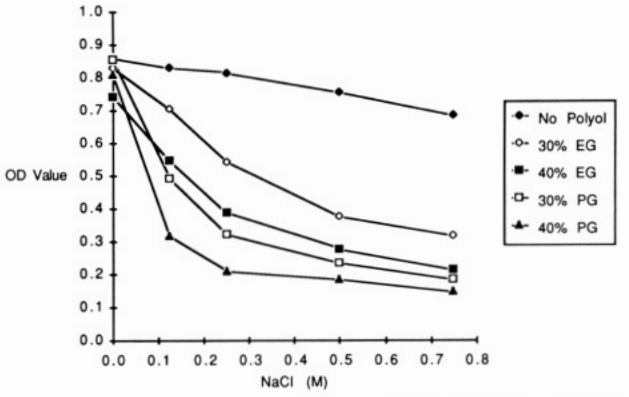

FIGURE 2: ELISA-elution assay using varying concentrations of salt and polyol. Core RNA polymerase was immobilized onto polystyrene and reacted with MAb NT73. The antigen–antibody complex was treated with TE containing 30% and 40% polyol (ethylene glycol or propylene glycol) in combination with 0–0.75 M NaCl.

was examined by the ELISA-elution assay to determine the effective levels of polyol and salt that would disrupt the antigen–antibody interaction. The concentrations of polyol and salt were varied to cover the range of 0–0.75 M salt in the presence of 30% and 40% polyol. Most salts were effective in the range of 0.5–0.75 M when combined with 30–40% polyol. A representative example using NaCl and two polyols is shown in Figure 2. In order to obviate any denaturing effect on the enzyme by using higher than necessary concentrations of polyol, we decided to use 30% polyol and 0.7 M salt in all further purification procedures.

Confirmation of Polyol-Responsiveness of NT73. To establish that the polyol/salt conditions that were effective in the ELISA-elution assay actually worked in a chromatographic situation, three different salts and two different polyols were tested for the ability to elute RNA polymerase from the immobilized MAb. *E. coli* extracts were prepared, and the RNA polymerase was adsorbed to the immobilized NT73 as described under Materials and Methods. After being extensively washed with TE containing 0.2 M ammonium sulfate, the immunoadsorbent was divided into 1-mL aliquots and eluted with 6 mL of TE containing salt (0.7 M) and polyol (30%). All eluates were dialyzed against SB. The SDS–polyacrylamide gel in Figure 3 shows the enzyme eluted with each polyol/salt combination tested. Sodium acetate was not as effective at eluting the enzyme as either NaCl or ammonium sulfate with either polyol tested. Activity assays showed that the sodium acetate/polyol combinations also had lower specific activity than the NaCl or ammonium sulfate/polyol combinations (data not shown). NaCl combined with ethylene glycol and ammonium sulfate combined with propylene glycol seemed to be the superior combinations in terms of amount of enzyme recovered and specific activity of the recovered enzyme. NaCl

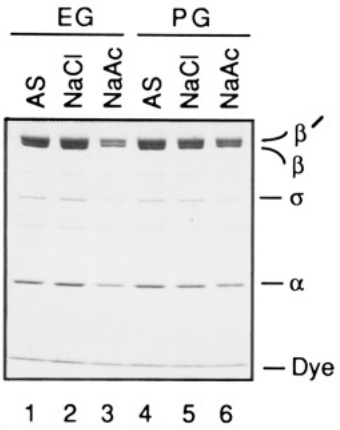

FIGURE 3: SDS–PAGE of *E. coli* RNA polymerase eluted from NT73 with different polyol/salt combinations. Lanes 1–3 contained material that was eluted with 30% ethylene glycol combined with 0.7 M ammonium sulfate (lane 1), 0.7 M NaCl (lane 2), or 0.7 M sodium acetate (lane 3). Lanes 4–6 contained material that was eluted with 30% propylene glycol combined with 0.7 M ammonium sulfate (lane 4), 0.7 M NaCl (lane 5), or 0.7 M sodium acetate (lane 6). Equal volumes were loaded in each lane. The gel was stained with Coomassie Blue.

and ethylene glycol were chosen for further studies because the ethylene glycol was available in a high-quality grade, packaged in sealed containers to prevent oxidation during storage.

When the control MAb NT63 was used in the immunoaffinity chromatography procedure and eluted with 30% ethylene glycol and 0.7 M NaCl, less than 10% of the RNA polymerase bound by the antibody column was recovered (data not shown).

Purification of Active RNA Polymerase by Immunoaffinity Chromatography. While the use of the immunoaffinity chromatography step seemed to provide a significant improvement in the speed of RNA polymerase purification, we needed to demonstrate that the holoenzyme could be purified using the immunoaffinity procedure with at least the yield, purity, and specific activity of the conventional procedure. *E. coli* K12 cells were processed as described under Materials and Methods through the ammonium sulfate precipitation step. One preparation (161) was processed through the conventional procedure (involving chromatography on DNA–cellulose, Sephacryl S-300, and Mono Q columns). Another identical preparation (170) was processed using the new immunoaffinity chromatography step followed by chromatography on the Mono Q column. The elution profile of the immunoaffinity column during elution with 30% ethylene glycol and 0.7 M NaCl is shown in Figure 4. The initial sharp peak (peak A) contained some RNA polymerase and most of the contaminating proteins (some of which were identified as noted under Discussion). The slowly eluting shoulder (peak B) contained RNA polymerase with very few contaminating proteins. The SDS–polyacrylamide gel in Figure 5 shows the starting material for the immunoaffinity chromatography step (lane 1) and the two pooled peaks (lanes 4 and 5). Peak B was further purified by Mono Q chromatography to separate the holoenzyme from the core polymerase (Hager et al., 1990). The holoenzyme eluted from the Mono Q column (Figure 5, lanes 6 and 7) was as pure as the holoenzyme prepared by the conventional procedure (Figure 5, lanes 8 and 9).

In Table II, we summarize yield and specific activity of RNA polymerase purified by the conventional and immunoaffinity chromatography procedures after the final Mono Q step. Two kinds of assays were performed: one used poly(dA-dT) as a template to measure the nonspecific transcription activity; the other assay used bacteriophage T7 DNA as the template to measure promoter-specific activity. In both assays, the final Mono Q-purified RNA polymerase from the immunoaffinity chromatography procedure was higher in specific activity than the RNA polymerase purified by the conventional method. The final yield of the holoenzyme from the immunoaffinity chromatography procedure was greater than that recovered from the conventional procedure.

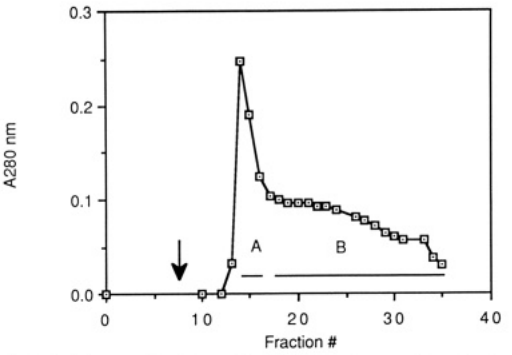

FIGURE 4: Elution profile from the NT73 column after elution with TE containing 0.7 M NaCl and 30% ethylene glycol. RNA polymerase was adsorbed onto the NT73-conjugated Sepharose in a batch mode at 4 °C. The immunoadsorbent was washed in a batch mode with TE containing 0.15 NaCl at room temperature, poured into a column, and eluted with TE containing 30% ethylene glycol and 0.7 M NaCl (started at arrow). Peak A (fractions 14–16) was discarded, and peak B (fractions 17–35) was applied to a Mono Q column to separate the core polymerase from the holoenzyme.

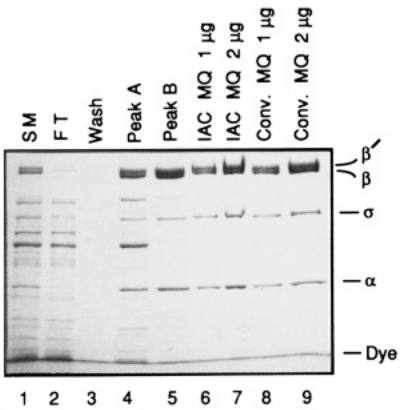

FIGURE 5: SDS–PAGE of material at different steps in the immunoaffinity chromatography purification of *E. coli* RNA polymerase. The gel was a commercially prepared 4–20% gradient gel and was stained with Coomassie Blue. Lane 1 contained 2 μL of the starting material for the immunoaffinity step (SM). Lane 2 contained 2 μL of the flow-through fraction from the immunoaffinity chromatography step (FT). Lane 3 contained 10 μL of the TE plus 0.15 M NaCl wash step. Lane 4 contained 10 μL of pooled peak A eluted from the immunoaffinity column (see Figure 4). Lane 5 contained 15 μL of pooled peak B eluted from the immunoaffinity column (see Figure 4). Lanes 6 and 7 contained 1 and 2 μg, respectively, of the holoenzyme peak from the Mono Q column from the immunoaffinity purification procedure. Lanes 8 and 9 contained 1 and 2 μg, respectively, of the holoenzyme peak from the Mono Q column from the conventional purification procedure.

DISCUSSION

Previously we reported a procedure whereby eukaryotic RNA polymerase II can be purified by immunoaffinity chromatography by the use of a polyol-responsive MAb (Edwards et al., 1990; Thompson et al., 1990). In the previous study, we fortuitously isolated a MAb that responded to this polyol-elution method. In this paper, we demonstrate that the

Table II: Comparison of Conventional and Immunoaffinity Purification of *E. coli* RNA Polymerase

prepn of fraction[a]	sp act. relative to 161 MQ: dAT	sp act. relative to 161 MQ: T7 DNA	yield (mg/10 g of cells)
conventional			
161 SE			3.76
161 MQ	1.00	1.00	0.46[b]
immunoaffinity			
170 IAC			2.16
170 MQ	1.42	1.76	0.78[b]

[a] 161 SE is the RNA polymerase fraction after chromatography on Sephacryl S-300. 161 MQ is the holoenzyme fraction after chromatography of the 161 SE fraction on Mono Q. 170 IAC is the fraction eluted from the immunoaffinity column. 170 MQ is the holoenzyme fraction after chromatography of the 170 IAC fraction on Mono Q.[b] Final purified RNA polymerase from the Mono Q column represents holoenzyme, containing σ^{70}. A core polymerase peak, lacking σ, contains an approximately equivalent or greater amount of polymerase protein, reflecting the fact that 30% or less of the core polymerase in the cell contains bound σ^{70}.

ELISA-elution assay can be used to identify polyol-responsive MAbs while the hybridomas are still in the original cell culture plates. Thus, the time-consuming cloning of many unwanted hybridomas can be avoided. Using the procedures described in this and the previous paper (Thompson et al., 1990), a polyol-responsive MAb that reacts with almost any protein of interest might be obtained. The polyol-elution procedure is gentle, and highly active enzyme can be recovered with high yields.

In this study, we obtained 19 presumptive-positive polyol-responsive MAbs from the 218 hybridomas tested in the ELISA-elution assay. On the basis of this figure, one might expect that 5–10% of MAbs are polyol-responsive. However, we have noted that false positives can be obtained. These MAbs seem to be polyol-responsive by the ELISA-elution assay, but they do not react with the protein in solution. We think that some antigens undergo a distortion when immobilized on polystyrene, causing some epitopes that normally are inaccessible to be exposed. The polyol/salt might relieve this distortion, causing the apparent polyol-responsiveness in the ELISA-elution assay. While the ELISA-elution assay can be used to identify polyol-responsive MAbs, and it can help to predict the elution conditions, it does not quantitatively indicate the exact conditions necessary to elute the antigen in an actual chromatographic situation.

It has been our experience that a polyol-responsive MAb generally responds to a variety of polyols and salts [Table I and Figure 3 and Thompson et al. (1990)]. However, unlike antibody 8WG16 (Edwards et al., 1990; Thompson et al., 1990), antibody NT73 does not respond well to combinations of salt and glycerol (Table I). Although the exact epitope for NT73 has not been determined, the β' subunit does not contain any sequence that resembles the unusual heptapeptide repeat of eukaryotic RNA polymerase II that is recognized by MAb 8WG16. Therefore, polyol-responsivity is not unique to the unusual structure of the heptapeptide repeat.

Ethylene glycol has been used in the past to elute antigen from immunoadsorbents (Andersson et al., 1979; Aerts et al., 1986; Bessos & Prowse, 1986; Croissant et al., 1986; Ganz et al., 1988; Lim, 1987). However, these reports all used high levels of ethylene glycol (50% or more), which we have found to be denaturing to eukaryotic RNA polymerase II (Thompson et al., 1990). In addition, elution was often carried out at pH 10 or higher (Andersson et al., 1979; Bessos & Prowse, 1986; Lim 1987), which is above the pH stability limits of many enzymes. Croissant et al. (1986) and Ganz et al. (1988) used 50% ethylene glycol at lower pH values (pH 6 and 7.4, respectively) and used NaCl in the elution buffer (0.1 and 1 M, respectively). However, none of these investigators systematically screened MAbs or optimized eluting conditions.

The mechanism by which polyol/salt gently but efficiently elutes the antigen from the antibody is not obvious. Intuitively, the two compounds should be working against each other—the polyol weakening the hydrophobic interactions and strengthening the electrostatic interactions—the salt weakening the electrostatic interactions and strengthening the hydrophobic interactions. Regardless of the mode of action, the result is a useful technique for rapidly, but gently, purifying very labile enzymes.

Antibody NT73 reacts with the β' subunit of RNA polymerase. Therefore, the immunoaffinity chromatography step isolates a mixture of the core polymerase and the holoenzyme. These forms can be separated by chromatography on a Mono Q column as described (Hager et al., 1990). For most purposes, the holoenzyme ($E\sigma^{70}$) is the desired form of the enzyme. By elution of the RNA polymerase from NT73–Sepharose in the column mode, two distinct peaks are obtained (Figure 4). Peak A contained the core subunits and several other bands, while peak B contained a mixture of the core polymerase and the holoenzyme with few contaminating proteins (Figure 5). In this study, we took only peak B and applied it to the Mono Q column to obtain holoenzyme (containing the major σ factor, σ^{70}). By analyzing the fractions from peak A with specific MAbs, we have observed that some of the extra protein bands are actually transcriptionally important proteins such as the nus A protein and σ^{54} (data not shown). Therefore, it is likely that NT73–Sepharose can be used to isolate alternative forms of the holoenzyme (those containing alternative σ factors).

Enzyme prepared by the immunoaffinity chromatography procedure was as active as enzyme prepared from the same batch of cells by the conventional purification procedure. The yield of holoenzyme recovered by both procedures was also comparable. The differences in recovery and specific activity noted in Table II are within batch to batch variation of holoenzyme preparations. The yield of the immunoaffinity-purified holoenzyme was surprisingly high in view of the large amount of polymerase that was contained in peak A from the immunoaffinity column and, consequently, excluded from the final yield of enzyme. However, selective pooling also occurred with the conventional procedure following chromatography on DNA-cellulose and Sephacryl S300.

Antibody NT73 reacts with the β' subunit of RNA polymerase from some of the other members of the enteric group of bacteria (data not shown), but it does not react with RNA polymerase from *Bacillus* sp. Therefore, it does not seem to be a widely cross-reacting MAb. However, it seems to be extremely useful for the rapid isolation of RNA polymerase from *E. coli* and possibly some other enteric bacteria. It will be very useful for examining RNA polymerase containing mutations in various subunits and for examining the distribution of σ factors associated with the core polymerase under different growth conditions.

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CORRECTIONS

Interaction of Oligonucleotides Containing 6-*O*-Methylguanine with Human DNA (Cytosine-5-)-methyltransferase, by Ngee-Wah Tan* and Benjamin F. L. Li, Volume 29, Number 39, October 2, 1990, pages 9234–9240

Page 9239. In the structure shown in the caption of Figure 6, the last two 5′ residues of the bottom strand should be GG.