Characterization of Effects of Anti-β and Anti-β′ Monoclonal Antibodies on the Activity of the RNA Polymerase from *Escherichia coli*†

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ABSTRACT: Monoclonal antibodies directed against antigenic determinants on the β and β′ subunits of the *Escherichia coli* RNA polymerase were characterized by using d(A-T)$_n$-directed transcription assays. Antibodies were prepared by using purified subunits as immunogens, and seven anti-β and five anti-β′ monoclonal antibodies were generated. Inhibitory anti-β monoclonal antibodies were found to affect RNA polymerase during synthesis of r(A-U)$_n$, abortive initiation of pApU and UpApU, and elongation by preformed ternary complexes. A comparative enzyme study of r(A-U)$_n$ synthesis showed the core polymerase to be more sensitive to inhibition by the anti-β monoclonal antibody than was the holoenzyme. In contrast, the inhibition effected by the anti-β′ monoclonal antibody was found to be 90% or greater for each of the d(A-T)$_n$-directed assays used. The different inhibitory patterns exhibited by the anti-β and anti-β′ monoclonal antibodies suggest that the β and β′ subunits engage in different roles during transcription. Kinetic analysis of the abortive initiation reaction in the presence and absence of the inhibitory antibodies resulted in distinctive but complex modes of inhibition. Inhibition by the anti-β monoclonal antibody 210E8 was noncompetitive with regard to UTP and competitive for UpA incorporation; at increased UpA concentration, the inhibition was completely reversed. Inhibition of the abortive synthesis of UpApU by the anti-β′ monoclonal antibody 311G2 was noncompetitive with regard to both UpA and UTP incorporation. When the preformed ternary elongation complex was used, inhibition by the anti-β monoclonal antibody was mixed with regard to the ribonucleoside triphosphate substrates. In reactions run as a function of the d(A-T)$_n$ concentration [for synthesis of r(A-U)$_n$], the apparent Michaelis constant was unchanged in the presence of the inhibitory anti-β monoclonal antibody 210E8 and reduced 3-fold for the inhibitory anti-β′ monoclonal antibody 311G2. The results obtained in this study are consonant with the role of the β subunit in catalysis by RNA polymerase. The data also point to an involvement of the β′ subunit in the catalytic function of RNA polymerase in addition to its previously defined role in template binding. The studies demonstrate that subunit-specific monoclonal antibodies are novel reagents with which to probe the relation of structure and function in RNA polymerase.

The *Escherichia coli* RNA polymerase is a large complex enzyme consisting of four different subunits, α, β, β′, and σ which engages in a complex sequence of steps required for the synthesis of RNA [for a recent review, see von Hippel et al. (1984)]. Involved are specific interactions with the DNA promoter sites and the attendent conformational changes in both the holoenzyme (Fisher & Blumenthal, 1980) and DNA (Gamper & Hearst, 1982) which precede RNA chain initiation. Thus far, the correlation of enzyme structure with function has not progressed much beyond the stage of assigning functions to particular subunits (Zillig et al., 1976). The enzyme has not yet been crystallized, and the three-dimensional structure of the *E. coli* RNA polymerase derived from neutron low-angle scattering (Stockel et al., 1980), chemical cross-linking (Hillel & Wu, 1977; Coggins et al., 1977), and immunoelectron microscopy (Tichelaar et al., 1983) have indicated the mode of subunit interactions. The proposed models do not define which regions of each subunit are involved in the interactions with adjacent subunits, catalytic site, DNA template, RNA product, or substrate ribonucleoside triphosphates.

Generation of monoclonal antibodies (Kohler & Milstein, 1975) against antigenic determinants on the individual subunits could provide a potentially powerful set of site-specific reagents with which to probe the relation of structure to function in RNA polymerase. In the present paper, we report on the characterization of monoclonal antibodies prepared against the purified β and β′ subunits.

Materials and Methods

Materials. Reagents were obtained as follows: [^{3}H]UTP and high-performance liquid chromatography grade (HPLC-grade) ribonucleoside triphosphates, ICN; uridylyl(3′–5′)-adenosine (UpA), adenosine 5′-monophosphate (AMP), radioimmunoassay-grade (RIA-grade) bovine serum albumin (BSA), rifampicin, and *p*-nitrophenyl phosphate, Sigma Chemical Co.; d(A-T)$_n$, P-L Biochemicals; fetal calf serum, Sterile Systems Inc.; Dulbecco's modified Eagle's medium (DMEN), GIBCO; poly(ethylene glycol) 4000 (PEG 4000), E. Merck, Inc.; phosphatase-coupled goat anti-mouse immunoglobulin, Kirkegaard and Perry Laboratories; Freund's complete and incomplete adjuvants, Pel-Freeze; protein A–Sepharose, Pharmacia; Liquifluor, New England Nuclear. Mice were obtained from Jackson Laboratory.

Buffers. PBS consisted of 10 mM potassium phosphate (pH 7.2) and 150 mM KCl, while storage buffer was composed of 50 mM potassium phosphate (pH 7.5), 150 mM KCl, and 0.05% sodium azide. ELISA wash buffer was PBS containing 2 mg/mL BSA, 0.05% Tween 80, and 0.02% sodium azide. P60-BSA buffer consisted of 20 mM potassium phosphate (pH 7.0), 1 mM ethylenediaminetetraacetic acid (EDTA), 60%

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glycerol, and 2 mg/mL BSA; TM buffer was 40 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) (pH 8.0), 1 mM dithiothreitol, and 10 mM $MgCl_2$. TGED was 20 mM Tris-HCl (pH 8.0), 1 mM $MgCl_2$, 0.1 mM dithiothreitol, 0.1 mM EDTA, and 5% glycerol, while TMM was composed of 50 mM Tris-HCl (pH 8.0), 15 mM $MgCl_2$, and 10 mM mercaptoethylamine. WASP solvent was water, saturated ammonium sulfate, and 2-propanol (18:80:2) adjusted to pH 8 with ammonium hydroxide (Hansen & McClure, 1979).

RNA Polymerase. E. coli RNA polymerase was prepared by a modification of the method of Burgess & Jendrisak (1975). Holoenzyme and core polymerase were resolved by chromatography on denatured calf thymus DNA–agarose as described by Lowe et al. (1979). Protein concentration was determined by using the following extinction coefficients: core polymerase, $E^{1\%}_{280nm} = 5.8$; holoenzyme, $E^{1\%}_{280nm} = 6.7$ (Levine et al., 1980).

RNA polymerase subunits were prepared by chromatography of urea-dissociated core polymerase on Bio-Rex 70 by the method of Yarbrough & Hurwitz (1974). The subunits were dialyzed against PBS prior to use.

Production of Monoclonal Antibodies. SJL/J female mice were injected intraperitoneally with 23 μg of the β subunit and 43 μg of the β' subunit emulsified in Freund's complete adjuvant. This was followed by three booster shots of similar doses of the subunits in Freund's incomplete adjuvant administered at approximately 15-day intervals. Spleens from two mice (immunized with β or β' subunit) were removed 3 days after the final injection. Fusion of spleen cells and P3×63Ag8.653 myeloma cells (Kearney et al., 1979) was carried out by a modification of the method of Oi & Herzenberg (1980). Cells fused with PEG 4000 were distributed into 96-well Costar culture plates containing 2×10^4 mouse macrophage cells per well. The microcultures were maintained in DMEM containing 20% fetal calf serum plus hypoxanthine, aminopterin, and thymidine. The production of antibodies to β or β' was determined by ELISA (see below). Positive antibody-producing cultures were subcloned 3 times by limiting dilution in DMEM containing 20% fetal calf serum and 2×10^4 mouse macrophage cells per well.

Antibodies were prepared from spent media of expanded cultures (200–500 mL) grown to stationary phase. Cells were removed by centrifugation at 4000*g* for 10 min. Immunoglobulin was concentrated by precipitation with 50% saturated ammonium sulfate, pH 7.0. The antibodies were purified by chromatography on protein A–Sepharose (Ey et al., 1978) or DEAE-cellulose (Parham et al., 1982). Immunoglobulin-containing fractions were concentrated to a volume of about 1 mL by negative-pressure dialysis (in an apparatus obtained from Bio-Molecular Dynamics) against storage buffer. The immunoglobulin isotype of each monoclonal antibody was determined by using the mouse immunoglobulin subtype identification kit purchased from Boehringer Mannheim Corp. Immunoglobulin concentration was determined by using the following extinction coefficient: $E^{1\%}_{280nm} = 14.0$ (Ey et al., 1978).

Solid-Phase ELISA. Costar 96-well EIA polystyrene plates were coated with subunit by a 3-h incubation at 37 °C followed by incubation overnight at 4 °C. Each well received 600 ng of β or β' in PBS. Remaining protein binding sites were blocked by incubation with 200 μL/well of wash buffer for 90 min at 37 °C. The plates were then washed twice with wash buffer. Following addition of 50 μL of culture supernatant per well, the plate was incubated for 60 min at 37 °C. After the plate was washed 3 times with wash buffer, 50 μL of phosphatase-coupled goat anti-mouse immunoglobulin (1:200 dilution in PBS + 1 mg/mL bovine serum albumin) was added and incubated for 60 min at 37 °C. After being washed 3 times with wash buffer, 100 μL of a solution containing 1 mg/mL *p*-nitrophenyl phosphate in 0.1 M diethanolamine (pH 9.0) + 2.5 μM $MgCl_2$ was added and incubated for 30–60 min at 37 °C. After addition of 100 μL of 1 M NaOH, the absorbance at 410 nm was determined for each well by using a Dynatech Microelisa reader.

Assay of $d(A\text{-}T)_n$-Directed $r(A\text{-}U)_n$ Synthesis. Monoclonal antibody–polymerase complexes were formed by incubation for 60 min at 0 °C or 30 min at 37 °C of 1 pmol of core or holoenzyme in 10 μL of P60-BSA with the indicated amount of antibody in 20 μL of storage buffer. After addition of 2 nmol of $d(A\text{-}T)_n$ in 10 μL of TM buffer, the mixture was incubated for 10 min at 37 °C. Synthesis of $r(A\text{-}U)_n$ was carried out in a reaction mixture (90 μL) which contained 40 mM Tris-HCl (pH 8.0), 13 mM potassium phosphate (pH 7.5), 33 mM KCl, 10 mM $MgCl_2$, 10 mM mercaptoethylamine, 1 mM sodium azide, 400 nmol of ATP, 100 nmol of [^{3}H]UTP (5000 cpm/nmol), 44 μg of bovine serum albumin, and 7% glycerol. After incubation for 20 min at 37 °C, the $r(A\text{-}U)_n$ was precipitated with 5% trichloroacetic acid, collected on glass fiber filters (Whatman GFC), and counted in Liquifluor–toluene.

Abortive Initiation Assay. Monoclonal antibody–polymerase complexes were formed by incubation of 10 pmol of holoenzyme in 5 μL of P60-BSA buffer plus the indicated amount of antibody in 20 μL of storage buffer for 30 min at 37 °C (for assay of pApU synthesis) or by incubation of 1 pmol of holoenzyme in 5 μL of P60-BSA buffer plus the indicated amount of antibody in 10 μL of storage buffer for 60 min at 0 °C (for assay of UpApU synthesis). The immune complex was then incubated for 10 min at 37 °C in a mixture containing 40 mM Tris-HCl (pH 8.0), 10 or 18 mM potassium phosphate (pH 7.5), 130 mM KCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, 0.1 mM EDTA, 1 or 2 mM sodium azide, 20 or 30 μg of bovine serum albumin, 5% or 6% glycerol, and 10 nmol of $d(A\text{-}T)_n$. Synthesis of pApU was assayed following the addition of 120 nmol of 5′AMP and 12 nmol of [^{3}H]UTP (15 cpm/pmol) (final volume 60 μL) and incubation for 10 min at 37 °C. After addition of 10 μL of 0.5 M EDTA (pH 7.6), a 50-μL aliquot of the reaction mixture was chromatographed in the WASP system of Hansen & McClure (1979). Synthesis of UpApU was assayed following the addition of UpA and [^{3}H]UTP (200 cpm/pmol) at the indicated concentrations (final volume 50 μL) and incubation for the indicated times at 37 °C after which a 10-μL aliquot was chromatographed.

Preparation of Ternary Complexes. Two procedures were employed to prepare $d(A\text{-}T)_n$–polymerase–$r(A\text{-}U)_n$ complexes. The ternary complex used to screen the effect of the monoclonal antibodies on elongation (Tables I and II) was isolated by a modification of the method of Rhodes & Chamberlin (1974). For the kinetic studies, the ternary complex was prepared by the method of Schmidt & Chamberlin (1984). To form the complex by the modified method, 50 pmol of holoenzyme was incubated for 1 min at 37 °C with 124 nmol of $d(A\text{-}T)_n$ plus 400 μM UTP in TMM buffer after which ATP was added to a final concentration of 120 μM. The reaction (250 μL) was incubated for 1 min at 37 °C, placed on ice, and terminated by the addition of 10 μL of 0.5 M EDTA (pH 7.6). The reaction mixture was immediately applied to a Bio-Gel P-100 column (2×25 cm) equilibrated

Table I: Properties of Anti-β Monoclonal Antibodies[a]

		effect of monoclonal antibody on d(A-T)$_n$-directed[b]			
mab	Ig class	r(A-U)$_n$ synthesis by core	r(A-U)$_n$ synthesis by holoenzyme	initiation by holoenzyme	elongation by holoenzyme
210E8	IgG1,κ	15	55	51	54
221C7	IgG2b,κ	100	93	91	89
222B10	IgG2a,κ	9	53	60	66
240D4	IgG1,κ	11	53	45	ND
241E6	IgG1,κ	11	54	62	ND
260E2	IgG1,κ	100	93	93	89
261D5	IgG2a,κ	14	55	58	ND

[a] RNA polymerase core (1 pmol) or holoenzyme (1 pmol) was preincubated with 20 pmol of the indicated monoclonal antibody for 30 min at 37 °C. The mab–polymerase complex was assayed for the indicated reactions as indicated under Materials and Methods. ND indicates that the effect of the antibody was not determined. Residual activity in the presence of the indicated monoclonal antibody is expressed as the percent of the following control values for incorporation of [^{3}H]UMP: r(A-U)$_n$ synthesis by core, 0.8 nmol; r(A-U)$_n$ synthesis by holoenzyme, 2.5 nmol; initiation by holoenzyme, 2.1 nmol; elongation by ternary complex, 0.13 nmol. [b] Values are the percent of residual activity.

with TGED at 4 °C. The mixture was eluted with TGED and the ternary complex located by assaying 50-μL aliquots of the fractions for elongation activity. Fractions containing the ternary complex were pooled and stored at 4 °C.

Assay of Ternary Complex Activity. A 50-μL aliquot of the ternary complex (100–200 elongation units; Rhodes & Chamberlin, 1974) was preincubated for 30 min at 37 °C with a 20:1 molar excess of monoclonal antibody in 20 μL of storage buffer containing 1 mg/mL bovine serum albumin. The ratio of antibody to complex assumed a 100% recovery of polymerase during preparation of the ternary complex. Assay for polymerase activity was carried out in a reaction mixture (100 μL) which contained 50 mM Tris-HCl (pH 8.0), 10 mM potassium phosphate (pH 7.5), 80 mM KCl, 10 mM $MgCl_2$, 10 mM mercaptoethylamine, 30 nM EDTA, 2.5% glycerol, 1 mM sodium azide, 400 μM ATP, 400 μM [^{3}H]UTP (60 cpm/pmol), and 1.0 μg of rifampicin. After incubation for 10 min at 37 °C, the reaction was terminated by addition of 5% trichloroacetic acid.

The kinetic studies were carried out by using the assay conditions described by Schmidt & Chamberlin (1984). The ternary complex (360 elongation units) in 120 μL was preincubated for 30 min at 37 °C with a 20:1 molar excess of monoclonal antibody in 60 μL of storage buffer containing 1 mg/mL bovine serum albumin. To initiate the reaction, 30 μL of the preincubation mixture was added to tubes containing (final volume 50 μL) 44 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, 14 mM mercaptoethylamine, 2% glycerol, 1 mM sodium azide, 14 μg of bovine serum albumin, and 0.5 μg of rifampicin plus ATP and [^{3}H]UTP (500 cpm/pmol) at the indicated concentration. Reactions were incubated for 5 min at 37 °C and then terminated by addition of 5% trichloroacetic acid.

Results

The effect on polymerase activity of increasing the molar ratio of monoclonal antibody at a fixed holoenzyme concentration is shown in Figure 1. The noninhibitory monoclonal antibodies were without effect on d(A-T)$_n$-directed r(A-U)$_n$ synthesis even at a high molar excess. The anti-β' monoclonal antibody (mab) 311G2 showed an 85% inhibition of polymerase activity at a 10 to 1 ratio of antibody to polymerase. The partial inhibition by the anti-β mab 210E8 (see Table I for other anti-β mabs) was retained even at a high antibody concentration. This is consistent with the presence in the β subunit of a single determinant for each monoclonal antibody. No difference in the extent of inhibition effected by the anti-β mab 210E8 was seen on varying the KCl concentration between 30 and 130 mM. The inhibition remained approximately 50% when the antibody–polymerase complex was formed by incubation for 30 min at 37 °C, 60 min at 0 °C, or overnight at 0 °C. However, the core polymerase was more sensitive to the effect of the inhibitory anti-β mab 210E8 then

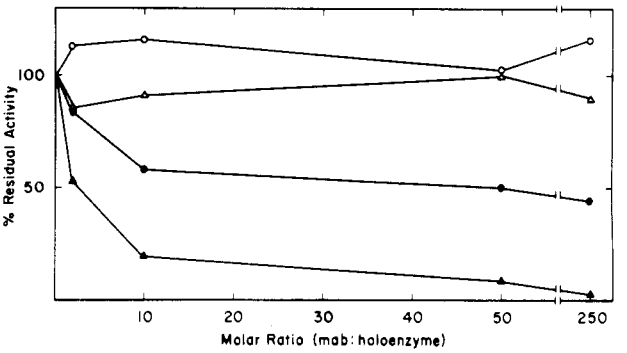

FIGURE 1: Effect of anti-β and anti-β' monoclonal antibodies on RNA polymerase. The d(A-T)$_n$-directed synthesis of r(A-U)$_n$ was assayed as described under Materials and Methods. Preincubation of 1 pmol of holoenzyme with and without the indicated amounts of antibody was for 60 min at 0 °C. mab 221C7 (○); mab 210E8 (●); mab 371D6 (△); mab 311G2 (▲). Activity is expressed as the percent of incorporation obtained with the holoenzyme control; 100% was 3.2 nmol of [^{3}H]UMP incorporated in 20 min at 37 °C.

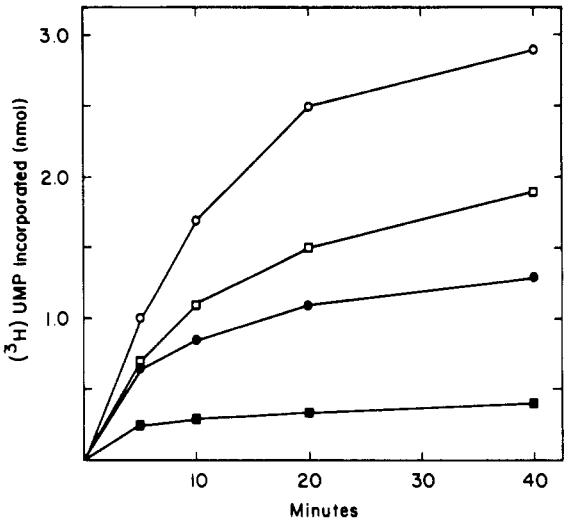

FIGURE 2: Effect of anti-β mab 210E8 on holoenzyme and core. The d(A-T)$_n$-directed synthesis of r(A-U)$_n$ was assayed as described under Materials and Methods. Preincubation of RNA polymerase holoenzyme (1 pmol) or core (1 pmol) in the presence and absence of mab 210E8 (20 pmol) was for 60 min at 0 °C. Holoenzyme (○); holoenzyme + mab 210E8 (●); core enzyme (□); core enzyme + mab 210E8 (■).

Table II: Properties of Anti-β' Monoclonal Antibodies[a]

mab	Ig class	effect of monoclonal antibody on d(A-T)$_n$-directed[b] r(A-U)$_n$ synthesis by core	r(A-U)$_n$ synthesis by holoenzyme	initiation by holoenzyme	elongation by holoenzyme
311G2	IgG1,κ	15	12	25	18
340C9	IgG2a,κ	103	106	133	ND
340F11	IgG2a,κ	99	101	79	96
370F3	IgG1,κ	80	97	100	136
371D6	IgG1,κ	104	85	96	132

[a] RNA polymerase core (1 pmol) or holoenzyme (1 pmol) was preincubated with 20 pmol of the indicated monoclonal antibody for 30 min at 37 °C. The mab–polymerase complex was assayed for the indicated reactions as indicated under Materials and Methods. See legend to Table I for 100% control values used. [b] Values are the percent of residual activity.

Table III: Effect of Monoclonal Antibodies on Kinetic Constants[a]

mab		K_m (μM)	V_{max} (pmol/min)	slope (±SE)
		UpApU Synthesis		
none	UpA	34	13	2.6 ± 0.27
210E8		83	13	6.5 ± 0.27
311G2		53	5	10.0 ± 0.84
none	UTP	53	19	2.8 ± 0.06
210E8		63	14	4.7 ± 0.86
311G2		32	3	12.6 ± 1.5
		r(A-U)$_n$ Synthesis		
none	ATP	12	7	1.7 ± 0.09
210E8		14	5	3.0 ± 0.14
none	d(A-T)$_n$	14	174	0.08 ± 0.003
210E8		13	75	0.17 ± 0.016
311G2		4	22	0.18 ± 0.03

[a] Assay conditions for the abortive initiation and r(A-U)$_n$ synthesis reactions are described under Materials and Methods and in the legends to Figures 3 and 4. The d(A-T)$_n$ titrations were performed with holoenzyme (1 pmol) preincubated with or without 20 pmol of the indicated monoclonal antibody using the assay conditions described in the legend to Figure 2. The K_m and V_{max} values were determined from lines fitted by linear regression analyses; the slopes are presented along with ±1 standard error.

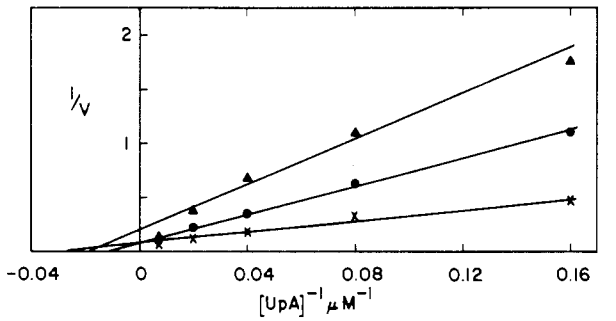

FIGURE 3: Effect of anti-β and anti-β' monoclonal antibodies on abortive synthesis of UpApU. Holoenzyme (1 pmol) was preincubated in the presence and absence of mab 210E8 (20 pmol) or mab 311G2 (20 pmol) for 60 min at 0 °C. Abortive synthesis of UpApU was assayed as described under Materials and Methods with 50 μM [^{3}H]UTP (200 cpm/pmol) and the concentration of UpA varied as indicated. Reciprocal initial velocities (picomoles per minute) are plotted vs. the reciprocal of the UpA concentrations. Holoenzyme (×); holoenzyme + mab 210E8 (●); holoenzyme + mab 311G2 (▲).

was the holoenzyme when assayed under the conditions used (Figure 2).

Summarized in Tables I and II are several properties of the seven anti-β and five anti-β' monoclonal antibodies isolated thus far. The monoclonal immunoglobulins contain G1, G2a, or G2b heavy chains and κ light chains. Subunit specificity of the monoclonal antibodies was verified by solid-phase ELISA using purified α, β, or β' subunits; the monoclonal antibodies cross-reacted only with the subunit used initially as the immunogen (data not shown). Five of the anti-β monoclonal antibodies (Table I) and one of the anti-β' monoclonal antibodies (Table II) inhibited the d(A-T)$_n$-directed synthesis of r(A-U)$_n$. The core polymerase was more sensitive to the effect of the inhibitory monoclonal antibodies than was the holoenzyme (see also Figure 2). Comparable results were obtained when the monoclonal antibodies were added before or after binding of holoenzyme to d(A-T)$_n$ (data not shown). Polymerase in the ternary elongation complex was inhibited by the monoclonal antibodies to approximately the same extent as was the holoenzyme. The abortive synthesis of pApU was used to determine the effects of the monoclonal antibodies on initiation by RNA polymerase. This reaction has been shown by Hansen & McClure (1979) to be strongly dependent on σ and represents a convenient assay for steps involved in initiation. The data presented in Tables I and II indicate that both initiation and elongation were affected to a similar extent by each of the inhibitory monoclonal antibodies.

The effects of monoclonal antibodies 210E8 and 311G2 on the kinetics of initiation and elongation are summarized in Table III. The results of abortive initiation experiments in which RNA polymerase activity was determined as a function of varied UpA concentration in the presence and absence of the inhibitory monoclonal antibodies are shown in Figure 3. Both the V_{max} and K_m^{UpA} were affected by the anti-β' mab 311G2, indicating a mixed mode of inhibition. In the presence of the anti-β mab 210E8, the K_m but not the V_{max} was changed for UpA. This competitive type of inhibition was also indicated by a reversal of the inhibition at UpA concentrations above 150 μM (data not shown). With UTP as the varied substrate, both the V_{max} and the K_m were altered in the presence of mab 210E8 and mab 311G2 (Table III). The anti-β' mab 311G2 is a potent inhibitor of RNA polymerase, and this was reflected in the low V_{max} when either the UTP or the UpA concentration was varied. The K_m^{UTP} in the presence of mab 311G2 was reduced while the K_m^{UpA} was increased compared to the control values.

The effect of the inhibitory anti-β and anti-β' monoclonal antibodies on the apparent K_m for d(A-T)$_n$ was determined in reactions containing a fixed concentration of ATP and UTP (Table III). As expected, each of the monoclonal antibodies affected the V_{max}. The apparent $K_m^{d(A-T)_n}$ was essentially unchanged in the presence of the anti-β mab 210E8 but was greatly reduced in the presence of the anti-β' mab 311G2 relative to the control value. None of the monoclonal antibodies inhibit binding of d(A-T)$_n$ by RNA polymerase (data not shown).

The effect of the anti-β mab 210E8 on the kinetics of ribonucleoside triphosphate incorporation by the ternary elongation complex is shown in Figure 4. Both the V_{max} and the K_m were slightly altered. The results indicated that even when the enzyme was in the elongation mode residual activity in the presence of the anti-β monoclonal antibody was 65% of the control value.

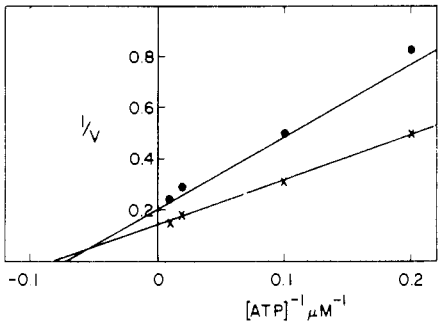

FIGURE 4: Effect of anti-β monoclonal antibody 210E8 on the kinetics of elongation. The reactions using the ternary complex prepared by the method of Schmidt & Chamberlin (1984) were carried out as described under Materials and Methods. The preformed ternary complexes were assayed in the presence and absence of mab 210E8 (20 pmol). Reciprocal initial velocities (picomoles per minute) are plotted vs. the reciprocal of the ATP concentrations. The concentration of [^{3}H]UTP (500 cpm/pmol) was 100 μM. Ternary complex (×); ternary complex + mab 210E8 (●).

DISCUSSION

In the present study, monoclonal antibodies raised against the purified β and β' subunits of the *E. coli* RNA polymerase were used as site-specific reagents. Previous studies using antibody preparations raised against the individual subunits demonstrated that such preparations could inhibit in vitro transcription by RNA polymerase (Fukuda et al., 1977; Gragerov & Nikiforov, 1980; Stender, 1981). Anti-subunit polyclonal antibodies have been used by Stender (1979) to demonstrate conformational changes in RNA polymerase. Although subunit specific, polyclonal antibodies consist of a heterogeneous population of immunoglobulins able to cross-react with many of the antigenic determinants present on the subunit. The development of hybridoma technology by Köhler & Milstein (1975) has made it possible to obtain antibodies directed against a single determinant present on a protein. Using the *E. coli* RNA polymerase core as the immunogen, Nikiforov et al. (1983) have raised monoclonal antibodies against determinants present on the β and β' subunits which were able to completely inhibit DNA-directed synthesis of RNA.

We have prepared monoclonal antibodies directed against the β and β' subunits (De Falco et al., 1983) and characterized their effects on RNA polymerase using the d(A-T)$_n$-directed reaction as a model system. Although anti-polymerase polyclonal antibodies have been reported to block DNA binding (Gragerov & Nikiforov, 1980; Stender, 1981), the binding of d(A-T)$_n$ is not inhibited by any of the anti-β or anti-β' monoclonal antibodies we have studied (unpublished results). The apparent $K_m^{d(A-T)_n}$ is unchanged for the inhibitory anti-β mab 210E8 and is markedly decreased for the inhibitory anti-β' mab 311G2. The inferred higher affinity for the template in the polymerase–anti-β' antibody complex may cause a lowered rate of translocation with a consequent inhibition of the rate of ribonucleotide incorporation. The β' subunit has been implicated in template binding by RNA polymerase (Zilling et al., 1976); the effect of the inhibitory anti-β' monoclonal antibody in decreasing the apparent K_m for d(A-T)$_n$ is consonant with a role of the β' subunit in template binding. The anti-β' monoclonal antibody is a potent inhibitor of both initiation and elongation reactions as indicated by the pronounced effects on the V_{max}. The observed inhibition is not a consequence of blocking template binding, suggesting that the β' subunit participates in the catalytic activity of the enzyme.

The preformed ternary elongation complex (Schmidt & Chamberlin, 1984) remains sensitive to the anti-β mab 210E8, showing both a decreased V_{max} and a moderate increase in the K_m, indicating a noncompetitive mechanism of inhibition. Inhibition of the abortive synthesis of UpApU by the anti-β mab 210E8 appears to be competitive with regard to the UpA primer and noncompetitive with regard to the UTP substrate. The competitive mode of inhibition effected by the anti-β mab 210E8 suggests that the antibody may bind to a region closely associated with the initiation nucleotide binding site of polymerase. Binding of the antibody would alter the affinity of polymerase for the initiation nucleotide (or dinucleoside monophosphate primer) without affecting the V_{max} of the reaction. It is difficult to ascertain whether the inhibition is a consequence of the antibody binding at the initiation site or an indirect effect resulting from an antibody-mediated distortion of the site. A direct inhibition by steric hindrance could result if the monoclonal antibody interacted with amino acid side chains involved in the catalytic domain. Conversely, binding of the antibody could indirectly lower enzymatic activity by freezing a conformation at the determinant with consequent effects transmitted to the catalytic domain. The interaction with the antibody could also prevent any subunit rearrangements or shifts which are necessary for efficient substrate utilization during catalysis. It is interesting to note that the inhibitor streptolydigin, which binds to the β subunit and inhibits polymerization, shows a pattern of inhibition which is opposite to that elicited by the anti-β mab 210E8. The inhibition of abortive initiation on a d(A-T)$_n$ template by streptolydigin is noncompetitive vs. the initiating nucleotide, AMP, and competitive vs. UTP (McClure, 1980).

Interpretation of the mechanism of antibody-mediated inhibition is further complicated by the complex nature of the events required for transcription to proceed. The RNA polymerase catalytic domain includes in close proximity the sites for initiation and elongation nucleotide binding, template binding, and helix unwinding (von Hippel et al., 1984). The present results indicate that the inhibitory anti-β and anti-β' monoclonal antibodies bind to antigenic determinants whose availability and conformation are conserved in both the free subunit and the assembled enzyme. The sensitivity of the ternary elongation complex indicates that the antigenic determinants are not directly involved in the template or product binding sites. An explanation of the mechanism of inhibition must also take into account the ordered nature of the polymerase reaction in which incorporation of the substrate ribonucleoside triphosphate is dependent on the prior binding of the nucleotide occupying the initiation/product terminus site (McClure et al., 1978). We consider it unlikely that the inhibitory antibodies act by directly binding to amino acid side chains in the nucleotide binding sites. Such sites are generally found to occupy clefts formed by folding or interaction of subunits and would be inaccessible to molecules as large as immunoglobulins. An antigenic determinant consisting of six to seven amino acid residues (Medgyesi et al., 1978; Lindstrom et al., 1978) covers a relatively small area. The actual area occluded by the arm of a bound antibody is about 35 Å (Tzartos et al., 1981). Tzartos et al. (1981) have pointed out that this area is about 5 times that of the antigenic determinant and refer to it as a "region". Because of the large area of the immunogenic region, it is not surprising that the nature of the inhibition effected by the binding of a monoclonal antibody is more complex than that exhibited by classical low molecular weight inhibitors. The interaction of σ with the core unit is accompanied by complex effects on the kinetic properties of RNA polymerase (Hansen & McClure, 1980). A differential inhibition as a function of the template employed has been

reported for RNA polymerase in the presence of the nusA gene product (Schmidt & Chamberlin, 1984).

Several of the monoclonal antibodies do not apparently inhibit any of the d(A-T)$_n$-directed reactions studied. The failure to inhibit can be a consequence of binding to determinants which are not present in critical enzyme domains. Conformational changes are involved in the assembly of RNA polymerase (Ishihama et al., 1979), and it is thought that antibodies are specific for a particular conformation of the antigenic determinant (Todd et al., 1982). It is also possible that the noninhibitory monoclonal antibodies may not bind to polymerase because their determinants are buried as a consequence of subunit–subunit interactions or because the determinants are in a nonreactive conformation.

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Registry No. UpA, 3256-24-4; UTP, 63-39-8; ATP, 56-65-5; d(A-T)$_n$, 26966-61-0; RNA polymerase, 9014-24-8.

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