required, so that binding of a substrate molecule at one site prevents reaction at the other. Also, reaction of iodoacetate at one site must reduce catalytic activity and reactivity toward iodoacetate at the other site. Possibly other models could be proposed. At this time, we prefer the model in which the active site is made up of two subunits. As far as we know, an active site of that type has not been conclusively established for any enzyme. Obviously, additional evidence is required to establish this type of active site for proline racemase.

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On the Role of Sulfhydryl Groups in the Structure and Function of the *Azotobacter vinelandii* RNA Polymerase†

Joseph S. Krakow

ABSTRACT: Exposure of sulfhydryl groups as indicated by titration kinetics is decreased under conditions where RNA polymerase exists as a dimer or higher aggregate (low salt), in the presence of Mn^{2+}, or when bound to d(A-T). Incubation of phenylmercurisulfonate with RNA polymerase above pH 9.0 results in loss of d(A-T) binding ability. Poly(U) binding is more sensitive to sulfhydryl modification and is lost at pH's above 8.0. The presence of 4 m*M* Mn^{2+} has an obvious effect in stabilizing the polymerase–poly(U) complex when incubated with 10 μM phenylmercurisulfonate + 1 *M* urea. Incubation of the enzyme with the mercurial and urea results in disaggregation to subprotomeric forms and release of the α subunit. Similar treatment in the presence of 4 m*M* $MnSO_4$ stabilizes the protomeric structure of the enzyme. During chain elongation the enzyme exists as a ternary $d(A\text{-}T)_n$–enzyme–$r(U\text{-}A)_n$ complex in which the bound $d(A\text{-}T)_n$ is refractory to the destabilizing effect of the mercurial; however, further phosphodiester bond formation is inhibited. The results are defined in terms of a role which reflects the involvement of polymerase sulfhydryl groups in the various conformations necessary for subunit–subunit interaction, tight template binding, and catalytic activity.

RNA synthesis by RNA polymerase is a result of a complex sequence of several substeps carried out by a complex enzyme consisting of several subunits (von Hippel and McGhee, 1972; Chamberlin, 1974). Although it is known that RNA polymerase is sensitive to a variety of sulfhydryl inhibitors, the large number of cysteinyl residues, 30–35, and their distribution among the enzyme subunits (β', β, $\alpha \pm \sigma$) makes the assignment of a specific functional role difficult. The following are affected by sulfhydryl modification: subunit–subunit interaction (Ishihama, 1972; Ito and Ishihama, 1973), template binding (Ishihama and Hurwitz, 1969), tight template binding (Smith et al., 1971; Krakow, 1972a), template recognition and specific initiation (Yarbrough and Wu, 1974), and phosphodiester bond formation (Krakow, 1966; Lee-Huang and Warner, 1969; Ishihama and Hurwitz, 1969; Sumegi et al., 1971; Smith et al., 1971; Harding and Beychok, 1973; Nicholson and King, 1973; Yarbrough and Wu, 1974). The published data and that to be presented in this paper do not exclude a direct involvement of sulfhydryl groups in the catalytic role of RNA polymerase but are also compatible with their involvement

† From the Department of Biological Sciences, Hunter College, New York, New York 10021. *Received March 18, 1974.* This work was supported by grants from the National Institutes of Health (GM 18673) and the Research Foundation of the City University of New York (1475). This paper is number XIV in the series *Azotobacter vinelandii* RNA polymerase. Preliminary accounts of these studies have been published (Krakow, 1972a,b).

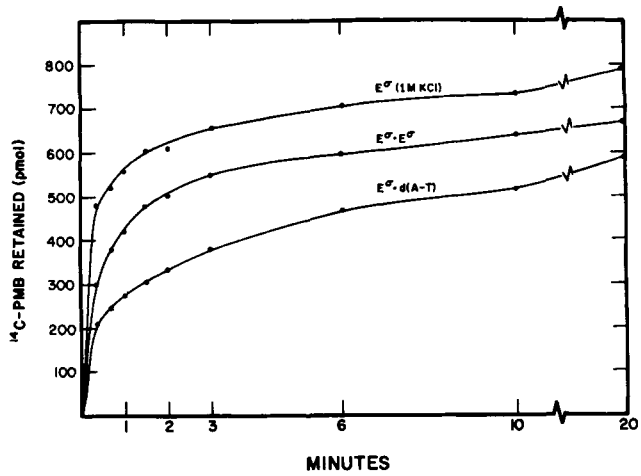

FIGURE 1: Effect of template and salt concentration on reaction of RNA polymerase sulfhydryl groups with [^{14}C]PMB. The reactions contained (final volume 2.0 ml): 80 m*M* Tris-HCl (pH 7.8), 5 m*M* $MgCl_2$, and 290 pmol of RNA polymerase holoenzyme. (Middle curve) No additions; (top curve) 1 *M* KCl; (bottom curve) 45 nmol of d(A-T)$_n$, and incubated 10 min at 37°. After addition of 15 nmol of [^{14}C]PMB (1 × 10^4 cpm/nmol) the rate of mercaptide formation was followed at 0° and at the time indicated 0.2-ml aliquots were removed and immediately filtered onto nitrocellulose membrane filters and washed with 10 ml of 0.02 *M* Tris-HCl (pH 7.8)-0.05 *M* NaCl (Krakow and Goolsby, 1971).

in the maintaining and attaining the various enzyme conformations necessary for the steps leading to RNA synthesis.

Methods and Materials

Dithiothreitol, Tris, bis-tris-propane,[1] PMB, PMS, ATP, and UTP were products of Sigma Chemical Co. and poly(U) and [^{3}H]poly(U) from Miles Laboratories; Ultra Pure urea and [^{14}C]PMB from Schwarz/Mann; [^{3}H]UTP and $^{32}PP_i$ from New England Nuclear. Labeled and unlabeled d(A-T)$_n$ were prepared using *Escherichia coli* DNA polymerase I (Jovin et al., 1969).

Nitrocellulose membrane filters (0.45-μ pore size, 25 mm diameter) were obtained from Matheson-Higgins, Woburn, Mass. Prior to use, the filters were soaked in 0.1 *M* KOH for 30 min at room temperature (Smolarsky and Tal, 1970) and then placed in 0.02 *M* Tris-HCl (pH 7.8)-0.05 *M* NaCl. This procedure was used to lower the blank adsorption of the labeled polymers.

Azotobacter vinelandii RNA polymerase was purified by a modification of the published procedure (Krakow and Horsley, 1968). RNA polymerase holoenzyme ($\beta'\beta\alpha_2$, σ) and core ($\beta'\beta\alpha_2$) were resolved by gradient elution from phosphocellulose (Whatman P-11); each form was essentially homogeneous as determined by sodium dodecyl sulfate acrylamide gel electrophoresis.

Acrylamide gel electrophoresis under nondenaturing condition was carried out by the method of Davis (1964) and stained with Coomassie Blue (Krakow and von der Helm, 1970).

Results

RNA polymerase is a complex enzyme and the holoenzyme has the subunit composition, 2α, β, β', σ; analysis of the *E. coli* RNA polymerase showed that cysteinyl residues are distributed among the subunits (Nicholson and King, 1973; Ito and Ishihama, 1973; Yarbrough and Wu, 1974). In addition to interaction between subunits the protomer itself undergoes reversible aggregation-disaggregation as a function of ionic strength (Berg and Chamberlin, 1970) and also template binding (Smith et al., 1967; Krakow and von der Helm, 1970). These various properties of the enzyme could play a role in the reactivity of the sulfhydryl groups with PMB. The time course of the reaction of the *A. vinelandii* RNA polymerase with [^{14}C]PMB (Figure 1) indicates at least two classes of sulfhydryl groups, rapidly reacting and slowly reacting including those which have not reacted within 20 min. In buffer containing 1 *M* KCl the enzyme exists as the protomer and as shown in Figure 1 there is an increase in the number of rapidly reacting sulfhydryl groups. Since the salt concentration used may have effects on the protomer itself in addition to dissociation from the dimer it is not certain that the observed increase in sulfhydryl reactivity is solely a consequence of protomer formation. One result of template binding is the dissociation of the polymerase aggregate to form the template–protomer complex. In contrast to the salt-induced disaggregation which increases sulfhydryl reactivity there is an obvious protective effect of d(A-T)$_n$ binding particularly with regard to the rapidly reacting class of sulfhydryl groups.

Table I: Effect of Divalent Cations on Sulfhydryl Reactivity.[a]

Additions	[^{14}C]PMB Bound (pmol)		
		Mg^{2+}	Mn^{2+}
None	231	223	140
KCl	384	329	171
d(A-T)$_n$	112	114	88

[a] The reactions contained (final volume 0.25 ml): 80 m*M* Tris-HCl (pH 7.8), 10 μg of RNA polymerase holoenzyme and as indicated 1 *M* KCl, 12 nmol of d(A-T)$_n$, 20 m*M* $MgSO_4$, and 4 m*M* $MnSO_4$. Assays containing d(A-T)$_n$ were incubated for 20 min at 37° and cooled prior to addition of the divalent cation and PMB. Each received 6 × 10^{-6} *M* [^{14}C]PMB (1 × 10^4 cpm/nmol) and, after 20 sec at 0°, 2 ml of cold 0.02 *M* Tris-HCl (pH 7.8)–0.05 *M* NaCl was added and immediately filtered onto a nitrocellulose membrane and washed with 10 ml of cold dilution buffer.

RNA polymerase has an absolute requirement for divalent cations, Mg^{2+} or Mn^{2+}, for phosphodiester bond synthesis; the effect of these metal ions on sulfhydryl reactivity is shown in Table I. Mg^{2+} has a relatively minor effect on the amount of [^{14}C]PMB which reacts within 20 sec at 0° with free enzyme in high or low salt or with the enzyme–d(A-T)$_n$ complex. There is a definite effect of Mn^{2+} in protecting some of the sulfhydryls of the rapidly reacting class especially in the assay run in 1 *M* KCl where only 45% of the [^{14}C]PMB has reacted relative to the control. The protective effect of Mn^{2+} is less pronounced with the polymerase–d(A-T)$_n$ complex. The results suggest that Mn^{2+} may alter (tighten) the conformation of RNA polymerase in a manner similar to that resulting from d(A-T)$_n$ binding.

Sulfhydryl groups are involved in maintaining the native quaternary structure of RNA polymerase. Ishihama (1972) showed that incubation of the *E. coli* RNA polymerase with PMB at neutral pH resulted in dissociation of the enzyme into subprotomeric forms and the release of the α subunit. Incubation of the *Azotobacter* RNA polymerase in the absence of the mercurial shows that d(A-T)$_n$ binding is relatively unaffected between pH 6 and 10 (Figure 2); there is a marked loss in enzyme activity (r(A-U)$_n$ synthesis) above

[1] Abbreviations used are: bis-tris, *N,N*-bis(2-hydroxyethyl)iminotris(hydroxymethyl)methane; PMB, *p*-chloromercuribenzoate; PMS, phenylmercurisulfonate; poly(U), poly(uridylic acid).

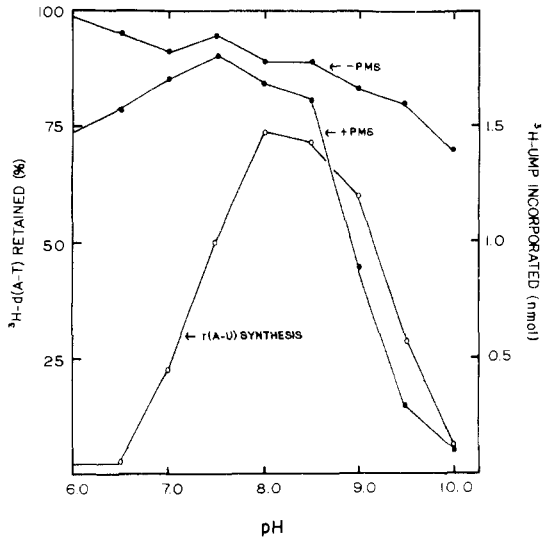

FIGURE 2: Effect of preincubation pH on d(A-T)$_n$ binding and r(A-U)$_n$ synthesis. The reactions contained (final volume 0.25 ml): 40 m*M* bis-tris-propane at the pH indicated, 5 μg of RNA polymerase core enzyme, and, where indicated, 4×10^{-6} *M* PMS. After incubating for 20 min at 37° synthesis of r(A-U)$_n$ and d(A-T)$_n$ binding were assayed as follows. r(A-U)$_n$ Synthesis. Add 1 μmol of $MgSO_4$, 100 nmol of ATP, 100 nmol of [^{3}H]UTP (1×10^3 cpm/μmol), and 2.3 nmol of d(A-T)$_n$. The reactions were incubated for 10 min at 37° and terminated by addition of 0.1 ml of 0.2 *M* $NaPP_i$, and 2 ml of cold 5% Cl_3CCOOH and filtered onto nitrocellulose membranes. Wash with 3 × 5 ml of cold 5% Cl_3CCOOH. [^{3}H]d(A-T)$_n$ Binding. Add 2.3 nmol of [^{3}H]d(A-T)$_n$ (1730 cpm/nmol). After incubating for 5 min at 37°, 1.75 ml of 0.05 *M* NaCl was added and the mixtures were filtered onto nitrocellulose membranes. The data are expressed as the percent [^{3}H]d(A-T)$_n$ retained relative to that retained at pH 7.5 (=2.1 nmol) when the enzyme was not preincubated for 20 min at 37° in the absence of PMS.

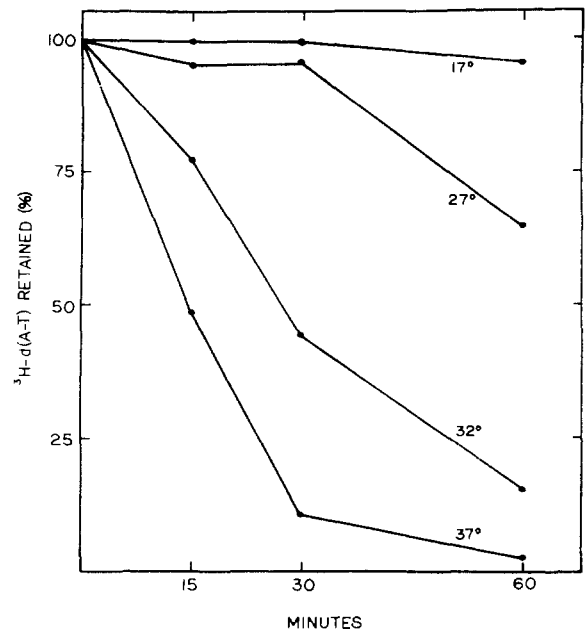

FIGURE 3: Rate of loss of d(A-T)$_n$ binding by mercurial modified RNA polymerase at different temperatures. The reactions contained (final volume 0.25 ml): 40 m*M* bis-tris-propane buffer (pH 9.0), 4×10^{-6} *M* PMS, and 5 μg of RNA polymerase core enzyme. Following an initial incubation at the temperature and time indicated 2.3 nmol of [^{3}H]d(A-T)$_n$ was added and incubated for 5 min at the same temperature previously used. Add 1.75 ml of 0.05 *M* NaCl and filter onto nitrocellulose membrane.

pH 9.0 relative to the controls assayed without prior incubation. Polymerase activity on the acid side of the curve is about the same whether or not the enzyme was preincubated. The results show that phosphodiester bond synthesis is a more sensitive property than is template binding. Incubation of polymerase with PMS for 20 min at 37° shows a marked loss of d(A-T)$_n$ binding capacity in the pH range above pH 8.5 suggesting that the protomeric structure of the mercurial-enzyme may have been altered.

The loss of template binding capacity by RNA polymerase incubated at pH 9.0 in the presence of 4×10^{-6} *M* PMS is both temperature and time dependent (Figure 3). Incubation of the enzyme with the mercurial for 60 min at 17° has only a minimal effect on [^{3}H]d(A-T)$_n$ binding capacity, while 30 min at 37° results in an almost complete loss of template binding and release of about 50% of the α subunit (results not shown). Incubation of the mercurial-polymerase adduct at temperatures above 27° at pH 9.0 results in a marked drop in d(A-T)$_n$ binding capacity. Incubation of the preformed binary complex with 4×10^{-6} *M* PMS for 30 min at 37° results in less than 10% dissociation of the [^{3}H]d(A-T)$_n$ from the complex. The enzyme-d(A-T)$_n$ complex once established is relatively stable to the effects of sulfhydryl modification although catalytic activity is blocked. Incubation of the polymerase-d(A-T)$_n$ complex at pH 9.0 does not result in α release presumably due to the stabilizing effect of the bound d(A-T)$_n$. Since the α subunit has not been directly implicated in template binding it would appear that formation of a tight enzyme-template complex results also in a tighter interaction among the polymerase subunits.

As shown in Figure 2, d(A-T)$_n$ binding by RNA polymerase is relatively unaffected even at pH 10.0 and the preformed enzyme-d(A-T)$_n$ complex is stable at this pH even in the presence of the mercurial. In contrast the binding of poly(U) by RNA polymerase progressively decreases as the pH is raised above 8.5 (Figure 4). Inclusion of 6×10^{-6} *M* PMS during the incubation results in almost complete loss of poly(U) binding capacity above pH 8.0. The loss of poly(U) binding above pH 8 suggests that the positively charged ϵ-amino groups of lysyl residues may be involved in binding of single-stranded polymers such as poly(U) to the enzyme, d(A-T)$_n$ binding may involve other types of interaction besides ionic as well as a second template site. Although RNA polymerase will bind poly(U) in the absence of divalent cations, synthesis of the complementary polynucleotide proceeds only in the presence of Mn^{2+}. Inclusion of 4 m*M* $MnSO_4$ results in an obvious stabilization of the polymerase-poly(U) complex incubated in the presence of the mercurial. The effect of Mn^{2+} is probably not directly on poly(U) binding but a consequence of a Mn^{2+} induced taut enzyme conformation (Table I).

While poly(U) (or d(A-T)$_n$) binding by RNA polymerase does not require Mn^{2+} or Mg^{2+}, the metal ions do have an obvious stabilizing effect when the complex is incubated under conditions known to cause conformational changes in the enzyme. Incubation of the polymerase-poly(U) complex in 1 *M* urea for 10 min at 37° results in dissociation of 75% of the complex formed in the absence of urea (Table II) and a minor effect of Mg^{2+} is seen. Addition of 4 m*M* $MnSO_4$ prior to incubating with 1 *M* urea has an obvious stabilizing effect, with 63% of the polymerase-poly(U) complex remaining intact. Incubation of the enzyme-poly(U) complex with 4×10^{-6} *M* PMS shows that it is least dissociated in the presence of Mn^{2+}. Mg^{2+} also has an appreciable effect compared to the assay run with the mercurial in the absence

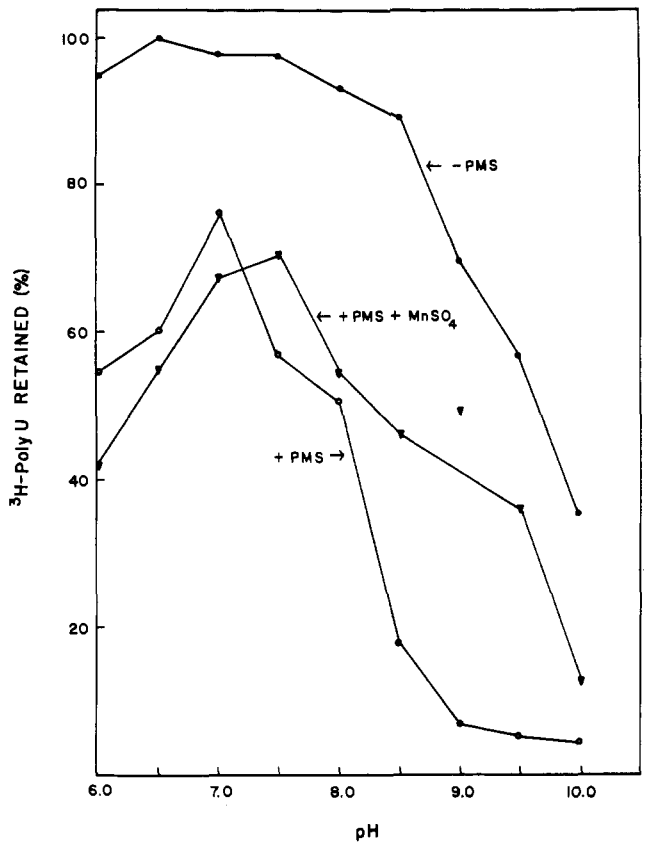

FIGURE 4: Mercurial induced loss of poly(U) binding in presence and absence of $MnSO_4$. The reactions contained (final volume 0.25 ml): 40 nm of bis-tris-propane buffer at the pH indicated, 6 nmol of [^{3}H]poly(U) (500 cpm/nmol), 10 μg of RNA polymerase core enzyme, and, as indicated, 4 mM $MnSO_4$ and 4 × 10^{-6} M PMS. Following incubation for 10 min at 37° 1.75 ml of 0.05 M NaCl was added and the mixtures were filtered onto nitrocellulose membranes.

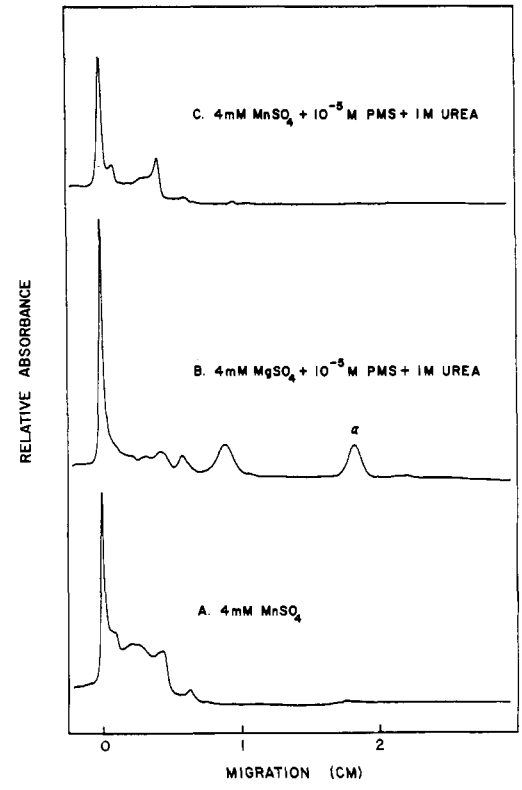

FIGURE 5: Prevention of PMS + urea induced disaggregation of polymerase by Mn^{2+}. The reactions contained (final volume 0.1 ml): 50 mM bis-tris-propane buffer (pH 7.5), 10% sucrose, and 5 μg of RNA polymerase core enzyme and the following: (A) 4 mM $MnSO_4$; (B) 4 mM $MgSO_4$, 1 × 10^{-5} PMS, and 1 M urea; (C) 4 mM $MnSO_4$, 1 × 10^{-5} M PMS, and 1 M urea. After incubating for 10 min at 37° the mixtures were resolved by electrophoresis on 6% acrylamide gels.

Table II: Effect of Divalent Cations on Stability of the Polymerase–Poly(U) Complex.[a]

Incubation I	[^{3}H]Poly(U) Retained (nmol) Incubation II			
		Urea	PMS	Urea + PMS
E + poly(U)	4.7	1.0	1.1	0.3
E + poly(U) + $MgSO_4$	4.5	1.3	2.4	0.4
E + poly(U) + $MnSO_4$	4.4	2.8	3.3	2.0

[a] The reactions contained (final volume 0.25 ml): 40 mM bis-tris-propane (pH 8.5), 6 nmol of [^{3}H]poly(U) (500 cpm/nmol), 10 μg of RNA polymerase core enzyme, and, where indicated, 20 mM $MgSO_4$ or 4 mM $MnSO_4$. After an initial incubation for 5 min at 37° the following were added as indicated: 1 M urea and 4 × 10^{-6} M PMS. After 10 min at 37° (incubation II) 1.75 ml of 0.05 M NaCl was added and the mixtures were filtered onto nitrocellulose membranes.

of the divalent cations. The combination of 1 M urea and 4 × 10^{-6} M PMS results in the almost complete dissociation of the polymerase–poly(U) complex in the presence or absence of Mg^{2+}; while the presence of 4 mM $MnSO_4$ stabilizes the complex resulting in 44% residual poly(U) binding. These results suggest that Mn^{2+} has a direct effect on the conformation of RNA polymerase.

The stabilizing effect of Mn^{2+} on RNA polymerase can be directly demonstrated by electrophoresis of enzyme incubated under conditions similar to those in Table II. The native core enzyme resolves as a predominantly dimer species with some higher aggregate forms also seen. Incubation of the enzyme in 1 M urea and 1 × 10^{-5} M PMS results in an altered pattern, the dimer band is lost and replaced by an higher aggregate along with the appearance of free α subunit plus another subprotomeric form containing β' and/or β at about 0.95 cm (Figure 5). This pattern is not affected by addition of 4 mM $MgSO_4$ to the incubation mixture. When the enzyme is incubated with urea and the mercurial in the presence of 4 mM $MnSO_4$, the resolved polymerase shows that the α subunit has not been released and that the other subprotomeric species have not formed. Although the higher aggregates predominate some residual dimer remains.

The polymerase–d(A-T)$_n$ complex incubated with PMS is more salt sensitive than the control complex. The results presented in Table III also show that the ternary complex incubated with PMS and then in 20 mM Tris-HCl (pH 7.8)–0.25 M NaCl shows 77% of the [^{3}H]d(A-T)$_n$ binding seen with the control not treated with the mercurial. The binary holoenzyme–d(A-T)$_n$ complex is sensitive to sulfhydryl modification and only 28% of the d(A-T)$_n$ is retained in the filter assay. The results indicate that following chain initiation, polymerase in the ternary complex is "locked" into a tight template binding state in which subsequent sulfhydryl modification has little effect on template binding. The data presented in Table III showing that 23% of the [^{3}H]d(A-T)$_n$ originally bound is lost after incubation with the mercurial is probably a reflection of the presence of inactive polymerase which can bind template but does not ini-

Table III: Stability of [^{3}H]d(A-T)$_n$ Bound as a Nascent Complex Against PMS.[a]

Preincubation Conditions	[^{3}H]d(A-T)$_n$ Retained (nmol)	
	−PMS	+PMS
ADP	1.87	0.46
ADP + UTP	2.03	1.44
ATP	1.82	0.50
ATP + UTP	2.02	1.56

[a] The reactions contained (final volume 0.25 ml): 80 m*M* Tris-HCl (pH 7.8), 4 m*M* $MgCl_2$, 4.5 nmol of [^{3}H] d(A-T)$_n$ (1000 cpm/nmol), 5 μg of RNA polymerase holoenzyme, and, when indicated, 4×10^{-5} *M* ADP, 4×10^{-5} *M* ATP, and 4×10^{-4} *M* UTP. Following incubation for 20 min at 37° the reactions indicated received 8×10^{-6} *M* PMS and the incubations continued for 2 min at 37°. After addition of 2 ml of 0.02 *M* Tris-HCl (pH 7.8)–0.25 *M* NaCl the mixtures were filtered onto nitrocellulose membranes and washed with 10 ml of the dilution buffer.

Table IV: PMS Inhibition of ^{32}PP Exchange by the Nascent Complex.[a]

Preincubation Additions	^{32}PP Incorporated (nmol)	
	−PMS	+PMS
ADP	8.0	0.13
ADP + UTP	14.0	0.70
ATP	29.6	1.00
ATP + UTP	12.8	0.70

[a] The reactions contained (final volume 0.25 ml): 80 m*M* Tris-HCl (pH 7.8), 4 m*M* $MgCl_2$, 5 μg of d(A-T)$_n$, 10 μg of RNA polymerase holoenzyme, and the indicated nucleotides (ADP, 4×10^{-5} *M*; ATP, 4×10^{-6} *M*; UTP, 4×10^{-4} *M*). Duplicate mixtures were incubated for 20 min at 37°, then one set received 8×10^{-6} *M* PMS and the incubations were continued for 2 min at 37°. To assay for pyrophosphate exchange UTP (4×10^{-4} *M*) (where previously omitted) and 1 m*M* Na^{32}PP$_i$ (5×10^5 cpm/μmol) were added and the reactions incubated for 5 min at 37° and processed by the method of Krakow and Fronk (1969).

tiate synthesis. Assay for [γ-^{32}P]ATP incorporation and alkaline-stable UMP and AMP incorporation (Nath and Hurwitz, 1974) shows that in *Azotobacter* holoenzyme preparations used only 50–60% of the enzyme protomers initiate r(A-U)$_n$ chains under the conditions used and that this is predominantly with pppAp (Kumar and Krakow, 1975).

If sulfhydryl groups are only involved in alterations in polymerase conformation prerequisite to tight template binding and consequent chain initiation then the preformed ternary d(A-T)$_n$–enzyme–r(A-U)$_n$ complex which is stable to mercurial and salt induced dissociation would still show polymerase activity. The results presented in Table IV indicate that even under conditions where there is tight template binding and a nascent product chain continued enzyme activity is almost completely inhibited by PMS. The results indicate that in addition to conformational effects relating to subunit interaction and tight template binding polymerase sulfhydryl groups may also play a role at a region close to the catalytic site of the enzyme.

Discussion

Amino acid analysis and direct titration with 5,5′-dithiobis(2-nitrobenzoic acid) and PMB shows that all of the cysteinyl residues are present as free sulfhydryl groups. Resolution of the polymerase subunits indicates that the sulfhydryl groups are present in β', β, and α (Nicholson and King, 1973; Yarbrough and Wu, 1974). In the native enzyme the sulfhydryl groups can be classified in terms of their relative reactivity and also their functional role. From the data presented in this paper and also by others working with the *E. coli* RNA polymerase (Sumegi et al., 1971; Harding and Beychok, 1973; Nicholson and King, 1973; Yarbrough and Wu, 1974) it is obvious that at least two classes of sulfhydryl groups can be distinguished based on their relative reactivity with PMB, 5,5′-dithiobis(2-nitrobenzoic acid), tetrathionate, or cystamine. This is a property of the native enzyme and following denaturation all sulfhydryl groups react rapidly. The reactivity of the sulfhydryl groups comprising the rapidly reacting class is sensitive to ionic conditions which increases the number within this group and especially to template binding which results in an apparent protection with regard to PMB modification. Sumegi et al. (1971) have shown that cystamine reacts to a lesser extent with the *E. coli* polymerase–DNA complex than with the free enzyme.

Results from two laboratories have indicated that modification of three or four sulfhydryl groups (presumably within the rapidly reacting class) has no effect on polymerase activity (Sumegi et al., 1971; Harding and Beychok, 1973) and that inactivation results after titration of 8 (Harding and Beychok, 1973), 12 (Sumegi et al., 1971), or 21 (Yarbrough and Wu, 1974) sulfhydryl groups. Harding and Beychok (1973) have presented data suggesting that ATP protects a small number of sulfhydryl groups from reaction with PMB while Sumegi et al. (1971) have shown that enzyme activity was protected and fewer sulfhydryl groups react with cystamine when polymerase is bound to DNA.

Although Ishihama and Hurwitz (1969) found that T7 DNA was not bound by PMB modified *E. coli* RNA polymerase, the results of Yarbrough and Wu (1974) and those using *A. vinelandii* enzyme (Krakow, 1966, 1972a,b) demonstrate that sulfhydryl modification does not prevent template binding. However, the complex formed between mercurial–polymerase and d(A-T)$_n$ is stable only at low ionic strength and dissociates at salt concentrations where the control enzyme–d(A-T)$_n$ complex is stable. These results indicate that sulfhydryl groups are not involved directly in template binding but are essential for the conformational changes leading to tight template binding. Formation of a tight template complex between RNA polymerase and DNA is a necessary prerequisite to chain initiation (Hinkle and Chamberlin, 1972).

Ishihama (1972) first demonstrated the involvement of sulfhydryl groups in maintaining the quaternary structure of RNA polymerase. Extensive modification with PMB resulted in release of the α subunit and formation of subprotomeric complexes of β' and β, α_2. In the present study the structural integrity of the PMS-modified enzyme as well as d(A-T)$_n$ binding (in low salt) is maintained after incubation in the pH range 6.0–8.5. At more alkaline pH d(A-T)$_n$ binding capacity is lost and the enzyme undergoes dissociation of its protomeric structure. While template binding is dependent on the structural integrity of the protomer, the binary complex is a much more stable structure then the free polymerase. This is indicated by the lower rate of sulfhydryl modification of the polymerase bound to d(A-T)$_n$ and also by the protection against the mercurial induced loss of template binding.

Stabilization of the enzyme against mercurial dissociation is only afforded by tight binding templates such as

d(A-T)$_n$ or d(I-C)$_n$ but not by poly(U). This may be a reflection of the involvement of two distinct template sites on RNA polymerases in which one site is involved in binding the informational strand with the second site holding the complementary strand following the proposed helix breaking event preceding chain initiation (Chamberlin, 1974; von Hippel and McGhee, 1972; Schafer et al., 1973; Saucier and Wang, 1972). In the case of poly(U)-directed transcription or the unprimed reactions carried out by RNA polymerase Mn^{2+} cannot be replaced by Mg^{2+} (Steck et al., 1968; Krakow, 1968). Since poly(U) and other templates will bind to the enzyme in the absence of added divalent cations the specific Mn^{2+} requirement has remained unexplained. Studies of the conformation of RNA polymerase by small-angle X-ray measurements indicated that the core enzyme is more loosely built than the holoenzyme protomer (Pilz et al., 1972). The results showing that sulfhydryl reactivity of free enzyme and polymerase–poly(U) complexes is lowered in the presence of Mn^{2+} suggests that this cation affects the conformation of RNA polymerase. In the presence of Mn^{2+} polymerase may shift to a taut conformation analogous to that resulting from d(A-T)$_n$ or promoter binding. Mn^{2+} has been shown to confer a taut conformation on glutamine synthetase (Shapiro and Stadtman, 1967; Hunt and Ginsberg, 1972) and RNA polymerase is apparently another example of this phenomenon. In the presence of Mn^{2+} the polymerase–poly(U) complex is more stable to incubation in the presence of PMS and 1 *M* urea than in the presence of Mg^{2+} or without added divalent cation. The presence of Mn^{2+} also prevents the dissociation of the quaternary structure of the protomer when incubated with PMS and 1 *M* urea.

The role of sulfhydryl groups in RNA polymerase is complicated by the complex nature of the enzyme itself (β', β, α_2, σ) and also because of the relatively large number of sulfhydryl groups present. The results obtained with the *Azotobacter* enzyme and by others with the *E. coli* RNA polymerase suggest multiple roles for sulfhydryl groups including subunit–subunit interaction, tight template binding, and catalytic activity all of which may reflect the role of sulfhydryl groups in the conformation of RNA polymerase. The data do not exclude a direct involvement of sulfhydryl groups in synthesis of phosphodiester bonds by RNA polymerase.

Acknowledgment

I thank Mr. Earl Fronk for his expert technical assistance.

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