Steady-state Kinetic Analysis of ATP Hydrolysis by the B Protein of Bacteriophage Mu

INVILOVEMENT OF PROTEIN OLIGOMERIZATION IN THE ATPase CYCLE*

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The DNA strand-transfer reaction of bacteriophage Mu requires Mu B protein and ATP for high efficiency. These factors facilitate the capture of target DNA by the donor protein-DNA complex. To understand the mechanism of the Mu B ATPase cycle in the Mu DNA strand-transfer reaction, we undertook a steady-state kinetic analysis of Mu B ATPase. The results reveal complex properties of the ATPase activity; Mu B protein oligomerizes in the presence of ATP, and ATP hydrolysis by the Mu B ATPase is stimulated by the protein oligomerization and shows a positive cooperativity with respect to ATP concentration. Mu B ATPase activity is also modulated by DNA and Mu A protein. DNA alone suppresses the catalytic activity of Mu B ATPase, whereas DNA enhances the apparent binding affinity for ATP. In the presence of Mu A protein together with DNA, however, the catalytic activity is greatly stimulated. Based on these results, we propose a working hypothesis in which oligomerization of Mu B protein plays a key role in its ATPase cycle.

Bacteriophage Mu utilizes transposition as an essential step in its life cycle and is the most efficient transposon known (for a review, see Symonds et al., 1987). A central step of Mu transposition is the transfer of Mu DNA strands to the target DNA strands, which has been amenable to extensive biochemical studies (for a review, see Mizuuchi and Craigie, 1986). The Mu DNA strand-transfer reaction in vitro contains, as DNA substrates, the donor mini-Mu DNA, which carries both (left and right) end sequences of Mu genome DNA as well as the internal activation sequence (Mizuuchi and Mizuuchi, 1989), and the target DNA. The reaction requires, as essential protein factors, Mu A protein (transposase) and Escherichia coli HU protein, and also requires Mu B protein and ATP for high efficiency. In the absence of either Mu B protein or ATP, the reaction efficiency can be very low (about 100-fold lower under normal reaction conditions) and the resulting products are mostly those in which donor Mu DNA strands are joined to the DNA sites within the same donor DNA molecule. Another host protein, IHF, also stimulates the reaction if the superhelical density of the donor DNA is relatively low (Sur et al. and Chaconas, 1989).

Mu B protein is an ATPase. The ATPase activity can be stimulated by DNA and Mu A protein (Maxwell et al., 1987). Mu B protein is also a nonspecific DNA binding protein (Chaconas et al., 1985). The stable association of Mu B protein with DNA requires binding of ATP (Adzuma and Mizuuchi, 1988b). Mu B protein stimulates the Mu DNA strand-transfer reaction primarily by associating with potential target DNA in the presence of ATP. DNA molecules associated with Mu B protein are, compared with free DNA molecules, much more efficient targets in the Mu DNA strand-transfer reaction. The binding of Mu B protein to DNA does not show any strong sequence specificity (there are, however, region(s) of DNA where Mu B protein appears to bind preferentially, which may be related to regional hot spot(s) of the Mu DNA strand-transfer). In the presence of Mu A protein, however, Mu B protein preferentially dissociates from DNA molecules carrying Mu end sequence(s), to which Mu A protein binds. This Mu A-induced dissociation requires hydrolysis of ATP. Therefore, if the Mu reaction mixture contains two kinds of potential target DNAs, one with and the other without a Mu end sequence, Mu B protein accumulates on the DNA molecules lacking a Mu end sequence. As a result, this DNA species is preferentially used as a target for strand-transfer (Adzuma and Mizuuchi, 1988b). DNA molecules carrying Mu end sequence(s) are thus protected against insertion of the mini-Mu donor DNA in vivo, a phenomenon called target immunity. This phenomenon is also observed with the Mu transposition in vivo (Reyes et al., 1987; Darzins et al., 1988). The in vivo transposition of the Tn3 family of transposons (for a review, see Grindley and Reed, 1985) and of Tn7 (Hauer and Shapiro, 1984) also exhibits a similar immunity phenomenon.

Thus, while ATP and Mu B protein are dispensable for the transfer of Mu DNA strands itself, these factors are crucial for efficient interaction of donor with the target DNA. ATP hydrolysis by Mu B ATPase is considered to play an essential role in maintaining the unequal distribution of Mu B protein between DNA molecules with and without Mu end sequence(s), presumably to avoid self-destruction. Understanding of these intricate protein-DNA and protein-protein interactions requires the detailed knowledge of not only structural but also kinetic aspects of these interactions. We are particularly interested in how Mu A protein might be involved in the apparent coupling of Mu B dissociation from DNA with the hydrolysis of ATP. Although there are many DNA-binding proteins which also possess ATPase activity, the analysis of their ATPase cycles remains mostly rudimentary, which limits our understanding of the kinetic and dynamic aspects of the reactions involving these proteins. Hoping to gain an insight into the mechanism of the Mu B ATPase cycle in the Mu DNA strand-transfer reaction, we initiated a kinetic analysis of Mu B ATPase. In this paper, we present the results of the analyses carried out at steady-state.

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1 M. Mizuuchi and K. Mizuuchi, unpublished observation.
The ATP-charcoal by centrifugation, the supernatant was centrifuged again to remove any residual charcoal, and a portion of this supernatant was used for measurements of $^{32}P$ and $^3H$ counts. The recoveries were normalized by the $^3H$ counts. The background $^3H$ count (without Mu B protein) was negligible (about 0.1-0.2% of total input, dependent on the batch of [y-3H]ATP). Since the recovery of [y-P]ATP was slightly dependent on the phosphate concentration itself, sodium phosphate (100 μM) was included in the charcoal suspension to keep the recovery constant.

Under these conditions, the hydrolysis of ATP is almost linear up to 2 h, without showing a noticeable lag. We thus use the measured hydrolysis rates to approximate the initial velocities at steady-state. Throughout this paper, we use $v_0/E_0$ (initial velocity normalized by total Mu B concentration) to describe the ATPase activity for easier comparison at different Mu B concentrations. ATP concentration always refers to initial ATP concentration, which is accurately detectable in concentrations of ≥ 0.1 μM (ATP).

**EXPERIMENTAL PROCEDURES**

**Chemicals**—The sources of chemicals are as follows: ATP, from Pharmacia, LKB; proteolytic index ATPs, from Boehringer Mannheim; [γ-32P]ATP, [γ-3H]lucine, [3H]glycine, and [3H]glycerol, Du Pont-New England Nuclear; BS, Pierce Chemical Co.; activated charcoal (Norit A), Sigma. These chemicals were used without further purification.

**DNA and Enzymes**—The DNA used in this study is, unless otherwise noted, the supercoiled plasmid pKN2 (3 kilobase-pairs in length), which carries the Mu right end sequence in pUC18 (Adzuma and Mizuuchi, 1988a, 1988b). DNA concentrations are expressed as moles of nucleotide.

Mu A protein was purified as described by Craigie and Mizuuchi (1985). We noticed, however, that a significant fraction (as much as 50%, depending on the preparation) seemed to have become inactivated during purification. Such a Mu B preparation (soiluted in a buffer containing 1 mM NaCl) was further purified by dialyzing the protein against a low salt Mu B buffer (LSB buffer: 30 mM Hepes-KOH (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol, and 30% (v/v) glycerol) at 4°C for several hours, followed by removing the buffer by centrifugation. Although Mu B protein has a tendency to precipitate at low ionic strength (such as 150 mM NaCl in the LSB buffer) and is then often irreversibly denatured, addition of glycerol (20-30%) suppresses this precipitation. After removing the precipitate formed during the dialysis, the remaining Mu B protein in the LSB buffer has no significant turbidity either initially or after a 1-h incubation at room temperature and also retains most of the original activity both in ATPase and strand-transfer assays. We thus consider that the precipitate which is removed mostly contains materials that had been already denatured before dialysis. The concentration of Mu B protein was determined by its optical density, using the extinction coefficient at 280 nm of 0.77 for 1 mg/ml Mu B protein.

$^3H$-labeled Mu B protein was prepared as follows. E. coli strain MK209 (leucine-prototroph), which carries a cryptic λ prophage with the cl857 repressor and the plasmid pMK209 containing the Mu A and B genes under the control of λ P, promoter (Craigie and Mizuuchi, 1985), was grown at 30°C to OD$_{600}$ = 0.5 in 1 liter of M9 medium (Miller, 1972) supplemented with 5 mg/kg leucine and 20 mg/kg each of the other 19 amino acids. Expression of the Mu B protein was induced by adding 500 mL of the same medium (without leucine) prewarmed to 64°C. After 15 min induction at 42°C, 5 mL of [3H]leucine was added and the incubation was continued at 37°C for an additional 2 h. The $^3H$-labeled Mu B protein was purified as described above (including the dialysis step). The specific activity of Mu B protein thus obtained was 820 cpm/pmol. We did not notice any appreciable difference between the $^3H$-labeled Mu B protein and unlabeled Mu B protein in either ATPase or the Mu DNA strand-transfer activity.

**Assay of ATP Hydrolysis**—Hydrolysis of ATP by Mu B ATPase was assayed by a charcoal-adsorption method as described by Maxwell and Gellert (1984) with minor modifications. The ATPase assay mixture (25 μl) is essentially the same as in the standard Mu DNA strand-transfer reaction, consisting of 25 mM Tris-HCl pH 7.5, 10 mM MgCl$_2$, 150 mM NaCl, 1 mM dithiothreitol, 20% (v/v) glycerol, 100 μg/ml BSA, 40 μCi/ml [γ-32P]ATP, and DNA and/or Mu A protein where indicated. BSA was added to prevent the adsorption of Mu B protein to reaction tubes, and inclusion of glycerol is necessary to prevent gradual inactivation of Mu B ATPase activity. The reaction was initiated by adding 1 μl of Mu B protein (appropriately diluted with LSB buffer containing 100 μg/ml BSA), incubated at 25°C, and quenched by adding 2.5 μl of glacial acetic acid. To the quenched mixture, we immediately added 500 μl of ice-cold charcoal suspension (HCl-washed, 10% (v/v) in 100 μM sodium phosphate, pH 7.5) and incubated the samples on ice for 30 min with brief vortexing at 10-min intervals. The charcoal adsorbs ATP but not Pi. After removing

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1. The abbreviations used are: ATP, γ-32P; adenine-5′-O-(3-thiotriphosphate); BSA, bovine serum albumin; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; SDS, sodium dodecyl sulfate; BS, bis(sulfosuccinimidyl) suberate.
An assay of the oligomeric state of Mu B protein by gel filtration (Sephacryl S200) also gives qualitatively similar results (data not shown). In the absence of nucleotides, Mu B protein elutes as a single peak consistent with a monomeric state, regardless of the amount loaded. If the buffer contains ATP or ATPγS, however, the position of the protein peak becomes dependent on the amount loaded; the protein eluted as a peak with a broad leading edge when a small amount was loaded or eluted in the void volume when the amount was large.

The view that Mu B protein forms oligomer(s) in the presence of ATP or ATPγS is also supported by results of protein cross-linking experiments, where Mu B protein was incubated with a protein cross-linker, BS3, under various conditions (data not shown). In the absence of nucleotides, the majority of Mu B protein becomes intramolecularly cross-linked, judging by the resulting smearable bands that migrate slightly faster than the intact Mu B protein on SDS-polyacrylamide gel electrophoresis. Use of a higher concentration of the cross-linker or a longer incubation of Mu B protein with the cross-linker does not affect the results qualitatively. In the presence of ATP or ATPγS, however, extensive intermolecular cross-linking is observed, giving rise to a ladder of discrete bands on the gel. No significant accumulation of a single specific species was observed under these conditions.

Oligomerization of Mu B Protein Stimulates Its ATPase Activity—The υ₀/[E], initial velocity normalized by total enzyme concentration (see "Experimental Procedures" and Footnote 4), of ATP hydrolysis by Mu B ATPase exhibits a dependence on the concentration of Mu B protein. Fig. 2 shows the values of υ₀/[E], measured at various concentrations of Mu B protein ([E],) at two different ATP concentrations (100 μM, closed circles, or 1 mM, open circles). When the Mu B concentration is reduced, the υ₀/[E], begins to decrease drastically in either case; the initial velocity is not proportional to the enzyme concentration. This decrease is not due to gradual denaturation of Mu B ATPase at lower protein concentrations, since, regardless of the Mu B concentration, ATP hydrolysis is almost linear within the time period in which the assays were carried out (data not shown). The possibility that the observed dependence on protein concentration might have been caused by an initial loss of Mu B protein due to adsorption to the reaction tubes was tested by transferring a part of the reaction mixture successively into new tubes, and then assaying for the ATPase activity, or directly measuring the loss of 3H-labeled Mu B protein. The results (data not shown) indicated that the fractional loss is slightly dependent on Mu B concentration, but is not substantial enough to fully explain the observed dependence of υ₀/[E], on Mu B concentration; only about 10–20% of the ATPase activity or 3H counts of Mu B protein was lost during transfer of the protein from one tube to another, even at a protein concentration of 0.15 μM. The fractional loss is not dependent on the presence or absence of ATP. From these results, we conclude that oligomerization of Mu B protein positively influences its ATPase activity. It should be noted that extrapolation of the data shown in Fig. 2 would indicate that, at an infinitely low protein concentration, Mu B protein may show very little or no ATPase activity in terms of υ₀/[E], implying that the Mu B protomer may be virtually incapable of binding and/or hydrolyzing ATP.
Hydrolysis—ATP hydrolysis by Mu B ATPase shows a sigmoidal response to ATP concentration (Fig. 3A). This non-hyperbolic behavior is more apparent upon plotting the data as an Eadie-Hofstee plot (Fig. 3B), which exhibits a characteristic curvature indicative of positive cooperativity (these non-hyperbolic behavior cannot be explained by the loss of Mu B protein due to adsorption to the reaction tubes at lower ATP concentrations; see above). The value of $k_{cat}$, $v_0/[E]$, at saturating concentration of ATP (see Footnote 4), is estimated to be $1.3 \times 10^{-2}$ s$^{-1}$ from the intercept on the ordinate of the Eadie-Hofstee plot. The ATP concentration that gives half $k_{cat}$, $[S]_{0.5}$, is approximately $150 \mu M$ under these conditions. However, $[S]_{0.5}$ varies with Mu B concentration as described below. Another important parameter worth recording here is the inflection point in the sigmoidal curve, which corresponds to the point whose value on the abscissa in the Eadie-Hofstee plot is maximal. The location of the inflection point, relative to $[S]_{0.5}$ or half $k_{cat}$, is a parameter which indicates the general contour of a sigmoidal curve (for example, in a symmetrically sigmoidal curve, the inflection point exactly corresponds to the $[S]_{0.5}$ and half $k_{cat}$) and is often related to the degree of the overall rate equation. As is seen, the inflection point comes slightly before the $v_0/[E]$, reaches half $k_{cat}$; it roughly corresponds to $[S]_{0.4}$ (the substrate concentration that gives 40% of $k_{cat}$).

The maximal slope on the Hill plot (Hill constant), another index of cooperativity, is graphically determined to be 2.0 (Fig. 4), implicating at least a second degree of the substrate concentration term in the overall rate equation. This can be explained by the presence of multiple active sites per active unit of Mu B ATPase. The notion of multiple active sites is also supported by the observation that nonhydrolyzable ATPγS does not behave as a simple competitive inhibitor. While ATPγS inhibits the ATP hydrolysis when the ATP concentration is high, it stimulates the hydrolysis at low concentrations of ATP (data not shown). We note, however, that the ATP hydrolysis in the presence of ATPγS may not be at steady-state, since dissociation of ATPγS from Mu B protein appears to be extremely slow (unpublished observation). Nonetheless, taken together with the sigmoidal dependence of ATP hydrolysis on ATP concentration, these results clearly indicate that description of the Mu B ATPase cycle requires consideration of multiple and nonequivalent states of the active sites.

Oligomerization of Mu B Protein Affects $[S]_{0.5}$ but Not $k_{cat}$—To understand the effects of oligomerization of Mu B protein on the ATPase activity, the dependence of $v_0/[E]$, on ATP concentration was compared at various concentrations of Mu B protein. As shown in Fig. 5, A and B, within the range of Mu B concentration tested (0.30–1.8 μM), the value of $k_{cat}$ (intercept on the ordinate in the Eadie-Hofstee plot; Fig. 5B) is not significantly affected by the concentration of Mu B protein. In contrast, the $[S]_{0.5}$ values differ drastically; the value increases from 90 μM at 1.8 μM Mu B protein to approximately 350 μM at 0.30 μM protein (Fig. 6). Thus, the low values of $v_0/[E]$, at low concentrations of Mu B protein are primarily attributable to larger values of $[S]_{0.5}$ (less apparent binding affinity for ATP), but not to smaller $k_{cat}$; if the ATP concentration is high enough, full activity can be restored. The Hill constant is always about 2 (± 0.2), regardless of Mu B concentration (data not shown).

DNA Modulates Mu B ATPase Activity either Positively or Negatively—We have so far described the characteristics of the Mu B ATPase activity in the absence of cofactors. However, this activity is known to be affected by DNA and Mu A protein (Maxwell et al., 1987), factors which are of central importance to understanding the role of ATP hydrolysis in
Kinetic Analysis of Mu B ATPase

**Fig. 6.** Dependence of [S]_{0.5} on Mu B concentration. The value of [S]_{0.5} is determined by dividing each of half k_{cat} by the corresponding value on the abscissa in Eadie-Hofstee plot shown in Fig. 5B. Obtained values were further compared with those obtained by other types of plots (standard, Hans, and Lineweaver plots) to confirm their identities. The values at lower Mu B concentrations are likely to contain larger standard errors because of more ambiguity with respect to the corresponding k_{cat}.

![Figure 6](image-url)

**Fig. 7.** DNA can either stimulate or inhibit ATP hydrolysis. Mu B ATPase activity ([E]_f = 1.8 μM) was measured at an ATP concentration of either 500 μM (closed circles) or 20 μM (open circles), in the presence of various concentration (plotted logarithmically) of DNA. Note that the right ordinate (activities at 20 μM ATP) is a 10-times expansion of the left ordinate (at 500 μM).

![Figure 7](image-url)

The Mu DNA strand-transfer reaction. In the following sections, the effects of these cofactors are addressed.

DNA can either stimulate or inhibit ATP hydrolysis, depending on the ATP concentration used. At a high concentration of ATP (500 μM, closed circles in Fig. 7), DNA inhibits ATP hydrolysis, whereas DNA significantly stimulates the activity at low ATP concentration (20 μM, open circles). The dependence of [E]/[E]_f on ATP concentration in the presence of saturating concentrations of DNA (open circles in Fig. 8, A and B) was compared with that in the absence of DNA (closed circles). It reveals two effects of DNA; DNA enhances the apparent binding affinity of Mu B ATPase for ATP (indicated by decreased [S]_{0.5}) but decreases k_{cat}. Thus, when the ATP concentration is limiting, DNA stimulates ATP hydrolysis, while, when the ATPase is saturated with ATP, DNA inhibits. At 0.45 μM Mu B protein and a saturating concentration of DNA, the k_{cat} is 2.0 × 10^{-3} s^{-1} and [S]_{0.5} is 25 μM.

Mu B ATPase activity in the presence of DNA appears to follow a simple Michaelis-Menten (first-degree) kinetics within the range of ATP concentrations examined (≥5 μM), where it gives rise to a nearly straight line in the Eadie-Hofstee plot (Fig. 8B). In fact, the Hill plot of the activity in the presence of DNA gives the Hill constant of about 1 (±0.2; data not shown). Since we could not reliably measure ATP hydrolysis at ATP concentrations less than 5 μM, there remains a possibility that the ATP hydrolysis in the presence of DNA might still be sigmoidal at very low concentrations of ATP. However, the ATP concentration at the possible inflection point would have to be lower than [S]_{0.5}, since the [E]/[E]_f at 5 μM ATP shows about 20% of the k_{cat}, but without any sign of inflection point. Thus, taking into consideration that the inflection point in the absence of DNA is around [S]_{0.5} (see Figs. 3 and 5), Mu B ATPase activity exhibits less, or possibly no, sigmoidal behavior in the presence of DNA.

Mu B ATPase activity in the presence of DNA is also virtually unaffected by the concentration of Mu B protein within the range of protein concentrations tested (0.15–1.8 μM; data not shown). The Mu B concentration dependence in the presence of DNA was examined at relatively low (30 μM) as well as high (300 μM) ATP concentrations, since [S]_{0.5} is small in the presence of DNA and the dependence of [E]/[E]_f on Mu B concentration is more obvious at ATP concentrations near [S]_{0.5}. In either case, the [E]/[E]_f is nearly constant, regardless of Mu B concentration.

Oligomeric states of Mu B protein was also investigated in the presence of saturating concentrations of DNA by protein cross-linking method (data not shown). In the presence of DNA alone, majority of the cross-linked products are intramolecular ones, while, in the presence of ATP or ATPγS together with DNA, extensive intermolecular cross-linking is observed. Thus, ATP-dependent oligomerization of Mu B protein most probably occurs even in the presence of saturating concentrations of DNA.

**Fig. 8.** DNA affects both [S]_{0.5} and k_{cat} of Mu B ATPase activity. The dependence of [E]/[E]_f on ATP concentration were examined in the absence (○) or presence (□) of 400 μM DNA at 0.45 μM Mu B protein. A, standard plot. B, Eadie-Hofstee plot.

![Figure 8](image-url)
binding of ATPγS outweighs the loss from the competitive inhibition by ATPγS.

Our results suggest that Mu B protein may be a monomer in the absence of ATP. However, we do not know whether the oligomer(s) formed in the presence of ATP are specific or heterogeneous in size. The results of sucrose gradient centrifugation, gel filtration, and protein cross-linking experiments are in favor of the latter view. However, to address this question more rigorously, it is necessary to know not only the apparent molecular weight but also the shape of the oligomer(s).

Models for Mu B ATPase Cycle in the Absence of Cofactors—There are in principle two simple models to explain the observed positive cooperativity of ATP hydrolysis and its dependence on protein oligomerization. We will briefly summarize these two models (Schemes I and II) here and discuss the details further in the Appendix (in miniprint). In the following arguments, we assume a dimer of Mu B protein as the oligomeric unit of Mu B ATPase; further possible oligomerization is assumed to have no effect on the kinetic parameters of this unit. This assumption is based on the results that the Hill constant of Mu B ATPase activity is about 2, which means that the degree of the overall rate equation can be approximated to 2, and also based on the observation that Mu B protein contains only one consensus amino acid sequence for a presumptive ATP binding site (Teplow et al., 1988). Although this assumption is merely the simplest that is consistent with the kinetic data, it is impractical, at this early stage of investigation, to invoke higher degrees of oligomerization, which could result in unnecessarily complicated schemes.

The first model (Scheme I) assumes that binding of ATP is a prerequisite step for dimerization of Mu B protein and dissociation of the dimer to monomer can occur only after it has lost ATP either by simple release or hydrolysis of ATP.

In Scheme I, $E$, $S$, and $P$ represent Mu B monomer, ATP, and the product (ADP and P$_i$), respectively. In this model, the positive cooperativity of ATP hydrolysis and its dependence on protein concentration are simultaneously explained by the assumption that dimer species are more efficient in binding and/or hydrolyzing ATP than monomer species. The factors $\alpha$, $\beta$, and $\gamma$ are assigned to indicate these kinetic difference(s) ($\alpha > 1$ and/or $\beta < 1$ and/or $\gamma < 1$). We note that the two active sites within a dimer molecule itself are assumed to be kinetically independent as well as equivalent (no coop-

As described later, our preliminary nucleotide binding studies suggest that simple release of ATP may be much slower than the hydrolysis of ATP. Therefore, although we assume that the nucleotide-free form of Mu B dimer ($E_0$ species) is eligible for the dissociation of the dimer to monomer in our schemes, it is also quite possible that some other form(s), such as ADP-bound form, may be the eligible form(s).
operative interaction between these two sites). Positive cooperativity is thus coupled to protein oligomerization in this scheme.

Preliminary results of ATP and ATPγS binding experiments suggest that the nucleotide binding itself appears to be positively cooperative, with the affinity dependent on the Mu B dimer approximation, that the first binding of ATP to Mu B monomer may be very slow or that the monomer may be very inefficient in hydrolyzing ATP (γ ≈ 0). Either of the two possibilities is consistent with the results that Mu B protein shows little or no ATP hydrolysis at very low concentrations of the protein (see Fig. 2).

Scheme I predicts that, regardless of the protein concentration, all of the Mu B protein would eventually become a dimer species (ES2) at an infinite concentration of ATP, which explains the results that Mu B concentration does not affect kcat. It is also worth noting that, according to our preliminary results of nucleotide binding experiments, simple dissociation of ATP may be much slower than the hydrolysis of ATP. If the loss of ATP is prerequisite to dissociation of Mu B oligomer as is assumed in Scheme I, these preliminary data agree with the results from sucrose gradient experiments, which suggest that dissociation of Mu B oligomer may be a very slow process in the absence of ATP hydrolysis (see Fig. 1).

One may consider an alternative model that assumes an equilibrium between monomer and dimer even in the absence of ATP (in favor of the monomer), in which only the dimer can bind ATP (Scheme II). In this scheme, ATP binding causes a shift of the monomer-dimer equilibrium toward the dimer species. Higher concentrations of ATP thus counteract the disadvantage of lower concentrations of Mu B protein, which explains the results that kcat does not depend on Mu B concentration. Because of the assumed difference in ATP binding ability between monomer and dimer, this scheme can explain the results of the ATPase activity on protein concentrations and also can produce a slightly sigmoidal curve. However, to explain the significantly sigmoidal curve that is observed in actual data, it is necessary to consider, in addition to the difference between the monomer and dimer in ATP-binding ability, a cooperative interaction between the two active sites within a dimer molecule itself (α > 1 and/ or β < 1 and/or γ < 1; see Fig. 11F in the Appendix). In this respect, cooperativity and protein oligomerization is not as intimately coupled in Scheme II as in Scheme I.

In the Appendix, we present the rate equations and theoretical curves based on Scheme I and II. As demonstrated in Fig. 11 (in the Appendix), these schemes can produce the curves that fit the data obtained in the absence of cofactors reasonably well.

DNA is a Modulator of Mu B ATPase—How might the models described above be applicable for the Mu B ATPase activity in the presence of cofactors? It is certainly possible (and, to some extent, justifiable) to consider that these factors act as allosteric effectors, permitting the Mu B ATPase to follow the pathway that may be virtually irrespective of the kinetic characters observed in the absence of cofactors. However, it is important to first examine whether, or to what extent, the schemes for the ATPase activity in the absence of cofactors can or cannot explain the data in the presence of cofactors, without invoking a gross alteration of the schemes. The following arguments will address this question.

DNA has two primary effects on Mu B ATPase activity, decrease of kcat and decrease of [S]0.5. The former effect corresponds to the inhibitory effect of DNA at high ATP concentration, and the latter underlies the stimulatory effect at low ATP concentration. Also, we have previously shown that stable association of Mu B protein with DNA requires binding of ATP and the dissociation from DNA requires hydrolysis of ATP. The simplest way to accommodate these results to Scheme I is to assume that, in the presence of saturating concentrations of DNA, the binding of Mu B protein to DNA is coupled to protein oligomerization, regarding all the dimer species (E, E*, E2S, E2S2) as unbound forms. In fact, the majority of Mu B protein can be intermolecularly cross-linked in the presence of saturating concentrations of DNA and ATP, whereas only intramolecular cross-linked products are obtained in the presence of DNA alone (data not shown). In this view, the catalytic rate constant of the dimer species (k0) should be smaller in the presence of DNA than in the absence of DNA, because kcat is reduced by DNA (kcat is equal to k0 in both Scheme I and II). If hydrolysis of ATP is much faster than the simple release of ATP (as suggested by our preliminary nucleotide binding studies), the decrease of k0 by itself could lead to a decrease of [S]0.5, as shown in Fig. 11C, curve 7 (in the Appendix). In order to explain a significant stimulation by DNA at low concentrations of ATP, however, additional alteration of other kinetic parameters may also have to be considered. We speculate that, for example, the oligomer of Mu B protein associated with DNA may be less likely to dissociate to monomer (smaller k0), compared with the oligomer formed in the absence of DNA (see Fig. 11C, curve 8, in the Appendix). Our results also show that the dependence of ATP hydrolysis on Mu B concentration is barely detectable in the presence of DNA and that the dependence on ATP concentration appears to be nearly hyperbolic in the presence of DNA (see Fig. 8). In Scheme I, these results can be most simply explained by the view that Mu B ATPase in the presence of DNA has the very small k0, so that the ATPase turns over essentially without a protein oligomerization cycle, thus without dissociating from DNA. In fact, dissociation of Mu B protein from DNA seems to be considerably slower than the rate of ATP hydrolysis (Adzuma and Mizuuchi, 1989). It should be recalled here that, in Scheme I, the two active sites within a dimer molecule are assumed to be kinetically equivalent as well as independent. Therefore, if the ATPase does not undergo the monomer state, the overall rate of ATP hydrolysis would become hyperbolic with respect to ATP concentration as well as independent on Mu B concentration.

In brief, Scheme I could explain the kinetic data in the presence of DNA by a relatively simple view that, compared with Mu B ATPase in the absence of DNA, the ATPase

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In a simple Michaelis-Menten equation:

\[ \frac{v_0}{[E]} = k_{cat}[S]/(K_a + [S]), \]

\[ k_{cat}/K_a \] approximates the apparent second-order rate constant at low [S], since

\[ v_0 = (k_{cat}/K_a)[E][S] \]

\[ ([S] \ll K_a, [E] \approx [E]). \]

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This is because the value of [S]0.5 is primarily determined by the ratio of \( k_{cat} + k_{i} \) versus \( k_{i} \), which would become equal to \( k_0/k_{i} \) if \( k_{i} \gg k_{cat} \).
associated with DNA may have less catalytic activity (smaller $k_3$) and may be slower to dissociate to monomer (smaller $k_d$). In contrast, more complicated interpretations are necessary to explain the data in the presence of DNA with Scheme II, since this scheme presupposes a positive cooperativity between the two active sites within a dimer molecule itself.

**Mu A Protein Is an Activator for Mu B ATPase in the Presence of DNA**—Our results indicate that Mu A protein primarily affects $k_{on}$ (equal to $k_3$ in our schemes) of Mu B ATPase in the presence of DNA. If ATP hydrolysis is significantly faster than the simple dissociation of ATP, a possibility that is suggested by our preliminary ATP binding studies, an increase of the catalytic rate constant ($k_3$) would result in an increase of $|S|_0$, which agrees with our data (see Fig. 11D, curve 9, in the Appendix; see also Footnote 10).

Mu A protein is also known to enhance the dissociation of Mu B protein from DNA, which requires hydrolysis of ATP (Adzuma and Mizuuchi, 1988b). How might this enhanced dissociation of Mu B protein from DNA be coupled with the stimulation of ATP hydrolysis by Mu A protein? The most naive view is that Mu A protein simply stimulates the ATP hydrolysis of DNA-bound Mu B ATPase, hereby indirectly increasing the time-average occupancy of some form (for example, the nucleotide-free form or ADP-bound form) of Mu B protein that is eligible to dissociate from DNA. This type of enhancement of the Mu B dissociation from DNA, however, should in theory be counteracted by higher ATP concentrations, if the Mu B ATPase can exchange ADP with exogenous ATP without leaving DNA as we suppose in our model. Although more precise measurements are essential, we have hitherto not noticed any sign that higher ATP concentrations suppress Mu A-induced dissociation of Mu B protein from DNA. Therefore, we currently favor the view that Mu A protein not only stimulates the catalytic activity of Mu B ATPase in the presence of DNA, but also somehow directly dissociate Mu B protein from DNA by forming Mu A-Mu B complex. We must emphasize that the Mu B ATPase cycle in the presence of DNA and Mu A protein most probably represents an intricate mixture of the activities exhibited by various species, such as free Mu B protein, DNA-bound Mu B protein, and Mu A-Mu B complex with or without DNA associated. It will require much more rigorous kinetic analysis to sort out these activities.

**CONCLUSIONS**

In this study, we examined the overall characteristics of the Mu B ATPase activity in the absence and presence of cofactors, by using steady-state kinetic analyses. The results may be summarized as follows. 1) Mu B protein oligomerizes in the presence of ATP, which results in an increase of apparent binding affinity of the ATPase for ATP. 2) ATP hydrolysis by Mu B ATPase in the absence of cofactors shows positive cooperativity with respect to ATP concentration. 3) DNA reduces the catalytic activity of the ATPase but increases its apparent binding affinity for ATP. (4) The ATPase activity in the presence of DNA is nearly hyperbolic. 5) Mu A protein stimulates the catalytic activity of the ATPase in the presence of DNA. From these results, we propose a working hypothesis, in which oligomerization of Mu B protein plays an integral role in the ATPase cycle. As shown in Fig. 11 (in the Appendix), it is not difficult to obtain theoretical curves, based on these models (Schemes I and II), which fit the kinetic data in the absence of cofactors reasonably well. Scheme I (but not Scheme II) can also produce the curves which qualitatively resemble the activities in the presence of cofactors. We view this consistency between the theoretical curves and the actual data, though by no means a proof of the model, as a good basis for further refinement of the picture of the Mu B ATPase cycle.

The Mu B ATPase cycle, especially in the presence of DNA and Mu A protein, is undoubtedly a very complex system and perhaps cannot be easily solved by steady-state kinetic analyses alone. Clearly, separate measurements of individual kinetic steps, which may only be possible by pre-steady-state kinetic analyses, are essential to refine or even refute our current scheme. We also must emphasize that, although kinetic analyses are certainly powerful in that they can limit the possible mechanism(s) and can provide a model which has a predictive value, the model would have to remain speculative unless it is complemented by physical information on the important kinetic intermediates.

Finally, we note apparent similarities between the Mu B ATPase and the E. coli recA ATPase, with respect to their major kinetic characteristics. The recA protein has a well known tendency to form oligomers and even polymers. Its ATPase activity shows a strong positive cooperativity with respect to ATP concentration, seems to exhibit a significant dependence of $|S|_0$ (but not the $k_{on}$) on the protein concentration (for example, see Table I in Weinstock et al., 1981; also see Kowalczykowski, 1986, for a possible interpretation for the sigmoidal behavior of recA ATPase), and is affected (though positively) by DNA (for a review, see Cox and Lehman, 1987). Further analyses of these ATPases are expected to provide a coherent view of the ATPase cycle of these self-assembling DNA-binding ATPases.

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**REFERENCES**


Kinetic Analysis of Mu B ATPase

**Semenal Material to:**

**Steady-state kinetic analysis of ATP hydrolysis by the B protein of bacteriophage Mu: Involvement of protein polymerization in the ATPase cycle**

*Kemp, Andrea and Kunain Mikaelen*

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**DISCUSSION**

**Purpose:** To examine the kinetic properties of the B protein of bacteriophage Mu in relation to its role in ATP hydrolysis and polymerization.

**Key Points:**
- The B protein is involved in the polymerization process.
- The kinetic analysis reveals the presence of multiple states.
- The polymerization process is kinetically distinct from the ATPase activity.

**Materials and Methods:**
- ATPase activity was measured using a stopped-flow apparatus.
- Polymerization was monitored using gel electrophoresis.
- Kinetic parameters were determined using substrate concentrations ranging from 10 μM to 1 mM and ATP concentrations from 0.1 mM to 1 mM.

**Results:**
- The B protein exhibits a high affinity for ATP, with a dissociation constant (Kd) of 0.1 mM.
- The turnover number (kcat) is 100 s⁻¹.
- The Vmax value is 1 μmol/min/mg.
- The Michaelis-Menten constant (Km) is 0.1 mM.

**Conclusion:**
- The B protein of bacteriophage Mu is a highly efficient ATPase.
- The polymerization process is an important regulator of its activity.

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**Table I: Kinetic parameters used for calculating the theoretical curves**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[B]</td>
<td>1</td>
</tr>
<tr>
<td>[ATP]</td>
<td>1</td>
</tr>
<tr>
<td>[Pi]</td>
<td>1</td>
</tr>
</tbody>
</table>

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**Figure 1:**
- The curves were obtained by numerically calculating the rate equations presented in Table I.
- The experimental data points are represented by squares.
- The fit of the theoretical curves to the data is shown.

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**Figure 2:**
- A, B, and C represent different stages of the polymerization process.
- The transition from B to C is accompanied by a change in the B protein's conformation.

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**Figure 3:**
- The B protein's activity is regulated by the presence of DNA.
- The polymerization process is triggered by the binding of DNA to the B protein.
- The rate of polymerization increases with increasing DNA concentration.

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**Figure 4:**
- The B protein's activity is inhibited by the presence of MgATP.
- The inhibition is concentration-dependent.
- The inhibition constant (Ki) is 0.5 mM.

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**Figure 5:**
- The B protein's activity is modulated by the presence of other proteins.
- The interaction between the B protein and other proteins affects its ATPase activity.
- The dynamic range of the B protein's activity is broad.