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The Role of S-Adenosylmethionine in the Cleavage of Deoxyribonucleic Acid by the Restriction Endonuclease from *Escherichia coli K**

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SUMMARY

The restriction endonuclease from *Escherichia coli* K specifically cleaves foreign DNA in the presence of *S*-adenosylmethionine, ATP, and Mg^{2+}. The role of *S*-adenosylmethionine in this reaction has been studied by following the specific binding of the enzyme to unmodified DNA. The results indicate that *S*-adenosylmethionine acts as an allosteric effector. However, the rate-limiting step in the activation of the enzyme is not the binding of the effector itself, but an event subsequent to it. The interaction of the *S*-adenosylmethionine with two mutant *K* restriction endonucleases isolated previously has also been investigated. One of them, which is defective in restriction, can be activated in a manner similar to the wild type enzyme, while the other one, which lacks both restriction and modification activities (due to a mutation in the subunit responsible for DNA recognition), shows no such effect.

Restriction endonucleases are enzymes that recognize DNA synthesized in other bacterial strains and hydrolyze it by making a limited number of double-stranded breaks. Besides a restriction activity, each one of these bacterial strains also possesses a specific DNA methylation activity that renders its own DNA insensitive to the corresponding restriction enzyme.

One type of restriction enzyme is exemplified by those produced by *Escherichia coli* strains carrying the resistance transfer factors R_I and R_{II}. These enzymes appear to be oligomers of identical subunits that require only Mg^{2+} for activity and do not seem to methylate unmodified DNA (1, 2). These properties are in marked contrast to those of another type of restriction enzyme such as those isolated from *E. coli* K and B,[1] which have a complex oligomeric structure, and requirements for ATP, *S*-adenosylmethionine, and Mg^{2+} for nuclease activity (3, 4, 5, 6). Furthermore, they are able to methylate unmodified DNA when only Ado-Met is present (7, 8).

Genetic studies with *E. coli* K and B are in agreement with a three-gene model for this type of restriction and modification system (9, 10). One gene, *hsdM*, is responsible for modification; another one, *hsdR*, codes for the endonuclease activity, and the third one, *hsdS*, allows recognition of the host specificity sites on the DNA. The purified K restriction enzyme contains three different kinds of subunit and can either restrict or modify unmodified DNA depending on the incubation conditions.

The role of Ado-Met in the restriction reaction catalyzed by the enzyme from *E. coli* K has been studied using the wild type enzyme and two mutant enzymes previously characterized. The first one of these mutant proteins, endonuclease R.K-18, cannot restrict unmodified DNA, but has normal methylase activity. On the other hand, endonuclease R.K-19 neither restricts nor modifies, due to a defect in the subunit responsible for site recognition on the DNA. These two mutant proteins complement each other *in vitro*, yielding the same properties as the wild type enzyme (11). In this paper, Ado-Met is shown to act as an allosteric effector, and some characteristics of the activated enzyme are described.

EXPERIMENTAL PROCEDURES

Materials

Bacteria and Bacteriophages—The wild type *Escherichia coli* K was 1100 KLF1, an endonuclease I-deficient strain that carries a second copy of the restriction and modification genes. The *E. coli* strains K-18 and K-19 were the strains 802 and 803 described by Wood (12) and have the phenotype $r_K^- m_K^+$ and $r_K^- m_K^-$ respectively. The λ phage was λ $cI_{857}sus$ S 7 and was prepared by heat induction of suitable lysogens. The phage was purified by standard procedures.

DNA and Enzyme Preparations—[^{3}H]thymidine, λ.K DNA, and

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[1] The abbreviations used are: K and B, restriction and modification specificities associated with *Escherichia coli* K and B, respectively; λ.0, phage λ grown on a nonmodifying strain; λ.K, phage λ grown on a K strain; r^- and m^-, refer to restriction and modification mutations, respectively; sK and sB refer to the genetically defined host specificity sites for the K and B restriction and modification systems;
endonuclease R.K, restriction endonuclease from *E. coli* K;
endonuclease R.B, restriction endonuclease from *E. coli* B;
endonuclease R.K-18, restriction endonuclease from K mutant K-18;
endonuclease R.K-19, restriction enzyme from mutant strain K-19;
Ado-Met, *S*-adenosylmethionine;
TES, *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid.

λ.0 [32P]DNA were prepared as described previously (3). The restriction enzymes from wild type K-18 and K-19 strains of *E. coli* K were purified according to procedures published elsewhere (3, 11). In all of these experiments, the purified glycerol gradient fractions were used.

Other Materials—Biochemical reagents were obtained from Sigma and, with the exception of Ado-Met, were used without further purification. Ado-Met was purified as described earlier (3) and stored frozen at a concentration of 6.7×10^{-3} M in 0.05 N acetic acid. The nitrocellulose filters used in the DNA binding assay were Sartorius SM 11306 with a diameter of 25 mm.

Methods

It has been previously shown that the wild type restriction enzyme forms a specific complex with unmodified λ DNA that can be stabilized with EDTA and detected by binding to nitrocellulose filters (13). The same procedure has also been used to detect complementation between endonucleases R.K-18 and R.K-19 (11).

RESULTS

Dependence of DNA Binding on S-Adenosylmethionine Concentration—The binding of λ.0 DNA to nitrocellulose filters by endonuclease R.K was measured at Ado-Met concentrations ranging from 10^{-8} to 10^{-6} M (Fig. 1*a*). The apparent K_m for Ado-Met in this DNA binding reaction was estimated to be 3×10^{-7} M, in agreement with the value obtained for cleavage of DNA (3). The curve was clearly sigmoidal. The cooperativity index is defined as the amount of ligand required to give 90% of maximal activity (A_{max}) divided by the amount that would give 10% of A_{max} (14). The value estimated from this curve was approximately 10. In the absence of cooperativity this value would be 81. This finding, along with earlier results showing that the Ado-Met isolated from an Ado-Met-enzyme complex had not undergone any chemical change (11), was consistent with the role of Ado-Met as an allosteric effector.

To test this hypothesis further, the data of Fig. 1*a* are shown replotted in Fig. 1*b* according to Hill (15). This method is generally applicable to cases of cooperative binding. The slope of the resulting straight line should yield the number of Ado-Met binding sites on the enzyme if cooperativity is absolute. In practice, absolute cooperativity is never observed, and the slope gives the minimum number of interacting binding sites. The number determined from Fig. 1*b* was 2.4. Therefore, the enzyme has at least three Ado-Met binding sites.

The characteristics of the interaction of the enzyme with Ado-Met can be summarized as follows. the dependence of DNA binding on Ado-Met concentration resulted in a sigmoidal curve, the reciprocal plot of these data was concave upwards (not shown), the cooperativity index was low and the slope of the Hill plot was greater than unity. These are four of the criteria described by Koshland (15) for an allosteric protein, and they allow us to conclude that Ado-Met is a positive allosteric effector of the K restriction endonuclease.

Activation of Enzyme by Ado-Met—The preceding results suggested that the interaction of the enzyme with Ado-Met might occur early in the sequence of reactions leading to restriction, perhaps even being the first event. Accordingly, experiments were performed in which the enzyme was incubated for 2 min at 30° with Ado-Met at a concentration several times higher than the apparent K_m, usually 5 times higher. The enzyme was then diluted 100-fold into a DNA reaction mixture containing λ.0 [32P]DNA, ATP, and Mg^{2+}, but no additional Ado-Met. In these experiments, DNA is in excess, while the Ado-Met concentration after dilution has become 20 times lower than the apparent K_m. Further incubation was carried out, and aliquots were removed at 10-s intervals and filtered through nitrocellulose filters after

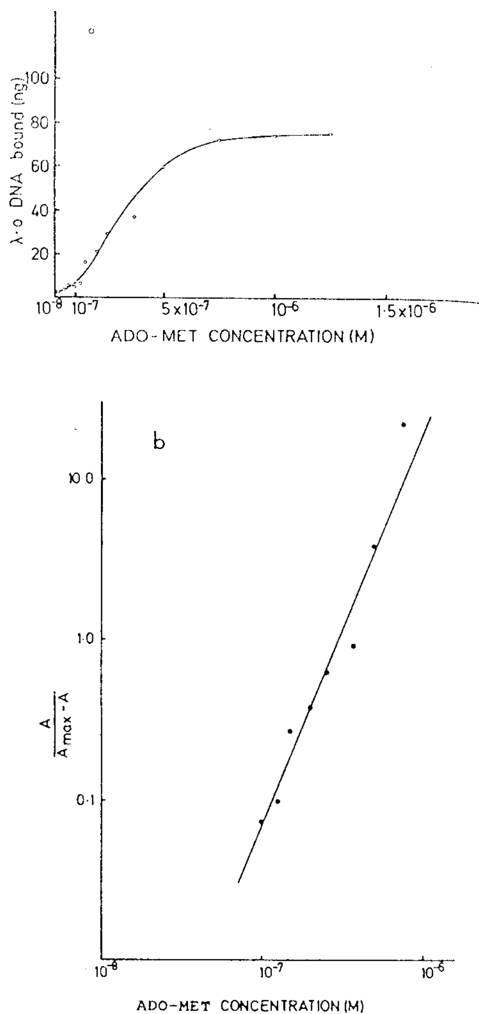


FIG. 1. Effect of Ado-Met concentration on DNA binding by endonuclease R.K. *a*, reaction mixtures with a volume of 0.25 ml contained 25 μmol of TES, pH 8.0, 70 μmol of EDTA, 1.6 μmol of $MgCl_2$, 3 μmol of mercaptoethanol, 0.1 μmol of ATP, 2×10^{6} phage equivalents of λ.0 [32P]DNA, and the indicated concentrations of Ado-Met. The reaction was started by the addition of 2.6 μg of endonuclease R.K. After incubation at 30°, the reactions were stopped by the addition of 0.03 ml of 0.5 M EDTA, pH 8.0. The mixtures were then filtered through nitrocellulose filters. *b*, plot of binding data from Fig. 1*a* according to Hill (14).

the reaction had been stopped by the addition of EDTA. The amount of λ.0 [32P]DNA bound to the filters was then measured. As control experiments, regular binding assays were done, mixing the enzyme with complete reaction mixtures containing Ado-Met at either the high (5 times the K_m) or the low (20 times lower than the K_m) concentrations. The results (Fig. 2) show that enzyme that has been preincubated with Ado-Met at the higher concentration and is then diluted to give an Ado-Met concentration 20 times lower than the apparent K_m is still able to bind DNA fully. However, incubation of the enzyme with complete reaction mixtures containing Ado-Met at the lower concentration yielded almost no DNA binding to the filters. Therefore, preincubation of the enzyme with Ado-Met produces an enzyme species that is now capable of binding unmodified DNA in the virtual absence of free Ado-Met. This shows that the reaction of Ado-Met with the enzyme is an early step in the mechanism of restriction that can take place in the absence of DNA and ATP.

In experiments similar to this one, it has also been observed that this reaction of Ado-Met with the enzymes does not require

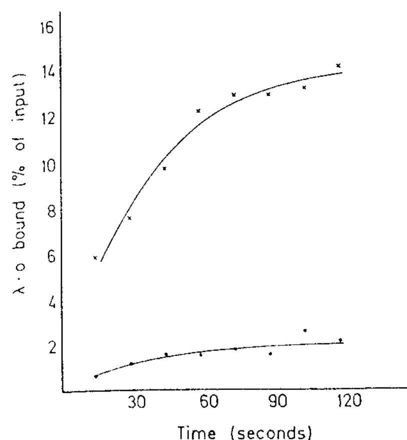
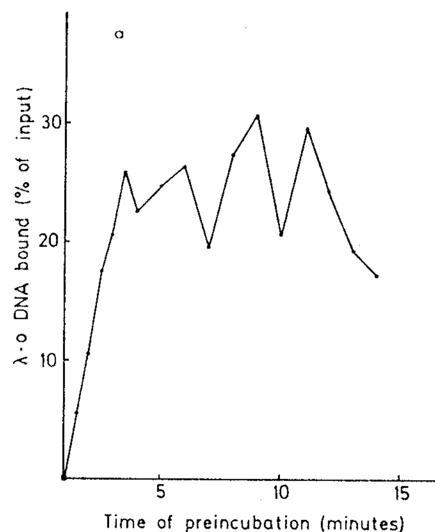


FIG. 2. Effect of preincubation of endonuclease R.K. with Ado-Met. The reaction mixture contained in a volume of 15 μl: 1.5 μmol of Tris, pH 8.0, 3.7 nmol of EDTA, 0.1 μmol of $MgCl_2$, 0.18 μmol of mercaptoethanol, and 15 pmol of Ado-Met (final concentration 1×10^{-6} M). Preincubation with 10 μl (10 μg) of endonuclease R.K was carried out in this solution for 2 min. at 30°. Then 1 ml of a DNA binding reaction mixture containing 100 μmol of Tris, pH 8.0, 0.25 μmol of EDTA, 6.4 μmol of $MgCl_2$, 11.9 μmol of mercaptoethanol, 0.5 μmol of ATP, and 3×10^{10} phage equivalents of λ.0 [^{32}P]DNA was added, bringing the Ado-Met concentration down to 2.7×10^{-8} M. The incubation was continued at 30°, and aliquots of 100 μl were taken out at the times indicated and added to 50 μl of 0.5 M EDTA to stop the reaction. The samples were then passed through nitrocellulose filters. In the control reaction, 10 μl of enzyme were added directly to 1 ml of the DNA binding reaction mixture which contained 2.7×10^{-8} M Ado-Met, incubation was carried out at 30°, and aliquots were removed just as indicated above. X——X, enzyme preincubated with Ado-Met; ●——●, DNA binding carried out at low Ado-Met concentration.

Mg^{2+}, and that the activated enzyme actually cleaves unmodified λ.0 DNA in the presence of ATP and Mg^{2+} without free Ado-Met. Therefore, free Ado-Met does not appear to be required for the actual DNA cleavage.

Kinetics of Enzyme Activation by Ado-Met—Since the K restriction endonuclease activated by Ado-Met is able to bind unmodified DNA to nitrocellulose filters in the virtual absence of free Ado-Met, it is possible to measure the amount of activated enzyme as a function of time of preincubation with Ado-Met. Only that fraction of the enzyme that has been activated by Ado-Met will be able to bind unmodified DNA after the Ado-Met has been diluted out.

A reaction mixture containing the enzyme and Ado-Met at a concentration 5 times higher than the K_m was incubated at 30°. Aliquots of 3 μl were removed after various incubation times and mixed rapidly with 200 μl of a reaction mixture that contained λ.0 [^{32}P]DNA, ATP, and Mg^{2+}. The DNA-enzyme complex was allowed to form, was stabilized by the addition of EDTA, and then passed through a nitrocellulose filter. The results (Fig. 3*a*) show that the amount of activated enzyme does not reach a stable plateau value, but rises to an initial peak, and then drops, only to rise again later. This phenomenon occurs with a periodicity of 3 to 4 min for at least 15 min and has been observed numerous times. This might be explained by the fact that the activated enzyme is unstable (as is shown in the following section). Unless the reaction proceeds beyond activation to DNA binding and ultimately DNA cleavage, the activated enzyme decays, giving a species that no longer binds, but which can be reactivated by Ado-Met to start the cycle over again. Periodicity might be explained in a number of ways. In this system, periodicity can be observed because both activation and decay are slow.



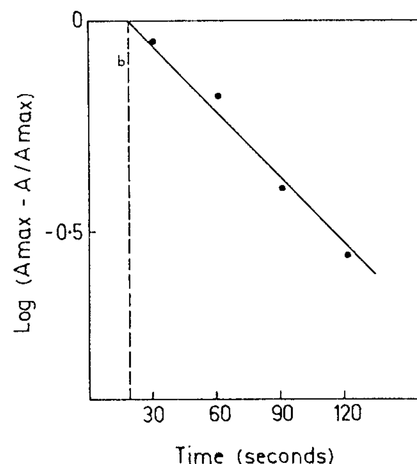


FIG. 3. Kinetics of enzyme activation by Ado-Met. *a*, preincubation of 40 μl (40 μg) of endonuclease R.K was carried out at 30° after adding it to a reaction mixture of 22 μl containing 6 μmol of Tris, pH 8.0, 0.015 μmol of EDTA, 0.38 μmol of $MgCl_2$, 0.72 μmol of mercaptoethanol, and 0.13 nmol of Ado-Met. At the times indicated, aliquots of 3 μl were removed and diluted into 200 μl of a DNA binding reaction mixture containing 20 μmol of Tris, pH 8.0, 0.05 μmol of EDTA, 1.3 μmol of $MgCl_2$, 2.4 μmol of mercaptoethanol, 0.1 μmol of ATP, and 5.9×10^9 phage equivalents of λ.0 [^{32}P]-DNA. Each sample was then further incubated for 2 min, and 50 μl of 0.5 M EDTA were added to stop the reaction. The samples were then filtered through nitrocellulose filters. *b*, plot of the initial rate of enzyme activation as a first order reaction.

Given the fact that the activation of the enzyme is a relatively slow process that does not reach its first maximum until approximately 3 min after the beginning of incubation with Ado-Met, one can ask the following question. Is the activation of the enzyme due to the binding of Ado-Met or to some subsequent event? The role of Ado-Met as an allosteric effector, and its slowness in activating the enzyme pointed to the second possibility, since one would expect the primary interaction between Ado-Met and the enzyme to be diffusion limited and thus a very rapid event. The answer can be found by determining the order of the reaction. The primary interaction of Ado-Met with the enzyme should be second order, while any conformational change subsequent to the Ado-Met binding would be a first order reaction. When the data from the activation experiments described in Fig. 3*a* were plotted according to the first order rate equation, the points fell on a straight line, which, however, did

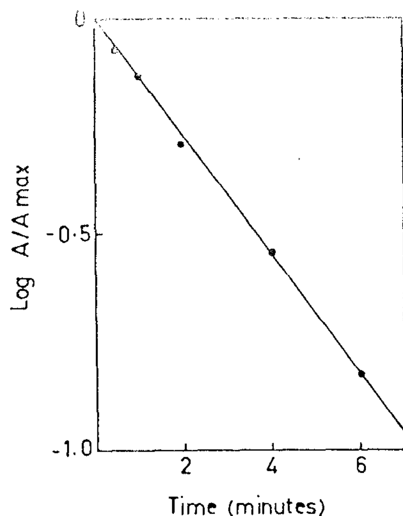


FIG. 4. Stability of the activated enzyme. A reaction mixture of 20 μl containing 5 μmol of Tris, pH 8.0, 0.013 μmol of EDTA, 0.6 μmol of mercaptoethanol, and 0.13 nmol of Ado-Met was mixed with 50 μl (50 μg) of endonuclease R.K. This mixture was preincubated for 5 min at 30° and kept on ice. Aliquots of 3 μl were then diluted into 300 μl of dilution buffer containing 30 μmol of Tris, pH 8.0, 0.08 μmol of EDTA, 1.9 μmol of $MgCl_2$, and 3.6 μmol of mercaptoethanol. The diluted enzyme was incubated for varying periods of time, then 5 μl of a mixture containing 80 nmol of ATP and 1.6×10^9 phage equivalents of λ.0 [^{32}P]DNA were added, and incubation was continued for a further 2 min. The reaction was terminated by the addition of 50 μl of 0.5 M EDTA and then filtered through a nitrocellulose filter.

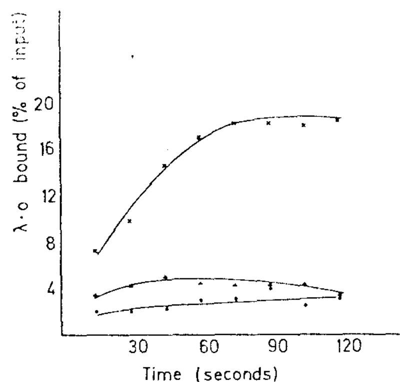


FIG. 5. Effect of preincubation of endonucleases R.K-18 and R.K-19 with Ado-Met. Endonuclease R.K-18 (4 μg) or endonuclease R.K-19 (12 μg) was preincubated in a volume of 0.03 ml containing 1.5 μmol of TES, pH 8.0, 4.5 nmol of EDTA, 0.09 μmol of $MgCl_2$, 0.017 μmol of mercaptoethanol, and 30 pmol of Ado-Met for 5 min at 30°. At the end of this period, 0.5 ml of a DNA binding reaction mixture containing 50 μmol of TES, pH 8.0, 1.4 μmol of EDTA, 3.2 μmol of $MgCl_2$, 6.0 μmol of mercaptoethanol, 0.2 μmol of ATP, and 6×10^9 phage equivalents of λ.0 [^{32}P]DNA were added to the preincubated enzyme. The DNA binding reaction was started by the addition of the complementing mutant enzyme. In the control reaction, Ado-Met was present in the DNA binding mixture at a concentration of 5×10^{-8} M, and the reaction was started by the addition of a mixture of endonucleases R.K-18 (4 μg) and R.K-19 (12 μg). Aliquots of 0.05 ml were taken out at the intervals indicated in the figure and added to 0.01 ml of 0.5 M EDTA to stop the reaction. ×——×, endonuclease R.K-18 preincubated with Ado-Met; ▲——▲, endonuclease R.K-19 preincubated with Ado-Met; ●——●, combined mutant enzymes assayed at low Ado-Met concentration.

not intersect the ordinate at zero as a simple first order reaction should (Fig. 3*b*). Instead, it intersected the zero line at a point corresponding to 15 to 20 s on the abscissa. This would correspond to a rapid binding of Ado-Met to the enzyme followed by a slow transition leading to the appearance of the activated species. The first order rate constant calculated from the slope of Fig. 3*b* is 1.3×10^{-2} s^{-1}, which gives a half-life of 54 s for the activation reaction.

Stability of Activated Enzyme—The stability of the activated enzyme was measured by preincubating the enzyme with Ado-Met and then diluting into buffer without the other substrates and incubating at 30°. Aliquots of 300 μl were removed at varying time intervals, and λ.0 [^{32}P]DNA and ATP were added. The DNA binding was allowed to go to completion, and the complex was stabilized with EDTA and measured on nitrocellulose filters. The decay of the activated enzyme is plotted as a first order reaction with a rate constant of 5.3×10^{-3} s^{-1} and a half-life of 130 s (Fig. 4).

Activation of Mutant Restriction Enzymes by Ado-Met—Two mutant restriction enzymes from restriction-deficient mutants of *Escherichia coli* K have been recently purified and characterized in this laboratory (11). One of them, endonuclease R.K-18, lacks the endonuclease activity, but is still capable of modifying DNA. The other one, endonuclease R.K-19, has lost both restriction and modification activities due to a defect in the *hsdS* subunit that is responsible for DNA site recognition. Both of these enzymes lack all of the restriction activities that have been detected: binding and cleavage of unmodified DNA, and the DNA-dependent ATPase activity. However, they complement each other fully to express all of these activities.

Experiments were done involving preincubation of one mutant enzyme with Ado-Met, followed by dilution of the effector below its K_m, and addition of the other substrates and the second mutant enzyme (Fig. 5). Preincubation of endonuclease R.K-19 at a high concentration of Ado-Met followed by dilution below the K_m along with the addition of λ.0 [^{32}P]DNA, ATP, and Mg^{2+} and the complementing endonuclease R.K-18 did not lead to significant DNA binding. In the converse experiment, however, full DNA binding was observed when endonuclease R.K-18 was preincubated at a high Ado-Met concentration. In a standard DNA binding assay at an Ado-Met concentration corresponding to that after dilution, a mixture of the two mutant enzymes gave negligible DNA binding. In a separate control experiment, it was ascertained that the endonuclease R.K-19 was not inactivated during preincubation with Ado-Met. The conclusion from these experiments is that endonuclease R.K-18 is activated by Ado-Met in a manner similar to the wild type enzyme. Therefore, the defect in its restriction mechanism occurs at some point later than the activation. Endonuclease R.K-19 can bind Ado-Met (11) but is not activated by it, indicating that the DNA recognition mechanism must be closely associated with the activation process. However, even in the absence of bound Ado-Met, it can complement the activated endonuclease R.K-18 in the restriction reaction, indicating that it can catalyze the steps following the enzyme activation.

DISCUSSION

S-Adenosylmethionine acts as the methyl group donor in most biological methylation reactions. However, it does not appear to act in this fashion in the cleavage of unmodified DNA by the restriction endonuclease from *E. coli* K. The experiments described in this paper are consistent with the role of Ado-Met as an allosteric effector in the restriction reaction. The enzyme binds Ado-Met rapidly, but the actual activation is a slow event. In the absence of λ.0 DNA and ATP, this activated species de-

... and has a half-life of 130 s, but it can be reactivated in the presence of Ado-Met. If DNA and ATP are present, the activated enzyme can then proceed through a complex series of steps leading up to the final scission of the double-stranded DNA without any further need for free Ado-Met. However, this does not rule out a more active role for enzyme-bound Ado-Met in some later step, though this would appear unlikely. Inevitably, these observations pose some interesting questions. Is the activation of the enzyme due to a conformation change in the protein or to some larger alteration such as the loss of a subunit? Is this same activated enzyme species also responsible for the methylase activity?

The results with the mutant enzymes corroborate those previously reported by us (11). The restriction-deficient endonuclease R.K-18 can be activated by Ado-Met in a manner similar to the wild type enzyme, and the activated species can be complemented by endonuclease R.K-19 without the addition of further Ado-Met. On the other hand, endonuclease R.K-19 cannot be activated by Ado-Met. The fact that both enzymes can bind Ado-Met (11) makes it tempting to think that the modification subunit binds Ado-Met, since it is the only intact subunit that both enzymes have in common. The binding of Ado-Met would then conceivably alter the relationship between the recognition and modification subunits, making it possible for the enzyme to recognize specific DNA sequences.

It is important to emphasize at this point that though we refer to this endonuclease as an enzyme, this is more from habit than from certainty. Our evidence and that of other investigators (16) indicates that this endonuclease and that from *E. coli* B do not act catalytically in the restriction reaction. In other words, each enzyme molecule can cleave only once. On the other hand, the ATPase activity which parallels the cleavage of DNA (17–19) is a catalytic event with thousands of ATP molecules hydrolyzed by each enzyme molecule. No information is available about the turnover of the methylase activity. Whether this makes these proteins eligible to be called enzymes or assigns them to the ranks of regulatory proteins remains an open question.

Finally, our experimental data and that from other laboratories on the related endonuclease from *E. coli* B allow us to draw a rough outline of the chain of events catalyzed by these endonucleases.

Enzyme Activation—The restriction endonuclease binds Ado-Met, and a slow activation process takes place. The characteristics of this reaction have been described above.

Interaction with DNA—The activated enzyme can then interact with DNA in the absence of ATP. This can only be shown indirectly by the protective effect that DNA has on the activated enzyme,[2] since it does not bind DNA to filters in the absence of ATP (13). The nature of this interaction is probably nonspecific as it can be demonstrated with either modified or unmodified DNAs.

Interaction with Host Specificity Site on DNA—The activated enzyme then proceeds to interact specifically with the host specificity site(s) on the DNA. It is likely that this reaction requires ATP and Mg^{2+}, though no evidence is available. It is at this point that the enzyme can choose to go into a restricting or a modifying mode. Originally, it had been thought that the conversion of the restriction enzyme into either of these two forms was regulated by the presence or absence of ATP.

However, the recent work of Vovis *et al.* (8) has shown that methylation of heteroduplex fl DNA (one strand modified, the other one unmodified) is sharply stimulated by ATP and Mg^{2+}. These heteroduplexes are resistant to cleavage by the restriction enzyme (3, 20). Since both restriction and modification require Ado-Met, ATP, and Mg^{2+}, we would like to propose that it is the nature of the host specificity site(s) that determines which activity the enzyme will express. The site can exist in three possible forms: fully modified, heteroduplex, and fully unmodified. If the site is fully modified, nothing will happen and the enzyme will come off the DNA. In the case of heteroduplex DNA, the enzyme is unable to restrict it, but would then go into a modifying mode, which would rapidly methylate the DNA converting it into fully modified. It should be pointed out that heteroduplex DNA would be the natural substrate, since it is the product of semiconservative DNA replication. The sequence methylated would be at the host specificity site or a site adjacent to it. The evidence for this is that phage mutants that have lost their host specificity sites also lose (*in vivo* and *in vitro*) the ability to be methylated (21, 22). Fully unmodified DNA can also be methylated by the enzyme when ATP is omitted though this is an extremely slow reaction (7, 8). To complicate matters further, it is apparent that there are differences between the various host specificity sites for a given restriction-modification system on the same DNA. It has been reported that endonuclease R.B methylates the sB_2 site on fl DNA at a faster rate than the sB_1 site (8), while the endonuclease R.K binds to the sK_2 site on DNA with a higher affinity than sK_1.[2] Whether this is due to a difference in the nucleotide sequence or to other structural features of the DNA is unknown.

Interaction with Cleavage Site—It has been shown that endonuclease R.B cleaves at a different site than the sB specificity site, and that the number of possible cleavage sites is larger than the number of sB sites though only one cleavage is made per sB site (23, 24). The activated enzyme upon finding a fully unmodified host specificity site would then be altered to its restricting mode and move along the DNA or, alternatively, it would bring a distal portion of the DNA into contact with it. It is probably this complex of the restricting enzyme with the cleavage site that can be stabilized with EDTA and trapped on nitrocellulose filters (11).

DNA Cleavage—The restricting enzyme then proceeds by first making a single-stranded break followed some time later by a break on the opposite strand (3, 24). Since nicked circles are stable products upon digestion of supercoiled DNA with a limiting amount of enzyme, it appears that the double-stranded scission is the result of action by two enzyme molecules.

ATP Hydrolysis—The DNA-dependent hydrolysis of ATP which takes place during and after restriction, is probably due to the dissociation of the enzyme yielding a species that remains stably bound to the DNA. This complex would be responsible for the ATPase activity. The alteration of the enzyme would prevent it from turning over in the restriction reaction. Three types of experiments lend support to this: detection of a stable DNA-enzyme complex that catalyzes ATP hydrolysis (16), the lack of turnover (measured in terms of DNA binding or cleavage)[2] (16), and the complementation of a limit digest by the mutant enzymes to give a new round of enzyme activity.[2]

In conclusion, though many of the biochemical details of the reaction mechanism of these enzymes are ill defined, it is to be expected that further experiments will reveal them in their full complexity. Of particular interest in this respect is the interaction of the host specificity site with the enzyme and the manner in which it can determine which activity will be expressed.

[2] R. Yuan and T. A. Bickle, unpublished results.

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