DNA (Cytosine-N⁴)- and -(Adenine-N⁶)-methyltransferases Have Different Kinetic Mechanisms but the Same Reaction Route

A COMPARISON OF M.BamHI AND T4 Dam[†]

Ernst G. Malygin‡, Victor V. Zinoviev‡, Alexey A. Evdokimov‡, William M. Lindstrom, Jr.§, Norbert. O. Reich§, and Stanley Hattman¶

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We studied the kinetics of methyl group transfer by the BamHI DNA-(cytosine-N⁴)-methyltransferase (MTase) from Bacillus amyloliquefaciens to a 20-mer oligodeoxynucleotide duplex containing the palindromic recognition site GAATTC. Under steady state conditions the BamHI MTase displayed a simple kinetic behavior toward the 20-mer duplex. There was no apparent substrate inhibition at concentrations much higher than the Km for either DNA (100-fold higher) or S-adenosyl-L-methionine (AdoMet) (20-fold higher); this indicates that dead-end complexes did not form in the course of the methylation reaction. The DNA methylation rate was analyzed as a function of both substrate and product concentrations. It was found to exhibit product inhibition patterns consistent with a steady state random bi-bi mechanism in which the dominant order of substrate binding and product release (methylated DNA, DNAMe, and S-adenosyl-L-homocysteine, AdoHcy) was AdoMet | DNA | DNAMe | AdoHcy. The M.BamHI kinetic scheme was compared with that for the T4 Dam (adenine-N⁶)-MTase. The two differed with respect to an effector action of substrates and in the rate-limiting step of the reaction (product inhibition patterns are the same for the both MTases). From this we conclude that the common chemical step in the methylation reaction, methyl transfer from AdoMet to a free exocyclic amino group, is not sufficient to dictate a common kinetic scheme even though both MTases follow the same reaction route.

DNA methylation plays an important role in expression of genetic information. In various eukaryotes methylation is involved in regulating transcription, mutation, recombination, parental imprinting, chromatin structure, and other important cellular events (1). In prokaryotes, a role in regulating transcription of certain genes has been established for the Dam

[†] The abbreviations used are: MTase, methyltransferase; AdoMet, S-adenosyl-L-methionine; AdoHcy, S-adenosyl-L-homocysteine; DNAMe, methylated DNA; MSC, model selection criterion.

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[§] The on-line version of this article (available at http://www.jbc.org) contains kinetic equations for Schemes 1 and 2 and an estimation of an effective rate parameter for BamHI MTase release from the reaction products.

[¶] To whom correspondence should be addressed. Tel.: 855-275-8046; Fax: 855-275-2070; E-mail: modDNA@mail.rochester.edu.

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Comparison of DNA (Amino)-methyltransferases

of the target base? To approach this question, we began a study of methylation by the BamHI (cytosine-N⁶)-MTase, which catalyzes methyl group transfer to the internal cytosine residue in the palindromic recognition site GGATCC (10). BamHI MTase can also methylate the modified sequence GGm6ATCC, but not the palindromic recognition site GGATCC (10). Analysis methyl group transfer to the internal cytosine residue in

and product release (methylated DNA, DNAMe, and S-adenosyl-

mechanism in which the dominant order of substrate binding

MTase reactions. We observed product inhibition patterns with

(adenine-N⁶)-MTase. We decided to compare the BamHI MTase with the T4 Dam (adenine-N⁶)-MTase, which has been the object of detailed studies (15–18). Because T4 Dam modifies the adenine residue in the palindromic sequence GATC, which is contained within the BamHI target sequence GGATCC, it was possible to use the same oligonucleotide duplexes to investigate methylation by the two MTases. Such a comparison is of significant interest because the target exocyclic amino groups are on different target bases. In this regard, there appears to be a difference in the kinetic behavior of the (adenine-N⁶-) and (cytosine-N⁶-)-MTases (17–19). For example, the rate constant of methyl group transfer from AdoMet to the (cytosine-N⁶-) position catalyzed by BamHI MTase is one order of magnitude lower than that for T4 Dam methylation of the (adenine-N⁶-) position (17, 19). In addition, T4 Dam has a higher affinity for substrate DNA duplex and AdoMet compared with BamHI MTase. However, the results of pre-steady state experiments showed that both enzymes catalyze effective transfer of the methyl group to DNA independent of which substrate was preincubated with the enzyme (18, 19). These results are consistent with random formation of productive ternary enzyme-substrate complexes. Thus, despite several important distinctions in their kinetic parameters, it was possible that BamHI and T4 Dam MTases share a common kinetic mechanism.

Recently, we carried out a detailed investigation of T4 Dam methylation of a 20-mer DNA duplex (20). Unexpectedly, contrary to the pre-steady state experimental results, which indicated random binding of substrates, the steady state kinetic data best fit a strictly ordered mechanism. This could be explained by the supposition that, initially, free T4 Dam is capable of binding randomly to AdoMet and DNA in the first turnover, but after ternary complex formation the enzyme adopts a conformation with altered substrate binding capabilities and specifically adapted to catalysis. During the following turnovers T4 Dam acts according to a strictly ordered reaction mechanism of substrate binding and product release according to the sequence AdoMet ‖ DNA ‖ DNA⁶⁶ ‖ AdoHey ‖ .

The present work further compares the BamHI and T4 Dam MTase reactions. We observed product inhibition patterns with BamHI that are consistent with a steady state random bi-bi mechanism in which the dominant order of substrate binding and product release (methylated DNA, DNA⁶⁶, and S-adenosyl-L-homocysteine, AdoHey) was AdoMet ‖ DNA ‖ DNA⁶⁶ ‖ AdoHey ‖ . Although the overall reaction is similar to that for T4 Dam, the two kinetic schemes differ; viz. with respect to an effector action of substrates and in the rate-limiting step of the reaction. Thus, a common chemical step in the methylation re-

action, methyl transfer from AdoMet to a free exocyclic amino group, is not sufficient to dictate a common kinetic scheme.

EXPERIMENTAL PROCEDURES

Enzymes and Chemicals—H-Ch₃-labeled S-adenosyl-L-methionine (15 Ci/mmol; 1 mCi/ml) was purchased from Amersham Biosciences. AdoHey and selenfungin were from Sigma. Unlabeled AdoMet (Sigma) was purified further by chromatography on a C₄ reverse-phase column as described previously (21). The synthetic 20-mer duplex used as substrate had the following structure (the recognition sequence GGATCC is italicized): 5’-CAAGGAAGTACCGATCCG-3’; 3’-GTCAGTCCCCCAGAGGT-5’.

A modified duplex containing N⁶-methylcytosine (on both strands) in place of the target Cs (underlined) was used in the product inhibition studies. It was obtained by complete M.BamHI in vitro methylation of the unmodified 20-mer using unlabeled AdoMet as a methyl group donor. Oligonucleotides were synthesized on an Applied Biosystems 380A/380B DNA synthesizer, and their concentrations were determined spectrophotometrically. Duplexes were obtained by heating and annealing stoichiometric amounts of complementary oligonucleotides from 90 to 20°C over 7–12 h. BamHI MTase was purified to homogeneity as described previously (19). Protein concentrations were determined by the Bradford method (22). These values were in close agreement with those determined spectrophotometrically at 280 nm (in 6.0 M guanidinium hydrochloride, 0.02 M phosphate buffer, pH 6.5) from the known composition and molar extinction coefficients of individual aromatic amino acid residues (23).

DNA Methylation Assay—The DNA MTase assay was similar to that previously described (19). Briefly, BamHI MTase reactions (final volume = 25 μl) were carried out at 37°C in buffer containing 100 mM Tris-HCl, pH 8.0, 1 mM EDTA, 1 mM diethiothreitol, 5% glycerol, and 0.2 mg/ml bovine serum albumin (21). A low concentration of BamHI MTase (10 nm) was used in most of the experiments. The concentrations of AdoMet, DNA duplex, and inhibitors varied according to the experiment. Reactions were initiated by the addition of prewarmed BamHI MTase to preincubated mixtures of [H-Ch₃]AdoMet and substrate DNA (with or without added inhibitor). The reaction times used in steady state experiments were selected to ensure linear initial velocity conditions; i.e. during the time of the reaction, terminal product formation was less than 10% of the initial substrate and added product inhibitor concentrations.

Kinetic data were analyzed using the program Sci-
maximum reaction rate. In contrast to T4 Dam (20), BamHI MTase methylation was not inhibited by high concentrations of substrate DNA nor was it activated by high concentrations of AdoMet without visible signs of saturation (20).

The absence of substrate inhibition at concentrations much higher than $K_s$, values for either DNA (100-fold higher) or AdoMet (20-fold higher) indicates that dead-end complexes did not form in the course of the reaction. In the case of T4 Dam, inhibition by high concentrations of substrate DNA was explained by the formation of dead-end complexes (Dam-DNA and Dam-AdoHcy-DNA), which slow down progress of the reaction (20). It should be noted that both T4 Dam (21) and M.BamHII can form dimers. However, they remain as monomers under steady state conditions, viz. [enzyme] $<$ [DNA] and AdoMet present in excess.

Inhibition by Reaction Products—To determine the order of BamHI MTase substrate binding and product release, we studied DNA methylation rate as a function of the substrate and product concentrations (Fig. 2, A–D). The character of all the double-reciprocal plots were analogous to those obtained earlier with the T4 Dam MTase (20). In summary, AdoHcy was a competitive inhibitor with regard to AdoMet (Fig. 2A) and a non-competitive inhibitor with respect to substrate unmethylated 20-mer duplex (D) (Fig. 2B). The other reaction product, fully methylated 20-mer duplex (P), was a non-competitive inhibitor with regard to both AdoMet and unmethylated 20-mer duplex (Fig. 2, C and D).

To confirm the non-competitive nature of the inhibition by fully methylated duplex, we also measured the dependence of the reaction velocity on total DNA concentration. In this experiment, increasing equimolar amounts of (P) and (D) were introduced in the reaction mixtures. Analysis of standard equations for competitive and non-competitive inhibition types (25) showed that the non-competitive inhibition equation predicts that the initial rate will reach a maximum and then decline with increasing total DNA concentration. As predicted, the results in Fig. 3 showed a maximum at [DNA] $\approx$ 0.6 mM and then declined at higher concentrations. Thus, as for the T4 Dam MTase, these results (summarized in Table I) are consistent with a steady state, BamHI MTase substrate binding, and product release that obey the scheme AdoMet $\downarrow$ DNA $\downarrow$ DNA${}_{\text{Me}}$ $\downarrow$ AdoHcy.$^\dagger$

Models of the Bam Reaction Mechanism—Scheme 1 (Fig. 4) represents the minimal kinetic scheme needed to describe all the effects of substrates and products on the reaction rate (Figs. 1–3). The reaction route in this scheme corresponds to the order of substrate binding and product release predicted above. Although possible dead-end complexes are included in this scheme, they do not contain substrate DNA (D) because no reaction inhibition was observed at elevated DNA concentrations (up to 20 mM). It should be noted that unmethylated 20-mer substrate DNA duplex D is initially converted to the hemi-methylated product, mD (19), and not to fully methylated duplex, P, which is an inhibitor of the reaction. Therefore, the step EHmD $\rightarrow$ EH + mD is irreversible. Scheme 1 contains only the dead-end complexes EP and ESP but not a complex EHP. This will be discussed further below.

A complete set of kinetic data, presented in Figs. 1–3 and derived from additional experiments, was analyzed to determine how well they fit the kinetic model of Scheme 1. The equations were derived using an approach developed earlier (26) and are presented in the Supplement at http://www.jbc.org. It is seen from Scheme 1 kinetic equation that some of the reaction rate constants ($k_p$, $k_{\gamma}$, $k_{\delta}$, see Table II, Scheme 1 column) can not be determined independently. Table II presents the results of the most successful and stably reproducible fitting of the experimental data to Scheme 1 kinetic equation (MSC = 4.29). Only two individual rate constants, which characterize the interaction of the free enzyme with AdoMet and the decomposition of this binary complex, fit Scheme 1 kinetic equation. The remaining constants form combinations, which perform as independent kinetic and equilibrium parameters as a part of Scheme 1 kinetic equation. Kinetic and equilibrium parameters for Scheme 1 were calculated with satisfactory precision (standard deviation $\leq$10%) except for the rate constant $k_{-1}$, which was determined with a significant standard deviation. Additional support for this fitting is the fact that the curve in Fig. 3 is perfectly described by Scheme 1 kinetic equation at $S = 10 \mu$M, $H = 0 \mu$M, and [D]o = [P]. The maximum for the equation is $D_{\text{max}}^2 = K_{\text{FP}} \cdot h_S \cdot Q_{\text{EP}}$. If we put into this expression the values of parameters from Table II and $S = 10 \mu$M, we obtain $D_{\text{max}}^2 = 0.098 ((0.039) (10) + 0.019)/0.41 = 0.098$, where $D_{\text{max}} = 0.31 \mu$M. Multiplied by factor of 2, $[\text{DNA}]_{\text{max}} = 0.62 \mu$M, which coincides perfectly with the experimentally observed maximum value.

In parallel, we fit the experimental data to a variant of Scheme 1, where EHP complex formation from complex EH was introduced. Accordingly, Scheme 1 kinetic equation was modified (variant Scheme 1 and equation are not shown). The MSC for the modified scheme had the same value of 4.29, but all the kinetic constants related to EH complex conversions, including $K_{\text{EHP}}$, had high standard deviations (from 10- to 100-fold the mean value). It must be noted that the calculated

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The mean value of $K_{EHP}$ is much higher than the values of $K_{EP}$ and $K_{ESP}$, which were determined at the same time with a high degree of precision (Table II). Hence, the elimination of the weak affinity complex EHP from Scheme 1 does not detract from the description of the experimental data, but it permits determination of the remaining reaction kinetic parameters with satisfactory standard deviations.

Since earlier pre-steady state kinetic data (19) did not exclude a random route for the productive complex ESD formation, we attempted to describe the steady state experimental data by Scheme 2 (Fig. 4), where BamHI MTase binding with both substrates is permissible. Scheme 2, with addition of an alternative route of enzyme binding with substrate, in the order DNA $\rightarrow$ AdoMet $\rightarrow$ DNA, had an MSC of 4.33; the calculated values of the kinetic and equilibrium constants are presented in Table II. Scheme 2 kinetic equation contains several new constants that are not present in the first equation. Table II shows that values for most of the parameters, which characterize identical reaction steps or their combinations (and can be determined for both schemes), coincided within standard deviation limits.
Equation 2.

reaction velocity is determined by the expression \((26)\) in the differential route of central complex ESD formation, as in Scheme 2. In addition, the sequence of substrate binding is AdoMet.

The dominant route of reaction in which the sequence of substrate binding is AdoMet first comes from the coincidence in the two-step parameter values \((K_{\text{cat}}/K_{\text{cat}})/S(1 + k_{-1}/k_{2})\) for the both schemes. This parameter was found as a result of the fitting for Scheme 1 and was calculated for Scheme 2 using the calculated values for constants \(k_{-1}\) and \(k_{-2}\). Taken together, the results of fitting are best described by a dominant route of reaction in which the sequence of substrate binding is AdoMet → DNA ↓.

Similarly, we tried to determine the relative contributions of possible routes of reaction product release. For this, we introduced into Scheme 2, EHmD ↔ EmD → E + mD. However, it was found that this resulted in such large increases in standard deviations of the calculated parameters that it was impossible to approximate the relative effectiveness of the corresponding routes. Evidently, the reason for such uncertainty is due to an insufficient amount of experimental data. However, on the basis of the graphic analysis and good fitting of the data to Scheme 2 kinetic equation (with one route of product release), the dominant route of product release appears to be DNA\(^{3\text{Me}}\) → AdoHcy ↓. This hypothesis is consistent with the fact that, as with most other enzymes, the order of the product release is a “mirror reflection” of the order of substrates binding (25).

The rate constant for the chemical step methyl group transfer from AdoMet to DNA is one order of magnitude lower for BamHI MTase, \(k_{\text{methyl}} = 0.085 \text{ s}^{-1}\) (averaged value of two values determined in Ref. 19) versus \(k_{\text{methyl}} = 0.56 \text{ s}^{-1}\) for T4 Dam (18). Yet, the value of \(k_{\text{cat}} = 0.052 \text{ s}^{-1}\) is ~3-fold higher than for T4 Dam (17). Earlier it was shown that \(k_{\text{methyl}}\) is ~1.5–2-fold greater in comparison with \(k_{\text{cat}}(0.053 \text{ s}^{-1})\) for the BamHI MTase (19). In addition, BamHI MTase catalyzed a burst in the reaction. These observations were consistent with the rate-limiting step being that of product dissociation. However, a more detailed analysis presented in this work leads us to alter that conclusion.

From the relationship between the \(k_{\text{cat}}\) and \(k_{\text{methyl}}\) values, it is possible to estimate an averaged parameter that characterizes an “effective” (27) rate parameter for BamHI MTase release from the reaction products, \(k_{\text{cat}}/k_{\text{cat}}(k_{\text{cat}} + k_{-1}) = 0.14 \text{ s}^{-1}\) (for details see Supplement 3 at http://www.jbc.org). Evidently, neither constant from this expression can be smaller than the effective parameter value. Hence, for the sequence of steps in Reaction 1,

\[
\begin{align*}
\text{ESD} & \rightarrow \text{EHmD} \rightarrow \text{EH} \rightarrow E \\
\text{REACTION 1}
\end{align*}
\]

the rate constant of the chemical step \(k_{\text{methyl}} = 0.085 \text{ s}^{-1}\) has the lowest value, so this step limits the overall reaction rate. In contrast, the release of AdoHcy from the complex EH is rate-limiting for T4 Dam (20) (Table II).

Kinetic mechanisms of DNA methylation reactions have been studied only for a limited number of (adenine-N⁶-) and (cytosine-N⁵-) DNA MTases; however, comparable studies with (cytosine-N⁴-) DNA MTases have not been done. Among (adenine-N⁶-) DNA MTases, an ordered reaction mechanism (where AdoMet is the first substrate bound) was derived for the EcoRI (28), EcoRV (29), and T4 Dam (20) MTases. In the case of Ccr MTase (30), an ordered mechanism was postulated with the enzyme binding to DNA first. A random mechanism of substrate binding has been reported for the EcoP15I MTase (23). Finally, a single kinetic mechanism was not found for the EcoI MTase (24).

(Cytosine-C5) DNA MTases form a covalent binary complex intermediate with DNA, where subsequent interaction with AdoMet results in transfer of the methyl group and splitting out of a C5-proton (6). Hence, an ordered kinetic mechanism with DNA first bound to the enzyme seems to be the logical pathway. Such a mechanism was shown for the HhaI (6, 9), MspI (31) and DnmI (mouse) (32) MTases. Recent studies on the HhaI (33) and DnmI (human) (34) MTases have led the authors to conclude that these enzymes catalyze methylation via a random kinetic mechanism. These experiments differed from one another in the ranges of substrate concentrations utilized (6, 9, 33).

The apparent capability of a DNA MTase to randomly bind
substrates and release reaction products does not always agree with the fact that there is really an ordered kinetic mechanism. We encountered such a problem studying the T4 Dam reaction mechanism (20). The parameters for random binding of substrates and release of reaction products did not agree with the steady state kinetic data, which favored a strictly ordered mechanism. However, satisfactory agreement could be obtained by hypothesizing that, initially, free enzyme is capable of random binding to the substrates. However, once the ternary MTase-AdoMet-DNA complex is formed, the enzyme acquires an altered conformation. After catalysis of methyl group transfer from AdoMet to DNA, the enzyme remains in a conformation that preferentially binds AdoMet, and hence, further catalytic cycles are carried out according to a strictly ordered reaction mechanism (20). Such a hypothesis would permit a satisfactory resolution of the apparent conflict between the different HhaI MTase studies (9, 33).

In conclusion, the kinetic schemes of the reactions catalyzed by the T4 Dam (adenine-N6)-MTase (30) and the BamHI (cytosine-N4)-MTase (this work) differ significantly. A comparison of the two MTases is summarized in Table III. Thus, the common chemical step of the reaction, methyl transfer from AdoMet to a free exocyclic amino group, is not sufficient to dictate a common kinetic scheme. Nonetheless, both enzymes

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**Table II**

Kinetic parameters of M.BamHI calculated for steady state ordered bi-bi (Scheme 1) and random two-route (Scheme 2) mechanisms

| Reaction steps | Parameter | Scheme 1 value | Scheme 2 value | T4 Dam value* |
|----------------|-----------|----------------|----------------|---------------|
| E ↔ ES | $k_{-1}$ | 0.039 ± 0.002 | 0.035 ± 0.004 | 0.13 ± 0.04 |
| E ↔ ES | $k_{-2}$ | 0.019 ± 0.007 | 0.014 ± 0.007 | 0.15 ± 0.10 |
| E ↔ ES | $k_{-3}$ | 0.39 | 0.39 | 1.15 |
| E ↔ ES | $k_{-4}$ | 0.024 ± 0.013 | 0.0089 | Step is absent |
| E ↔ ES | $k_{-5}$ | 0.73 ± 0.07 | Can not be determined | Can not be determined |
| E ↔ ES | $k_{-6}$ | 0.37 | 0.086 | |
| E ↔ ES | $k_{-7}$ | 0.050 ± 0.009 | 0.005 | Step is absent |
| E ↔ ES | $k_{-8}$ | 0.10 | | |

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**Table III**

Differences between kinetic properties of M.BamHI MTase and T4 Dam

| Features | M.BamHI | T4 Dam |
|----------|---------|--------|
| General scheme of reaction | Random bi-bi | Ordered bi-bi |
| Inhibition of reaction by substrates | Absent | DNA at high concentration inhibits the reaction |
| Activation of reaction by substrates | Absent | Increasing the concentration of AdoMet leads to a progressive stimulation in the reaction rate |
| Limiting step of reaction | The chemical step of reaction is slowest (0.085 s⁻¹), although the effective rate of release of the products is only 2-fold higher (0.14 s⁻¹) | Release of the product AdoHcy from enzyme appears to be rate-limiting in the overall reaction (at least 20-fold lower comparing with the chemical step, 0.56 s⁻¹) |
| Enzyme isomerization | Not known | Repeating reaction cycles are catalyzed by isomerized T4 Dam form, F, which is specifically adapted for catalysis |
follow the same overall reaction route, AdoMet $\rightarrow$ DNA $\rightarrow$ DNA$^{\text{Me}}$ $\rightarrow$ AdoHcy $\rightarrow$.

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REFERENCES

1. Jaenisch, R., Beard, C., and Li, E. (1999) in Genomic Imprinting: Causes and Consequences (Ohiuson, E., Hall, K., and Itzen, M., eds) pp. 118–126, Cambridge University Press, Cambridge
2. Marinus, M. G. (1996) in Escherichia coli and Salmonella: Cellular and Molecular Biology, 2nd Ed. (Neidhardt, F. C., Curtiss, R., Ingraham, J. L., Lin, E. C. C., Low, K. B., Magasanik, B., Reznikoff, W. S., Riley, M., Schaechter, M., and Umbarger, H. E., eds) pp. 782–791, American Society for Microbiology, Washington, D.C.
3. Heithoff, D. M., Sinsheimer, R. L., Low, D. A., and Mahan, M. J. (1999) Crit. Rev. Biochem. Mol. Biol.
4. Ahmad, I., and Rao, D. N. (1996) Crit. Rev. Biochem. Mol. Biol. 31, 361–380
5. Malone, T., Blumenthal, R. M., and Cheng, X. (1995) J. Mol. Biol. 253, 618–632
6. Wu, J. C., and Santi, D. V. (1987) J. Biol. Chem. 262, 4778–4786
7. Allan, B. W., and Reich, N. O. (1990) Biochemistry 29, 14757–14762
8. Reich, N. O., and Mashhoon, N. (1993) J. Biol. Chem. 268, 9191–9193
9. Lindstrom, W. M., Flynn, J., and Reich, N. O. (2000) J. Biol. Chem. 275, 4912–4919
10. Hattman, S., Keister, T., and Gottehrer, A. (1976) J. Mol. Biol. 124, 701–711
11. McCleland, M., and Nelson, M. (1988) Gene 74, 169–176
12. Nardone, G., George, J., and Chirikjian, J. G. (1994) J. Biol. Chem. 269, 10357–10362
13. Nardone, G., George, J., and Chirikjian, J. G. (1986) J. Biol. Chem. 261, 12128–12133
14. Rubin, R. A., and Modrich, P. (1977) J. Biol. Chem. 252, 7265–7272
15. Tuzikov, F. V., Tuzikova, N. A., Naumochkin, A. N., Zinoviev, V. V., and Malygin, E. G. (1997) Mol. Biol. (Moscow) 31, 73–76 (English translation)
16. Malygin, E. G., Petrov, N. A., Gorbunov, Yu. A., Kossykh, V. G., and Hattman, S. (1997) Nucleic Acids Res. 25, 4393–4399
17. Zinoviev, V. V., Evdokimov, A. A., Gorbunov, Yu. A., Malygin, E. G., Kossykh, V. G., and Hattman, S. (1998) Biol. Chem. 379, 481–488
18. Malygin, E. G., Lindstrom, W. M., Jr., Schlagman, S. L., Hattman, S., and Reich, N. O. (2000) Nucleic Acids Res. 28, 4207–4211
19. Lindstrom, W. M., Jr., Malygin, E. G., Ovechchina, L. G., Zinoviev, V. V., and Reich, N. O. (2003) J. Mol. Biol. 325, 711–720
20. Evdokimov, A. A., Zinoviev, V. V., Malygin, E. G., Schlagman, S. L., and Hattman, S. (2001) J. Biol. Chem. 277, 279–286
21. Malygin, E. G., Evdokimov, A. A., Zinoviev, V. V., Ovechchina, L. G., Lindstrom, W. M., Jr., Reich, N. O., Schlagman, S. L., and Hattman, S. (2001) Nucleic Acids Res. 29, 2361–2369
22. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
23. Rao, D. N., Page, M. G., and Bickle, T. A. (1989) J. Mol. Biol. 209, 599–606
24. Szilak, L., Der, A., Deak, P., and Venetianer, P. (1993) Eur. J. Biochem. 218, 727–733
25. Cornish-Bowden, A. (1976) Principles of Enzyme Kinetics, Butterworths & Co., pp. 177–199, London
26. Malygin, E. G. (1977) Biofizika 22, 15–20
27. Varfolomeev, S. D., and Gurevich, K. G. (1999) Bioinformatics: Practical Course, p. 123, Moscow Fair-Press, Moscow
28. Reich, N. O., and Mashhoon, N. (1991) Biochemistry 30, 2933–2939
29. Gowher, H., and Jeltsch, A. (2000) J. Mol. Biol. 303, 95–110
30. Berdis, A. J., Lee, I., Coward, J. K., Stephens, C., Wright, R., Shapiro, L., and Benovic, S. J. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 2874–2879
31. Bhattacharya, S. K., and Dubey, A. K. (1999) J. Biol. Chem. 274, 14743–14749
32. Flynn, J., and Reich, N. O. (1998) Biochemistry 37, 15162–15169
33. Vilkaitis, G., Merkien, E., Serva, S., Weinhold, E., and Klimasauskas, S. (2001) J. Biol. Chem. 276, 20924–20934
34. Bacolla, A., Pradhan, S., Roberts, R. J., and Wells, R. D. (1999) J. Biol. Chem. 274, 33011–33019
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Ernst G. Malygin, Victor V. Zinoviev, Alexey A. Evdokimov, William M. Lindstrom, Jr., Norbert. O. Reich and Stanley Hattman

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