Mutational analyses of restriction endonuclease—HindIII mutant E86K with higher activity and altered specificity

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We have performed mutational analyses of restriction endonuclease HindIII in order to identify the amino acid residues responsible for enzyme activity. Four of the seven HindIII mutants, which had His-tag sequences at the N-termini, were expressed in Escherichia coli, and purified to homogeneity. The His-tag sequence did not affect enzyme activity, whereas it hindered binding of the DNA probe in gel retardation assays. A mutant E86K in which Lys was substituted for Glu at residue 86 exhibited high endonuclease activity. Gel retardation assays showed high affinity of this mutant to the DNA probe. Surprisingly, in the presence of a transition metal, \(\text{Mo}^{2+}\) or \(\text{Mn}^{2+}\), the E86K mutant cleaved substrate DNA at a site other than HindIII. Substitution of Glu for Val at residue 106 (V106E), and Asp for Lys at residue 125 (K125N) resulted in a decrease in both endonuclease and DNA binding activities of the enzyme. Furthermore, substitution of Leu for Asp at residue 108 (D108L) abolished both HindIII endonuclease and DNA binding activities. CD spectra of the wild type and the two mutants, E86K and D108L, were similar to each other, suggesting that there was little change in conformation as a result of the mutations. These results account for the notion that Asp108 could be directly involved in HindIII catalytic function, and that the substitution at residue 86 may bring about new interactions between DNA and cations.

**Keywords:** HindIII endonuclease/His-tag sequence/site-directed mutagenesis/star activity/transition metal

Introduction

Endonuclease HindIII is a type II restriction enzyme which recognizes and cleaves the palindromic sequence AAGCTT in Oligodeoxynucleotide primers for PCR-mediated cloning and the presence of Mg\(^{2+}\). The gene encoding HindIII endonuclease (R) is located upstream of its cognate methyltransferase gene (M). These genes were cloned together (designated RM in this paper), and the whole nucleotide sequence was determined (Nwankwo et al., 1994). Its open reading frame (ORF) consisted of 903 bp, encoding a protein of 300 amino acids with a predicted molecular mass of 34 950 Da, and the protein forms a homodimer in its active form.

In spite of its commercial importance as a tool in recombinant DNA technology, very little information is available about the mechanism of substrate recognition and phosphodiester bond cleavage by this enzyme (Takasaki, 1994; Smith and Wilcox, 1970). Also, the three-dimensional (3D) structure of the HindIII molecule is not available as yet.

In this study, we have employed mutational techniques to search for amino acid residues that might be involved in catalysis. More than a thousand species of type II restriction enzymes have been discovered and characterized (Wilson, 1991). However, there is little homology among these enzymes. Siksnys et al. (1995) described two conserved regions in restriction enzymes with 6 bp recognition (Silksny et al., 1995).

Target residues to be mutated were chosen according to the recent model proposed by Stahl et al. (1998). In this model, it is probable that many type II restriction enzymes follow a common mechanism of recognition and catalysis of DNA, with a potential motif of PD-----D/EXK. We prepared seven kinds of HindIII mutants by site-directed mutagenesis (Tang et al., 1999).

Recently, we purified four of the seven HindIII mutants by cloning the genes into the expression vector pET16b. The experimental results on mutant proteins are presented below. There is some evidence that Asp108 may be essential for the catalytic function of HindIII. We also found that the mutant E86K showed altered specificity in the presence of some transition metal ions.

Materials and methods

**Materials**

*Haemophilus influenzae* Rd, which produces the HindIII restriction–modification system, was a gift from Nippon Gene (Toyama, Japan). We had constructed our first plasmid, pUC19-RM, from DNA of this bacteria strain. Restriction enzymes were purchased from Nippon Gene and Takara Shuzo (Otsu, Japan). DNA ligation and LA PCR kits were purchased from Toyobo Biochemicals (Osaka, Japan). Plasmid pHAA47, which carries the human aldolase A cDNA (Sakakihara et al., 1989), and plasmids pUC19 and Bluescript II SK(−) were laboratory stock.

**Oligodeoxynucleotides**

Oligodeoxynucleotide primers for PCR-mediated cloning and sequencing were synthesized by Hokkaido System Science (Sapporo, Japan). The sequences of the primers used in PCR are listed in Table I.

**Construction of plasmids for protein purification**

For convenience, during protein purification the tandem *RM* gene for wild type or mutant HindIII in pUC19 (Tang et al., 1999) was cloned into pET16b. The *RM* gene had been cloned into pUC19 by inverse PCR; the size of this vector was large enough for the inverse PCR, where the whole size of plasmid could be obtained as a product (Jones and Howard, 1991). The *RM* gene for wild type or mutant HindIII was then excised and transferred into pET16b by PCR. Primers for this PCR were HistagNdI and H3MCMC (Table I). HistagNdI and
H3MCB possess a NdeI site at the 5’ end and a BamHI site at the 3’ end, respectively, so that the resultant PCR products could be cloned into pET16b at the NdeI and BamHI sites without a frame-shift.

An aliquot (50 μl) for PCR contained 1 ng wild type or mutant RM gene in pUC19 (as template), each 0.4 mM primers, each 0.25 mM dNTP and 2.5 U Taq DNA polymerase. The PCR was carried out in ASTEC Programme Temperature Control System PC 701. The conditions for PCR were as follows: denaturation, 95°C for 30 s; annealing, 55°C for 30 s and extension, 72°C for 2 min, for 25 cycles. After digestion with NdeI and BamHI, purified PCR products were ligated into pET16b, then transfected in E.coli plysS strain. pET16b plasmids containing wild type and the four different mutant RM genes were designated pHisRM and pHisMut (collectively named), respectively. 50 mM NaCl). The reaction mixture was electrophoresed in 10% PAGE and activities were monitored by SDS–PAGE and activity assays.

### Protein expression and purification

Protein expression was performed using E.coli BL21(DE3)-plysS strain. pET16b plasmids containing wild type RM and the four different mutant RM genes were designated pHisRM and pHisMut (collectively named), respectively.

Protein purification was carried out according to Laemmli (1970), and protein content was determined using a Bio-Rad (Hercules, USA) Protein Assay kit.

### Circular dichroism (CD) spectra

The CD spectra of proteins were obtained using a Jasco Model 700 spectrophotometer.

### HindIII activity assay

Plasmid pHAA47 (Sakakibara et al., 1989) containing the vector pBluescript SK(−) and human aldolase A cDNA (1 kb) was digested with EcoRI. Cold or 32P-labeled linear pHAA47 containing a HindIII site was used as the main substrate DNA in this study. The substrate DNA (0.5 μg) was incubated at 37°C for 1 h, with purified protein in 20 ml reaction mixture (10 mM Tris–HCl, pH 7.5, 10 mM MgCl2, 1 mM DTT and 50 mM NaCl). The reaction mixture was electrophoresed in 1% agarose and stained with 5 μg/ml ethidium bromide.

### End-labeling

For gel retardation assays and determination of enzyme activity, end-labeling of DNAs was carried out with [γ-32P]ATP and T4 polynucleotide kinase, according to the manufacturer’s instructions (Nippon Gene), and the products were purified on a ProbeQuant G-50 Micro column (Amersham Pharmacia Biotech, Tokyo, Japan).

A 210 bp PCR product was obtained from the HindIII R gene itself as a template where the HindIII site was located at the center of the fragment. It was labeled and used both as a substrate and a probe to investigate the effect of the His-tag sequence in the presence of Mg2+.

### Gel retardation assay

A 63 bp DNA fragment of pBluescript II SK(−) was used as a probe in the gel retardation assays. The fragment, containing a HindIII site at the center, was obtained by Xhol/BamHI digestion, and end-labeled with [γ-32P]ATP. The above 210 bp fragment was also used as a probe. Gel retardation assays were carried out as described previously (Takasaki, 1994). The end-labeled DNA was incubated at room temperature for 20 min with purified protein in a 20 ml reaction mixture which also contained 10 mM Tris–HCl (pH 8.0), 5 mM CaCl2, 10 mM DTT, 100 ng poly(dI–dC) and 50 mM NaCl. Native polyacrylamide (5%) electrophoresis was run at 4°C for 2 h at 150 V in TAE buffer (10 mM Tris–HCl, acetic acid, 1 mM EDTA, pH 7.4). After electrophoresis, the gel was dried and subjected to autoradiography using Fuji Medical X-ray film. The radioactivity was quantified using a Bio Imaging Analyzer BAS 2000 (Fuji Photo Film, Tokyo, Japan).
results and discussion

Introduction of mutant HindIII genes into pET16b system

The tertiary structures of seven type II restriction enzymes, EcoRI (Kim et al., 1990), EcoRV (Winkler et al., 1993), BamHI (Newman et al., 1995), PvuII (Cheng et al., 1994), Cfr10I (Bozic et al., 1996), FokI (Wah et al., 1997) and BgiII (Newman et al., 1998), have been determined successfully. On the basis of these structures, Stahl et al. (1998) proposed a model: many type II restriction enzymes share a common sequence motif, the PD----D/EXK motif, that is important for catalytic reactions.

Because of insufficient information about the structure of the HindIII protein, we at first treated HindIII gene with sodium nitrite to obtain a mutant gene, which resulted in the substitution of Asn for Asp at position 123 in the enzyme (Dahai and Takasaki, manuscript in preparation). The HindIII protein encoded by this gene lacked activity to double-stranded ds linear DNA, while it was still active to double-stranded circular DNA, and the protein could bind substrate DNA as well as wild type HindIII. We therefore regarded Asp123 as one of essential residues in catalysis, and the above motif would exist around the Asp123.

Tang et al. (1999) constructed an expression plasmid for HindIII, pUC19-RM. Site-directed mutagenesis was performed on this whole plasmid by employing inverse PCR (Tang et al., 1999). Seven kinds of mutant HindIII genes, P50S, E86K, P84Q, V106E, D108L, D123N and K125N, were prepared. Enzymatic properties of the proteins which were directed by these genes were examined. A mutant E86K, in which Lys was substituted for Glu at position 86, exhibited endonuclease activity that was higher than wild type. Replacement of Val with Glu at residue 108 (V106E) and of Lys with Asn mutant D108L showed no detectable activity. Interestingly, as the activity that was higher than wild type. Replacement of Val with Glu at residue 106 (V106E) and of Lys with Asn mutant D108L showed no detectable activity. Interestingly, as

Protein purification

We have cloned wild type gene RM and E86K, V106E, D108L and K125N into plasmid pET16b, which the presence of the lac operator and with the addition of chloramphenicol allows inducible overproduction of the protein in E.coli. Also, the

DNA binding assay

Gel retardation assays were carried out in order to examine the effect of the His-tag sequence and also to see whether the
Fig. 3. HindIII activities of purified enzymes. (A) Effect of the His-tag at the N-terminus of the wild type enzyme. Enzymes: lane 1, commercial enzyme; 2 and 3, His-tagged (0.06 and 0.12 µg, respectively); 4 and 5, unmodified (0.06 and 0.12 µg, respectively). (B) Activities of mutant enzymes. Enzymes: lane 1, commercial enzyme; 2, substrate alone; 3, wild type; 4, E86K; 5, D108L; 6, V106E; and 7, K125N (lanes 3—5, each 0.24 µg enzyme). (C) Comparison of activities of unmodified dilute enzymes. Lane 1, commercial enzyme; 2, E86K; and 3, wild type (each 2.4 ng).

Table II. Biochemical properties of HindIII mutants

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Relative activitya</th>
<th>$k_{cat}$ ($\times 10^2$/min)b</th>
<th>DNA binding activityc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>++</td>
<td>9.53</td>
<td>100</td>
</tr>
<tr>
<td>E86K</td>
<td>+++++</td>
<td>19.08</td>
<td>128</td>
</tr>
<tr>
<td>V106E</td>
<td>+</td>
<td>ND</td>
<td>43</td>
</tr>
<tr>
<td>D108L</td>
<td>−</td>
<td>0.00</td>
<td>0</td>
</tr>
<tr>
<td>K125N</td>
<td>+</td>
<td>0.19</td>
<td>42</td>
</tr>
</tbody>
</table>

aJudged by fluorescence intensities of ethidium bromide bound to product DNA.
bCalculated by radioactivities of product DNA (1.2 kb), assuming molecular masses of product and enzyme (as dimer) to be 775.5×10^3 and 69.9×10^3, respectively. The standard curve of product DNA was obtained using commercial HindIII.
cRepresented by radioactivity of complex band of wild type to be 100.

inactive mutants, such as D108L, lacked DNA binding activity. The presence of the His-tag sequence caused no gel shift: no DNA bound to enzyme (data not shown). It is possible that the positive charges of the N-terminal His residues might bind DNA in an arbitrary manner and hinder the correct interaction. Nonetheless, the enzyme can reach the HindIII site and cleave it in the presence of $\text{Mg}^{2+}$. Unmodified enzymes cause a gel shift, as illustrated in Figure 4A. The E86K mutant showed a higher affinity to DNA than wild type. Mutants V106E and K123N showed weak affinity in comparison with wild type. No DNA binding activity was detected in mutant D108L. In Table II, we summarize relative intensities (in other words, affinity to the probe) of the shifted bands.

The DNA binding and enzyme activities of the wild type in the presence of $\text{Mg}^{2+}$ were observed simultaneously, as illustrated in Figure 4B. A small amount of enzyme was present in the reaction, and it was terminated after 30 min: a shifted band and the reaction product could be observed at once. The His-tag sequence was again proven to have no effect on the activity; the radioactivity ratio of ‘cleavage DNA’ bands, which were produced by the His-tagged and unmodified wild type enzymes, was 1 : 0.9. However, even in the presence of $\text{Mg}^{2+}$, the His-tag sequence interfered with gel shift to a certain degree. Radioactivity measurement implied that the unmodified enzyme showed a threefold higher affinity to the probe (or substrate) than the His-tagged enzyme.

CD spectra of HindIII proteins

As described above, mutant D108L lost all activity. There is a possibility that the three-dimensional structure of the protein was destroyed in this mutant. Also, the extent of the structural difference between the E86K mutant and that of wild type should be investigated. We measured the CD spectra of the purified enzymes—wild type, E86K and D108L—as illustrated in Figure 5A. The spectrum of the wild type suggests poor
content of ordered structures such as α-helices and β-pleated structures in the *HindIII* molecule. CD measurements of all three samples in the presence of 5 mM Mg$^{2+}$ showed considerable similarity of conformation to each other. The curve gradually decreased from 260 down to 220 nm, showed a negative trough at 212 nm, then rapidly increased in regions below 210 nm. The mean residue ellipticity [$	heta$] at 212 nm was commonly $-11,350$. We therefore conclude that the *HindIII* protein did not change its conformation by site-directed mutagenesis, even if the mutant enzymes showed drastic changes in biochemical properties.

**Effect of divalent cations on *HindIII* activity**

It is well known that restriction enzymes require divalent cations as cofactors. The recombinant *HindIII* in this study also showed little activity when a powerful metal chelate EGTA was added (data not shown). We considered the possibility that the mutant E86K allowed water molecules to enter its catalytic site more easily. This would explain the high activity of this mutant. Some divalent cations, preferably Mg$^{2+}$, are believed to bind water molecules and carry them into the catalytic site of nucleases (Pingoud and Jeltsch, 1997). We examined the effects of several kinds of divalent cations on activities of the wild type enzyme and mutant E86K, as displayed in Figure 6. The substrate pHAA47 was treated with *Eco*RI previously; each cocktail contained 5 mM MgCl$_2$ (in the case of Mg$^{2+}$, its final concentration was 7 mM). In Figure 6A, the effects of five kinds of divalent cations were examined. It is evident that both enzymes were inhibited by the ions to a greater or lesser extent, except in the case of Mg$^{2+}$. Addition of Zn$^{2+}$ resulted in a strong inhibition to both enzymes. Transition metals, Mn$^{2+}$ and Mo$^{2+}$, showed weak inhibition of the wild type enzyme. The wild type enzyme could not cleave circular DNA in the sole presence of Mn$^{2+}$ (data not shown). These facts indicate that Mn$^{2+}$ cannot replace Mg$^{2+}$ as a cofactor for *HindIII*, differing from other type II restriction enzymes; it rather showed inhibitory effects on the *HindIII*. The unusual activity of *HindIII* in the presence of Mn$^{2+}$ has been reported previously (Hsu and Berg, 1978). Hsu and Berg (1978) assayed *HindIII* activity in 10 mM Mn$^{2+}$ for 3 h, high Mn$^{2+}$ concentration and prolonged reaction time. We repeated their experiment using commercial or our purified wild type enzymes, and obtained a loss of activity, again. *HindIII* is found to be an exceptional restriction enzyme in that it can barely accept Mn$^{2+}$ as a cofactor.

Interestingly, treatment by E86K with coexistent Mn$^{2+}$ or Mo$^{2+}$ generated new discrete bands of 2.55, 2.07, 0.77, 0.51 and 0.29 kb, in addition to the normal product bands, 3.4 and 1.18 kb, as illustrated in Figure 6A (magnified lane, far right). This anomalous result suggested that the mutant *HindIII* E86K also showed little activity when a powerful metal chelate EGTA could cleave DNA at sites different from the *HindIII* site. This additional activity of E86K was exhibited with the linear DNA, rather than of the circular DNA. Effects of several kinds of divalent cations on activities of the wild type enzyme and mutant E86K, as displayed in Figure 6. The substrate pHAA47 was treated with *Eco*RI previously; each cocktail contained 5 mM MgCl$_2$ (in the case of Mg$^{2+}$, its final concentration was 7 mM). In Figure 6A, the effects of five kinds of divalent cations were examined. It is evident that both enzymes were inhibited by the ions to a greater or lesser extent, except in the case of Mg$^{2+}$. Addition of Zn$^{2+}$ resulted in a strong inhibition to both enzymes. Transition metals, Mn$^{2+}$ and Mo$^{2+}$, showed weak inhibition of the wild type enzyme. The wild type enzyme could not cleave circular DNA in the sole presence of Mn$^{2+}$ (data not shown). These facts indicate that Mn$^{2+}$ cannot replace Mg$^{2+}$ as a cofactor for *HindIII*, differing from other type II restriction enzymes; it rather showed inhibitory effects on the *HindIII*. The unusual activity of *HindIII* in the presence of Mn$^{2+}$ has been reported previously (Hsu and Berg, 1978). Hsu and Berg (1978) assayed *HindIII* activity in 10 mM Mn$^{2+}$ for 3 h, high Mn$^{2+}$ concentration and prolonged reaction time. We repeated their experiment using commercial or our purified wild type enzymes, and obtained a loss of activity, again. *HindIII* is found to be an exceptional restriction enzyme in that it can barely accept Mn$^{2+}$ as a cofactor.

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As shown in Figure 6A and B, the normal product band of 1.18 kb (and maybe also the 3.4 kb band) seemed to disappear after E86K treatment in the presence of Mn$^{2+}$. However, as mentioned below, we found the existence of a normal product band even after treatment in the presence of Mo$^{2+}$. The bands corresponding to 1.18 kb were recovered from lanes of Mg$^{2+}$ and Mo$^{2+}$ treatments in Figure 6A, and the DNA fragments were purified. They were then ligated with pUC19-*Eco*RI-*HindIII*, and used for transformation of *E.coli* DH5α in the presence of X-gal. The number of white colonies produced in each case were as follows: 48 (wild type-Mg$^{2+}$), 55 (wild type-Mo$^{2+}$), about 200 (E86K-Mg$^{2+}$) and 6 (E86K-Mo$^{2+}$).
Divalent cation dependence of the activities of wild type and E86K. Substrate DNA, pHAA47, was previously treated with EcoRI in 5 mM MgCl₂. The activity was assayed for 1 h in the presence of 5 mM cations. Arrows represent a product DNA band of 1.18 kb. (A) Effect of five different cations. Enzymes (0.24 µg each): wild type and E86K. Cations: no further addition, MgCl₂, CaCl₂, MnCl₂, ZnSO₄ and MoCl₂. #, Magnification of the rightmost lane (E86K/H₁₁₀₀₁/Mo₂⁺/H₁₁₀₀₁) with molecular sizes (kb) of product bands. (B) Inhibitory effects of Zn²⁺ and Mo²⁺ on wild type and E86K. Cations (5 mM each): MgCl₂, ZnCl₂ and MoCl₂.

Respective five of the emerged white colonies yielded plasmids, which were all found to have inserts of 1.18 kb fragments when treated with EcoRI and HindIII. The colony numbers above are reasonable, because the presence of previously added Mg²⁺ directed the normal digestions. These results indicate that Mn²⁺ and Mo²⁺ inhibited E86K in generating the normal product.

The results in Figure 6B are similar to those in Figure 6A. The band marked with an asterisk (0.85 kb), present after E86K-Mo²⁺ treatment, was also recovered, and no white colonies emerged when ligation and transformation was performed by the same method as the other fragments, suggesting again that, in the presence of Mo²⁺ or Mn²⁺, E86K digests DNA at unique sites in addition to the normal HindIII site. This activity was attained only in E86K, with a coexistent transition metal. Figure 7 shows the similar effect of Mo²⁺ on E86K when the other DNAs were employed as substrates. At least one extra band (asterisked) was observed when E86K digested pBluescript in the presence of Mo²⁺. No such bands were found when pUC19 was used. We conclude here that E86K cleaved pBluescript DNA at different site(s) from the HindIII site, when a transition metal was present in the reaction cocktail.

The bands marked with an asterisk in Figures 6B and 7 were both recovered and purified. Both of them had UV absorption peaks at 260 nm. All the substrate DNAs were prepared by CsCl density gradient ultracentrifugation: no contaminating bacterial nucleic acids were present. We believe that these bands must be DNA fragments specifically derived from the substrate DNAs only by E86K treatment.

Although their sequences have not yet been determined, we think this activity by E86K would not be regarded as a so-called star activity. Star activity is induced under unusual conditions, such as high pH, addition of DMSO (in the case of HindIII; Nasri and Thomas, 1987) and high concentrations of glycerol. There should be several possible star sites in pUC19 or pHAA47, pUC19 or pBluescript DNA. There are nine star sites for HindIII in pBR322 (Nasri and Thomas, 1987), and following their estimation, we found five star sites in pHAA47, while there were no anomalous bands in pUC19 upon treatment with E86K and Mo²⁺ (Figure 7). These facts show that the altered specificity of E86K is different from star activity.

In general, the smaller the ion radius, the higher the hydration number. Because of its small radius (about 0.7 Å), Mg²⁺ is the best cation for carrying water molecules as substrates. On the contrary, the radius of Zn²⁺, which inhibits perfectly both wild type and E86K activities, is approximately 0.80 Å, less than that of Ca²⁺ (approximately 1.2 Å), which does not
inhibit E86K. In the case of Mn$^{2+}$ or Mo$^{2+}$, the ion radius is somewhat greater than Zn$^{2+}$. We therefore think that the novel action of E86K is not related to ion radius or hydration of cations: it is specifically caused by transition metals, which cannot be replaced with the usual divalent cations.

Structural changes of the wild type and mutant E86K in the presence of MoCl$_2$ were examined by CD spectra, as illustrated in Figure 5B. The spectra of both enzymes were analogous to each other, and no noticeable differences from those in Mg$^{2+}$ (Figure 5A) could be observed. The conformations of wild type and E86K are therefore similar; accordingly, the conformations are not influenced by Mg$^{2+}$ or Mo$^{2+}$.

Residues involved in active site of HindIII

A mutational analysis of the Klenov fragment of $E$.coli DNA polymerase I (Derbishire et al., 1988) revealed that the divalent metal ions are coordinated in the negatively charged pocket, which is formed largely by Asp and Glu. However, based on the three-dimensional structures of several restriction enzymes, it is said that the two acidic residues (both have negative charges) bind one Mg$^{2+}$ at the catalytic center (Pongud and Jeltsch, 1997). For example, a mutant of restriction enzyme EcoRI, in which Glu111 was replaced with Gly, showed a reduced activity (King et al., 1989). HindIII mutant D108L showed no enzyme activity (Figure 3B). One can strongly argue that Asp108 is directly responsible for the activity: it is one of the two acidic residues at the catalytic center, like Glu111 in EcoRI. Also, Asp108 in HindIII is likely to be the residue D/E in the D/EXK motif (Stahl et al., 1989). In the case of Glu86 in HindIII, the situation is complicated. This residue might chelate Mg$^{2+}$, and the Lys86 residue in mutant E86K might do the same. Nonetheless, we think that Lys86 in E86K also gets electrostatic interaction as a Lewis acid, with a phosphate group of DNA.

Our model as to the role of transition metals is as follows. In E86K, the transition metal, of course, competes with Mg$^{2+}$ in digestion of DNA in a regular manner. Besides, the transition metal ion would certainly be bound to a DNA phosphate group which permits the second cation, especially that of a transition metal, to bind another phosphate group(s) to be attacked by water.

This is the first report describing a change of substrate specificity for a restriction enzyme under moderate conditions. It is hoped that more optimal conditions for the E86K treatment will be discovered soon to shed light on the complete alteration of substrate specificity of HindIII.

Two mutants, V106E and K125N, were prepared to examine the effect of mutations introduced in the vicinity of Asp108 and Asp123. Introduction of negative charge (V106E) and the disappearance of positive charge (K125N) resulted in a decrease in both enzyme and DNA binding activities. However, these mutants are not as remarkably inactive as D108L. It may be the case that the Val106 and Lys125 residues are not members of the motif proposed by Stahl et al. (1998). Investigation of the three-dimensional structure of HindIII is necessary for further discussion.

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References


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