Note

Arginine-55 in the β-Arm is Essential for the Activity of DNA-Binding Protein HU from Bacillus stearothermophilus

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DNA-binding protein HU (Bst HU) from Bacillus stearothermophilus is a homodimeric protein which binds to DNA in a sequence-nonspecific manner. In order to identify the Arg residues essential for DNA binding, four Arg residues (Arg-53, Arg-55, Arg-58, and Arg-61) within the β-arm structure were replaced either by Gin, Lys, or Glu residues, and the resulting mutants were characterized with respect to their DNA-binding activity by a filter-binding analysis and surface plasmon resonance analysis. The results indicate that three Arg residues (Arg-55, Arg-58, and Arg-61) play a crucial role in DNA binding as positively charged recognition groups in the order of Arg-55 > Arg-58 > Arg-61 and that these are required to depress the dissociation rate constant for Bst HU-DNA interaction. In contrast, the Arg-53 residue was found to make no contribution to the binding activity of Bst HU.

Key words: Bacillus stearothermophilus; DNA-binding protein HU; site-directed mutagenesis; surface plasmon resonance

DNA-binding protein HU, being ubiquitous in the eu-bacterial kingdom, is a basic, sequence-nonspecific DNA-binding protein with 90-92 amino acid residues (Mr of 9,500 for the monomer) and occurs as a homotypic dimer in a solution. By analogy to histones, (e.g., its small size and nonspecific binding feature), the molecular mechanism for the interaction of HU with DNA has been extensively investigated by using the E. coli HU and bacteriophage Mu strand transfer reaction as a model. E. coli HU is a heterodimeric protein composed of two non-identical but homologous subunits, HU-1 and HU-2, encoded by the hupB and hupA genes, respectively. E. coli HU plays an important role in a variety of DNA metabolic reactions such as replication,39 inversion,45 transposition,46 and repair,7 possibly as a DNA chaperone.8

The three-dimensional structure of HU (Bst HU) from Bacillus stearothermophilus has been determined by both the X-ray diffraction10 and NMR spectroscopic methods.11 A nucleic acid binding model has been proposed, whereby the two exposed β-ribbon arms interact directly with DNA. A recent study, using E. coli HU coupled to a DNA cleavage reagent (iron-EDTA), has presented a snapshot of HU-DNA interaction.12 The three-dimensional structure of a complex consisting of the integration host factor (IHF), a member of the DNA-binding protein HU family, and an oligonucleotide has been resolved and demonstrates that the β-arm of IHF extensively interacts with the minor groove of the oligonucleotide.13 Despite a vast amount of structural information, the amino acid residue(s) responsible for the DNA binding of HU have not been precisely identified. In this study, in order to provide more insight into the mechanism for HU-DNA interaction, we employ site-directed mutagenesis of the HU protein (Bst HU) from B. stearothermophilus to evaluate the role of the arginine residues on the outward strand of the β-arm in DNA binding. This analysis identifies Arg-55 as the most essential amino acid for the DNA-binding activity of Bst HU. While Arg-58 and Arg-61 have a less essential role, Arg-53 is found not to contribute to the binding activity of Bst HU.

Most of the materials and methods used for the DNA manipulations in this study have been described in ref. 14. A CM5 sensor chip, HEPES saline buffer, and amine-coupling kit for BIACore™ were obtained from Pharmacia Biotech. Streptavidin was purchased from Sigma Chemicals, and a Biotin labeling kit was obtained from Gibco BRL. The filter-binding assay was principally carried out by the method of Goshima et al.,15 using the EcoRI-HindIII fragment of pUC18 labeled by α-32P-CTP. Data were fitted to curves by the non-linear least-squares method, using Kaleidagraph software after normalization of the retention efficiency, and the apparent binding constants (Ks) were calculated as described by Draper et al.16 A real-time analysis of Bst HU-DNA interaction was done with the BIACore™ instrument (Pharmacia Biosensor). The biotinylated EcoRI-HindIII DNA fragment of plasmid pUC18 was coupled to the CM5 sensor chip where streptavidin had previously been immobilized. Details of the methods for immobilizing a protein to the CM5 sensor chip and for the kinetic analysis with the BIACore™ instrument have been described in a previous paper.17

The β-arm structure that was predicted to be involved in the interaction of Bst HU with DNA includes four Arg residues, Arg-53, Arg-55, Arg-58, and Arg-61, as

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Abbreviations: Bst HU, DNA-binding protein HU from B. stearothermophilus; IHF, integration host factor; RU, resonance units; SPR, surface plasmon resonance
shown in Fig. 1, some of which are likely to interact with the backbone phosphates or bases of DNA. In the present study, to evaluate the role of these Arg residues in DNA binding, we substituted these Arg residues with Gln, Lys, and Glu residues, and then examined the effects of substitution on the binding affinity. Mutant genes were constructed by site-directed mutagenesis, overexpressed in E. coli cells, and the proteins were purified by procedures identical to those described for recombinant Bst HU.

Four Arg residues (Arg-53, Arg-55, Arg-58, and Arg-61) were first replaced by an uncharged Gln amino acid residue, and the resulting mutant proteins (R53Q, R55Q, R58Q, and R61Q) were characterized with a filter-binding assay. Figure 2 shows typical binding curves of the mutants, while Table 1 summarizes the apparent binding constants determined from these binding curves. It was found that the mutation of Arg-55 severely weakened the binding affinity to DNA, and a less severe decrease in DNA binding activity was observed with the Arg-58 and Arg-61 mutations. In contrast, the mutation of Arg-53 had no significant effect on the binding affinity. These results indicate that Arg-55 is essential for DNA binding, and that the other three Arg residues make only small contributions to the binding of Bst HU to the DNA. We next prepared eight mutant proteins, in which four Arg residues were individually replaced by either a positively charged residue (Lys) or a negatively charged residue (Glu). The resulting mutants were again analyzed by a filter-binding assay. The results showed that all four Lys mutant proteins (R53K, R55K, R58K, and R61K) completely retained their DNA-binding affinity to the same extent as the wild type of Bst HU (Fig. 2 and Table 1). Even a quadruple mutant (R53/55/58/61K), in which all four Arg residues were simultaneously replaced by Lys residues, retained considerable DNA-binding activity. In contrast, the Glu mutant proteins exhibited significantly less binding affinity to DNA than that of the wild type of Bst HU, although the R61E mutant retained slight DNA-binding activity (Fig. 2 and Table 1).

The interaction of Bst HU and its mutants with DNA was further quantitatively analyzed by SPR with the BIAcore™ system. Figure 3 shows typical sensorgrams of the association and dissociation of Bst HU and its Gln mutant proteins. The values for the association rate constant ($k_{on}$), dissociation rate constant ($k_{off}$), and apparent association constants ($K_a$) obtained are summarized in Table 1. Although the SPR measurements gave a similar relative binding strength to those obtained by the filter-binding assay, the $K_a$ values derived from real-time analyses by the BIAcore system were generally

![Tertiary Structure of the Dimer of Bst HU](Image)

**Fig. 1.** Tertiary Structure of the Dimer of Bst HU.

The protein model is from Tanaka et al., and side chains show only those residues where the contribution to DNA-binding activity was examined in this study. It should be noted that the residues in the distal region of the arm between strands 2 and 3 (59–70) are tentatively included in this figure.

![Saturation Binding Curves](Image)

**Fig. 2.** Saturation Binding Curves for the Interaction of Bst HU and Its Mutant Proteins with DNA.

Increasing amounts of proteins were incubated with the EcoRI and HindIII fragment of pUC18 and isolated by filtration. A, binding curves for Bst HU (○), R53Q (□), R55Q (△), R58Q (×), and R61Q (+); B, binding curves for Bst HU (○), R53K (●), R55K (◇), R58K (×), R61K (+), and R53/55/58/61K (△); C, binding curves for Bst HU (○), R53E (◇), R55E (◇), R58E (×), and R61E (+).
<table>
<thead>
<tr>
<th>Protein</th>
<th>Filter-binding assay $K_a$ ($\times 10^6$ M$^{-1}$)</th>
<th>Surface plasmon resonance measurement $k_{on}$ ($\times 10^3$ M$^{-1}$ s$^{-1}$)</th>
<th>$k_{off}$ ($\times 10^7$ s$^{-1}$)</th>
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<tr>
<td>Bst HU</td>
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<tr>
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<td>2.50</td>
<td>1.14</td>
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<tr>
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<td>R61Q</td>
<td>2.16</td>
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<tr>
<td>R55Q</td>
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<td>0.24</td>
<td>2.85</td>
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</table>

The values for association constant $K_a$ obtained from the filter-binding assay were estimated by the method of Draper et al.[10] The values for the apparent association constants obtained by BIA core were calculated from the relationship $K_a = k_{on} / k_{off}$.

The results indicate that the BIAcore measurements and the filter-binding assay both show that Arg-55, and possibly Arg-58 and Arg-61 as well, in Bst HU play a crucial role in binding to DNA. These measurements also revealed that mutant R55Q, as well as R58Q and R61Q, retained values for the association rate constant comparable to that of the wild type, while they showed increased values for the dissociation rate constants. This indicates that the lower affinity of each mutant was due almost exclusively to the increased dissociation rate constant. It is thus suggested that three Arg residues (Arg-55, Arg-58, and Arg-61), and particularly Arg-55, contribute to DNA binding primarily by keeping Bst HU in direct contact with the DNA once the complex has been formed.

Goshima et al. have previously shown that the simultaneous mutation of Arg-58 and Arg-61 in E. coli HU1 reduced the DNA-binding activity as determined by the affinity to a DNA cellulose column, and that the mutant E. coli cell harbouring this mutant HU was unable to maintain normal cell growth in a hupA-hupB-himA triple mutant at 42°C. It was therefore concluded that Arg-58 and Arg-61 were important for the DNA-binding ability and biological functions of E. coli HU.[10] The two Arg residues are conserved in Bst HU as Arg-58 and Arg-61. The result of the present study support the important role of Arg-58 and Arg-61, although they appeared to make relatively small contributions to the binding activity in Bst HU. The sum of a small contribution of the individual residues may probably result in some effects on the DNA-binding activity and function of Bst HU.

**Acknowledgments**

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**References**

DNA-binding Protein HU from *B. stearothermophilus*


