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Effects of Hin Recombinase Dimer Interface Mutants on DNA Binding and Recombination

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Abstract

Previous biochemical assays and a structural model indicated that the dimer interface of the Hin recombinase is composed of the two a-helices. To elucidate the structure and function of the helix, amino acids in the N-terminal end of the helix, where the two helices contact most, were randomized, and inversion-incompetent mutants were selected. To investigate why the mutants lost their inversion activities, the DNA binding, *hix*-pairing, invertasome formation, and DNA cleavage activities were assayed using *in vivo* and *in vitro* methodologies. Results indicated that the mutants could be divided into 4 classes based on their DNA binding activity. We proposed that the a-helices might place a DNA binding motif of Hin properly to the minor DNA groove of the recombination site. All the mutants except the non-binders were able to perform *hix*-pairing and invertasome formation, suggesting that the dimer interface is not involved in the process of *hix*-pairing or invertasome formation. The inversion-incompetent phenotype of the binders was caused by the inability of mutants to perform the DNA cleavage activity. The less binders exhibited wild-type level of *hix*-pairing activity because the *hix*-pairing activity overcomes the DNA binding defect of the less binders. This phenotype of the less binders suggests that the binding domains of Hin could mediate Hin-Hin interaction during *hix*-pairing.

Introduction

Hin (21 kDa) inverts a DNA segment flanked by two of 26-bp DNA sequence *hix* recombination sites (Johnson and Simon 1985). Hin exists as a homodimer in solution, and binds to *hix*. For efficient DNA inversion, an accessory protein known as Fis (Factor for inversion stimulation) is also required (Johnson *et al.*, 1984). The DNA inversion reaction can be performed *in vitro* on a negatively supercoiled plasmid-DNA substrate that contains two *hix* sites and the enhancer with purified Hin and Fis proteins (Fig. 1). Inversion reaction can be divided into several discrete biochemical steps (Fig. 1): 1) DNA binding of Hin and Fis, 2) Juxtaposition of the recombination sites is by protein-protein interaction between *hix*-bound Hin dimers (*hix*-pairing; Heichman and Johnson 1990), 3)The next step is to form a specific protein-DNA complex called invertasome, 4) Invertasome formation is followed by Hin cleavage at the middle of *hix* sites (Johnson and Bruist 1989). Exchange of the cleaved DNA strands and religation of the inverted DNA ends complete the inversion reaction (Fig. 1).

Implication of the dimer interface in DNA cleavage activity has been The role of the dimer interface in DNA cleavage activity of Hin has been studied extensively (Lim, 1994; Haykinson *et al.*, 1996). The dimer interface is composed of two long a-helices coming from each monomer (amino acid residue 101 to 135). In this study, a portion of *hin* gene that codes for amino acid residues in the dimer interface

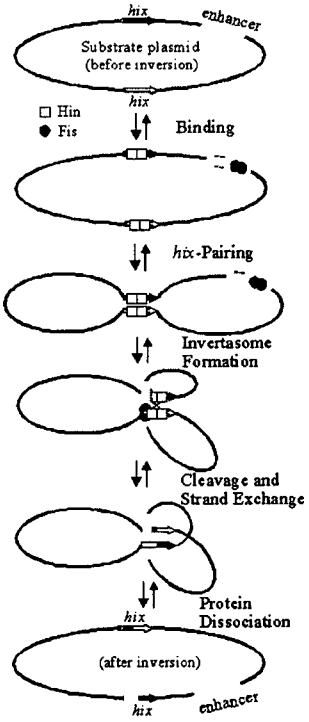

Fig. 1. A schematic representation of biochemical steps during Hin-mediated DNA inversion on plasmid DNA. DNA inversion reaction requires negative supercoiling of the plasmid DNA, which is excluded in this figure for clarity. Hin-Hin dimer interaction brings the two *hix* sites close together (*hix*-pairing). Proteins are removed from Cleavage and Strand exchange step for clarity. Note that after strand exchange the intervening DNA between *hix* sites is inverted.

permissible changes	Leu 58				Cys		Ile
	Met 101	**Gly 102**	**Arg 103**	**Phe 104**	**Phe 105**	**Phe 106**	**His 107**
impermissible changes	Arg	Val 8 Trp 8 Ser Glu 3 Arg 3	Leu 4 Gln	Ile Val Leu 6	Ser	Ile Leu 5 Ala Cys 2 Ser 4	Leu 8 Gln 9 Thr Pro 5 Arg 2

Fig. 2. Permissible and nonpermissible amino acid changes from 101 to 107. The wild type sequence is given in boxes. Permissible changes gave rise to inversion-competent phenotype while nonpermissible changes caused inversion-incompetent phenotype. Numbers next to each amino acid changed indicate the number of incident.

from 101 to 107 was randomly mutagenized. Those that showed inversion-incompetent phenotype were further assayed to identify which step caused inversion-incompetency. Results showed that the inversion-incompetent mutants were defective either in binding or in DNA cleavage.

Materials and Methods

Mutagenesis was performed with dopped oligos as described in Kunkel 1985. *In vivo* assays for DNA binding, *hix*-pairing, and invertasome formation were performed as described in Lee *et al.* (1998).

Results

Generation and characteristics of mutations at the dimer interface

A portion of *hin* gene coding for amino acid residues from 101 to 107 was randomly mutagenized using a doped oligonucleotide that was synthesized by contaminating each base with 3.3% of the other three nucleotides. Theoretically, this procedure should make a pool of oligonucleotides, each of which has a single base change.

Forty-eight hours after transformation, 59% of the transformants were completely white and the rest were either red or pink. To determine the amino acid changes in these mutants, *hin* genes isolated from 71 red colonies and 160 white colonies were sequenced. Among the *hin* genes from white colonies, 78 had single amino acid substitution (Fig. 2), 25 had double changes, and 2 had triple changes. The rest (55) had either deletions or insertions of one or more bases. DNA sequencing of the *hin* genes from red colonies revealed that 60 incidents had single amino acid change and 11 contained no changes. Surprisingly, out of 60 permissible changes, 58 incidents occurred at the amino acid residue 101, and all were changed to Leu. Thus, most substitutions, except Leu at 101, Cys at 105, and Ile at 107, caused inversion-incompetent Hin (Fig. 2).

Characteristics of the mutants assayed *in vivo*

The inversion-incompetent amino acid changes in the helix E of Hin proteins were further characterized. At which step the inversion reaction was blocked in these mutants was examined by *in*

Table 1. SF[a] of HB101 harboring following plasmids.

Hin-producing plasmid	*rpsL*-containing plasmid		
	pSingle	pDouble2	pTriple
pHinWT	0.12	0.44	0.56
pHinM101L[b]	0.07	0.17	0.40
pHinG102R	$<10^{-7}$	$<10^{-7}$	$<10^{-7}$
pHinG102V	$<10^{-7}$	$<10^{-7}$	$<10^{-7}$
pHinG102S	$<10^{-7}$	$<10^{-7}$	$<10^{-7}$
pHinG102W	$<10^{-7}$	$<10^{-7}$	$<10^{-7}$
pHinG102E	$<10^{-7}$	$<10^{-7}$	$<10^{-7}$
pHinR103Q	0.80	0.70	0.29
pHinR103L	1.1×10^{-5}	0.05	0.11
pHinF104L	$<10^{-7}$	0.07	0.38
pHinF104V	0.16	0.28	0.59
pHinF104I	0.23	0.50	0.68
pHinF105S	2.1×10^{-3}	0.30	0.38
pHinF106I	0.14	0.25	0.68
pHinF106N	$<10^{-7}$	$<10^{-7}$	6.5×10^{-5}
pHinF106A	$<10^{-7}$	$<10^{-7}$	5.1×10^{-5}
pHinH107Q	0.18	0.23	0.5
pHinH107P	1.0×10^{-4}	1.2×10^{-5}	0.37

[a]The SF values are averages from at least two independent experiments.
[b]M101L is inversion-competent.

vivo methodologies (Lee *et al.*, 1998). Hin proteins from the mutants were made more or less the same as the wild type (data not shown). The results were summarized in Table 1.

The mutants could be divided into 4 groups

The results of SF measurements enabled us to sort the mutants into 4 classes based on their DNA binding activity. The first class, the Bind^{+} (F104V, F104I, F106I, and H107Q), bound *hix*, paired *hix*, and formed invertasome as efficiently as WT. Thus, it is likely that the Bind^{+} class became inversion-incompetent because they were blocked in one of the steps after invertasome formation. The second class, Bind$^{+/-}$ (R103L, F104L, F105S, and H107P), showed reduced DNA binding activity, but their *hix*-pairing, or invertasome formation activity was comparably efficient as WT. F104L was added to this group, though it did not show any DNA binding activity, because the *hix*-pairing activity of F104L occurred and we believed that *hix*-pairing activity couldnt occur without DNA binding. Indeed, F104L showed DNA binding activity when measured *in vitro* (see below). The Bind$^{+/-}$ class appeared also blocked in steps after invertasome formation. The third class, Bind^{-} (G102R, G102V, G102S, G102W, G102E, F106N, and F106A), did not show binding activity. All of the Bind^{-} did not show any activity. It is likely that binding activity of the non-binders were so severely impaired that protein-protein interactions on DNA were not possible. The fourth class, Bind^{++} (R103Q) bound *hix* better than WT.

Discussion

The DNA binding activity of Hin and the helix E at the dimer interface

The binding specificity of the Hin recombinase results from the interactions of two structural motifs within Hin with the specific DNA sequence of *hix*. One is a helix-turn-helix (HTH) motif at the C-terminus of Hin from Glu 148 through Phe 180. This HTH motif interacts with the major DNA groove of *hix*. The other is the minor groove binding (MGB) motif that is composed of the three consecutive amino acid residues of Arg 140-Pro 141-Arg 142 (Feng *et al.*, 1994). For the current studies, both the *in vivo* and *in vitro* DNA binding assays showed that 75% of mutants have trouble in DNA binding. Because none of the mutants in this study have amino acid changes in these binding motifs, the DNA binding defect of those mutants could have been caused by allosteric effects on the DNA recognition regions resulting from structural changes at the N-terminal end of the helix E. It is possible that the amino acid changes in the Bind^{-} class could have destabilized the dimer interface, causing them to exist as monomers in solution. However, it is hard to imagine that all the mutations in the helix E disrupted the dimer interface. Besides, among the cysteines substituted at the helix E (from 101 to 125), G102C, F105C, F106C, H107C (Lim 1994), L112C, A113C, E116C, R117C, L119C, E122C, and R123C, showed no binding (H. M. Lim and H. J. Lee, unpublished data).

The structural model of Hin showed that the N-terminus of the MGB motif is immediately adjacent to the C-terminal end of the helix E. And there have been reports that emphasized the importance of the MGB motif of Hin in DNA binding. A single nucleotide change in the minor DNA groove of *hix* can abolish binding of Hin, while a base change in the major DNA groove results in a less severe binding defect (Hughes *et al.*, 1992). The binding affinity of a synthetic peptide of Hin (52-mer; from Gly 139 to Asn 190) to *hix* was completely abolished if Arg 140 was deleted (Sluka *et al.*, 1987). Based on these previous results and the structure of the MGB motif, the Bind^{-} class might not be able to position the MGB motif to the minor groove of *hix* properly, due to the improper positions of the

two E helices at the dimer interface. The binding activity of the Bind$^{+/-}$ class could be explained by the same way. Thus, the helix E may be involved in DNA binding activity of Hin by positioning the MGB motif properly to the minor groove of *hix*.

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