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Molecular Dynamics Simulations of a Helicase

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Abstract

Helicases are ubiquitous enzymes involved in nucleic acid metabolism. The PcrA DNA helicase is an essential bacterial protein involved in rolling circle plasmid replication and DNA repair. Recent crystal structures of PcrA bound to DNA indicate that a flexible loop mediates a functionally important rigid-body-domain rotation. In this study, we report stochastic boundary molecular dynamics simulations focused on this region for wild-type and mutants designed to increase the rigidity of the region. Residues in the region that were helix-disfavoring, such as glycine, threonine, and others, were mutated to alanine. The simulated dynamics, analyzed with a variety of measures of structure and mobility, indicate that a few point mutations will substantially increase helix formation in this region. Subnanosecond stochastic boundary molecular dynamics simulations at several temperatures offer a rapid protocol for assessing large numbers of mutants and provides a novel strategy for the design of experiments to test the role of this flexible loop region in the function of PcrA.

Keywords

DNA-binding protein; stochastic boundary; DNA helicase

INTRODUCTION

Proteins and DNA are biopolymers that form the basis of life. The interactions between the two types of molecules are crucial for maintaining and propagating life. The genetic information is locked within the DNA duplex, and to gain access to it, cells must be able to unwind the duplex into its single-strand DNA components. Ubiquitous enzymes known as helicases are responsible for this essential task. They are involved in all aspects of nucleic acid metabolism, ranging from DNA replication and repair to recombination, rescue of stalled replication, and translation (see reviews^{1,2}). The significance of these enzymes has become clear with the association of genes coding for helicases associated with premature aging disorders and increased susceptibility to cancers.³

There are many different types of helicases,⁴ but their oligomeric structures fall into two broad families: those that are hexameric, such as the T7 helicase; and those that are monomeric, such as Rep, UvrD, and PcrA. In this study, we focus on PcrA, a mainly α -helical protein consisting of 652 residues (Fig. 1). It is organized into two domains, each comprising two subdomains. The molecular mechanism by which PcrA unravels duplex DNA is still under investigation, but with recent advancements in structural and kinetic studies, the mechanism is becoming clearer. The “inchworm” or “Mexican wave” mechanism is the most favored at present.^{5,6} Three structures of PcrA have been solved with

the use of X-ray crystallography.^{7,8} They are the native form, a substrate form bound to 3'-tailed DNA, Mg²⁺ and ADPNP, and a product form bound to the same DNA, with a sulphate ion in the active site. The main differences between the product and substrate forms are the observation of ligand-induced conformational changes and the details of the ssDNA binding interactions.⁸ The binding of the single-stranded DNA (ssDNA) on top of subdomains 1A and 2A induces a rigid-body rotation of subdomain 2B by 130°, which is mediated by a flexible loop between I⁵⁴¹ and A⁵⁵⁸. Subsequent binding of adenosine triphosphate (ATP) in the active site, in a cleft between subdomains 1A and 2A, leads to the closure of this cleft and significant movement of the 1B and 2B subdomains relative to each other, which then causes the formation of a surface complementary to the shape and charge of the duplex DNA. The structure of the enzyme bound to a ss-dsDNA junction and ATP is represented by the substrate complex. The hydrolysis of ATP returns the helicase to a conformation represented by the product complex. Binding to ssDNA induces a rigid-body rotation of subdomain 2B that vacates the space into which dsDNA binds. This rotation is mediated by a flexible loop between residues I⁵⁴¹ and A⁵⁵⁸, and it is a crucial feature of the helicase function. In this study, we have designed a series of mutants aimed at reducing the flexibility of this region, using molecular dynamics simulations of wild-type PcrA helicase and these mutants to assess the flexibility of the region 541–558. The results from this study will enable us to design appropriate mutations and, subsequently, measure the biologic activity of these mutants experimentally.

METHODS

PcrA is a relatively large protein, consisting of 652 residues. To avoid the complexity and size of the protein-DNA complexes, our initial focus was on the native structure, which has no DNA cofactors. The macromolecular modeling software CHARMM⁹ was used to perform stochastic boundary molecular dynamics simulations,^{10,12} whereby the active region is focused on using explicit water molecules, and the rest of the protein is treated implicitly. Most of the molecule is treated implicitly, and the computational time required depends on the size of the sphere used to solvate the active region.

The first stage, before starting the simulations, was to build in the missing I⁵⁴¹-A⁵⁵⁸ loop region of the helicase protein. Because of the flexibility of the loop, X-ray crystallography could not determine the structure of the residues between I⁵⁴¹ and A⁵⁵⁸. Therefore, we modeled this region using the biomolecular modeling package, Sybyl from Tripos.¹³ The biopolymer loop setup searches a protein database for fragments of the indicated length (in our case, 17 residues) that fit well between the two flanking residues. The search is defined by certain parameters, such as the method for fitting the loops smoothly to the two window residues and the number of anchor residues on either side of the window region. We employed the smoothing function meld-anchor, which uses the exact database coordinates for the window region of the loop and a melding procedure at the anchor regions. The loops that we found exhibited two types of secondary structure: helix or irregular. Because the loop region structure is not known experimentally, the starting point for the simulation, from helix or coil, is ambiguous. Therefore, examples of each were selected as initial structures for simulations. It is unlikely that this region will be completely helical, because a well-defined helix would have been observed in the X-ray crystallography.

As mentioned above, we used stochastic boundary conditions to study the system, but other models of solvation were considered. A fully implicit treatment of solvent may have been undesirable, because of the neglect of potentially important interactions between the loop region and particular water molecules. A fully explicit treatment of solvent, with periodic boundary conditions, would have been beyond available computational resources, because of the size of the system. A compromise is to surround the protein, or just part of the protein,

with a sphere of water, and to maintain its shape with boundary potentials; this approach is known as stochastic boundary molecular dynamics (SBMD).^{10,12} This usually requires less water molecules and renders problems more tractable. The sphere is divided into two regions: an inner reaction region and an outer buffer region (Fig. 2). In the reaction region, the molecular dynamics simulations are propagated in the conventional manner, with the use of Newton's equation of motion. In the buffer region, the molecular dynamics are treated with the use of Langevin dynamics. This hybrid method couples the water molecules in the buffer region to a heat bath, which keeps the system at thermal equilibrium. A spherical boundary potential is employed to maintain the correct average distribution of water molecules and to prevent water from escaping into the vacuum. The stochastic boundary approach removes water molecules that are far from the area of interest, while still including, in an approximate manner, their effect on the molecules in the reaction region.

The Langevin equation is divided into three components. The first is the interatomic force, $F_i\{x_i(t)\}$, due to the interaction between the atoms in the system. This is the same force as that used in Newton's equation of motion. The second component, the frictional force, describes the drag on the particle due to the solvent. The magnitude of the drag is related to the frictional coefficient γ_i . The third component, $R_i(t)$, is the random or stochastic force due to thermal fluctuations of the solvent. The solvent is not explicitly represented, but its influence on the explicit atoms comes from the frictional and random forces. When the frictional and random forces are zero, the Langevin equation reduces to Newton's equation of motion:

$$m_i a = F_i\{x_i(t)\} - \gamma_i v_i m_i + R_i(t).$$

The Langevin equation generates classical Brownian dynamics that describe the motion of a particle under the influence of random collisions with the surrounding solvent. Friction coefficients of 250 ps^{-1} and 62 ps^{-1} were used for the protein atoms and the oxygen atoms of water, respectively, in the buffer region.

To solvate helicase completely with explicit solvent would require in excess of 10,000 water molecules and a sphere with a radius of $\sim 60 \text{ \AA}$. The main advantage of SBMD is that we can focus on the region of helicase containing the loop, without compromising the quality of the model for the solvent directly surrounding the flexible region. We have assumed that the motion of the solvent close to the flexible region is more important than the motion of the protein further removed from this region. This assumption probably warrants testing but is beyond the scope of the present study.

The simulation was set up as follows, starting from the protein structure with the model-built loop and 101 water molecules identified in the crystallographic structure. A sphere of water was centered on the region of interest. The water molecules that overlapped the protein were deleted. A brief minimization removed unfavorable contacts. The boundary potential was set up for the solvent, and the solvent friction was defined. All protein atoms and water molecules in the reservoir region were fixed. An initial dynamics run, with the protein atoms fixed, was performed to remove artifacts from the initial structure of the water. Another overlay of water molecules onto the system filled any holes that might have appeared after the initial dynamics run, as water settled into the surface of the protein. Further equilibration dynamics were performed on the water, with the protein atoms again fixed. The reaction and reservoir zones (and appropriate constraints) were defined with respect to the resulting configuration of the system.

Spheres of water with radii of 15 Å, 20 Å, and 25 Å were considered. Exploratory simulations of 100 ps on each solvated protein system were performed. We used the CHARMM22 forcefield¹⁴ and the TIP3P water model.¹⁵ The 15-Å radius sphere was too small and did not properly solvate the loop region. The simulations of the systems with 20 Å and 25 Å radius spheres appeared to be comparable. So we chose the 20 Å radius sphere for computational expediency. For the different mutants, the number of water molecules in the reaction region ranged from 648 to 660. The reaction region for the wild-type is shown in Figure 1. Minimization of the system after it was initially built was performed using 100 steps of steepest descent, followed by 500 steps of adopted-basis Newton–Raphson (ABNR). The steepest descent algorithm quickly alleviates unfavorable interactions but cannot distinguish between a transition point and a minimum, whereas ABNR can. SHAKE¹⁶ was used with the dynamics and minimization to allow a time step of 2 fs. The cutoffs were based on the recommendations in the CHARMM documentation. We used a nonbonded cutoff of 12 Å, employing an atom–atom shifting function. Atom-based Verlet lists were employed to process the nonbonded interactions and updated as necessary based on a 13-Å list cutoff distance.

Simulations were carried out on the wild-type and six mutants. The mutants were generated (Table I) through a series of cumulative, single-point mutations from the wild-type. The wild-type structure was taken from the Sybyl procedure, as described above. The loop region chosen was from the protein tyrosine phenol-lyase¹⁷ [Protein Data Base (PDB) code 1TPL] starting at His 317 of chain A, because this structure had the highest homology with our required sequence. The root-mean-square (RMS) difference of the fit was 0.86 Å, and the initial structure was half helical and half coil. Ala, which is helix-favoring, was used to substitute residues such as Gly and Asn, which are helix-disfavoring. By carrying out these mutations, we anticipated that the loop might become more helical and therefore more rigid. Simulations were run from two different initial structures: coil and helical. An alternative strategy would have been to devise mutations based on forming other rigid but nonhelical, structures, perhaps by the introduction of proline residues or more bulky sidechain groups. However, this approach has not been pursued in this work.

The flexibilities of the different sequences were assessed by several properties computed from the simulations. We examined RMS fluctuations of atomic positions and dihedral angles, comparing the difference between an initial structure and the structure at a given time. We followed structural changes by monitoring the evolution of the number of $i, i + 4$ α -helical hydrogen bonds over time. Such hydrogen bonds were identified based on an amide-nitrogen to carbonyl oxygen distance of 4 Å or less. The mobility of the protein backbones were also characterized with the generalized order parameter, a measure of the angular correlation for the dynamics of the N–H bond. The generalized order parameter was calculated from the final 500 ps of each trajectory as the plateau value of the autocorrelation function $\langle P_2[\mu(t) \cdot \mu(t + \tau)] \rangle$; where μ is a unit vector oriented along the N–H bond and $P_2(x)$ is the second Legendre polynomial.¹⁸ A value close to unity indicates little motion on the picosecond time scale; lower values indicate greater motion.

Most of the simulations were performed at 300 K. However, additional independent simulations of the wild-type and Mutant 6 were performed at 600 K and 800 K to probe further the kinetic stability of the two sequences. Such a strategy has been previously used to investigate helical fragments from myoglobin.¹⁹ One of the goals of our study is to work toward a simulation protocol that will permit the flexibility of the region 541–558 to be assessed rapidly and reliably for a large number of mutants. It may be that shorter simulations at higher temperatures will be useful in this regard.

RESULTS AND DISCUSSION

Helical Starting Structure

We begin by considering the 700-ps trajectories initiated from the helical structures. Figure 3 shows that the simulations of the wild-type and mutants all appear to converge to stable structures, typically 2–2.5 Å from their initial structures; the different simulations are not distinguished, because the point is simply that all the simulations are reasonably converged based on RMS deviation from the initial structure. To probe whether the designed mutants exhibit greater rigidity, we examined various trends in flexibility. The backbone atomic fluctuations computed from the 700-ps trajectories of the seven systems are in general accord with the anticipated result. Table II shows the largest backbone atomic RMS fluctuation for each mutant, which in each case occurs in the region 541–558 and gives a representative indication of its flexibility. The flexibility of the region 541–558 falls in the series: Wild type > Mutant 1 > Mutant 2 > Mutant 3 > Mutant 6 > Mutant 4 > Mutant 5.

The dihedral fluctuations for the mutants are greatest at residues 554, 556, and 558 (Fig. 4). The largest for the wild-type corresponds to the Ψ dihedral angle of residue 554, which has little conformational hindrance. Some of these data are shown in Table III. Mutants 2–6 have the Ala549Gly mutation, and the fluctuation of the Ψ dihedral angle at residue 548 is less than 10° in these sequences. The dihedral angles that seem most sensitive to mutation are those between residues ⁵⁵⁴Ala and ⁵⁵⁵Glu, with the fluctuation varying from 19° for Mutant 1 to 103° for wild-type. Interestingly, this is where the structure of the loop changes from helix to coil.

We assessed the helical structure of the 541–558 region by counting the number of hydrogen bonds (Table IV). Mutant 1 is the least helical, with the number of hydrogen bonds dropping from 17 to 13 over the course of the simulation. Unexpectedly, Mutants 4 and 5 appear to be more helical than Mutant 6. This may be due to the addition of the residue ⁵⁴³Ala or may reflect limited sampling. Making a series in order of decreasing helical structure in the region 541–558, based on the number of helical hydrogen bonds at 700 ps gives: Mutant 5 = Mutant 4 > Mutant 2 > Mutant 3 = Mutant 6 > wild-type > Mutant 1.

The generalized order parameters (Fig. 5) provide another measure of the flexibility of the region 541–558. The wild-type also shows some enhanced motion at residues 549–554, and the generalized order parameters suggest that most of the mutants are more rigid at these residues. However, the values of $\langle S^2 \rangle$ that are below 0.7 should be viewed with caution. In these cases, the angular autocorrelation function for the N–H vector did not converge to a well-defined plateau. Convergence was assessed based on the similarity of the values of the autocorrelation function at 200 ps, 300 ps, and 400 ps. Variations of greater than 0.1 indicated a lack of convergence, arising because the conformational motion of these residues was greater than that of the rigid residues and on a longer time scale. Adequate conformational sampling is a well-recognized issue in the estimation of generalized order parameters from simulations.^{18,20} Although longer simulations would be required to converge all the angular autocorrelation functions in the more flexible proteins, from the present simulations, it is nevertheless clear at a qualitative level that the mutants and wild-type show considerable motion at residues 555–558, which was also reflected in the fluctuations of the dihedral angles.

Coil Starting Structure

We performed simulations of the wild-type and the mutants, with the initial structure of the region 541–558 built as irregular coil (based on the structure of residues Glu107–chain B of Gmp Synthetase²¹ (PDB code: 1GPM) rather than helix. Here, our aim was to investigate

whether the mutants would adopt a helical structure in the region 541–558. We anticipated that the time scale of such a transition might well be significantly greater than the available simulation time, but perhaps the more helical mutants might exhibit some tendency toward helix formation. Indeed, Mutant 6 was observed, over the course of 700 ps, to adopt a partial helical conformation in the region of interest, which began to form in the region ⁵⁵²Gln–⁵⁵⁶Ala (Fig. 6). This is different than the most kinetically stable helical region, ⁵⁴²Ser–⁵⁴⁵Ala, identified in the simulation at 800 K, starting from the helical conformation.

Simulations of the other mutants and wild-type were also performed, with the initial irregular structure in the region 541–558. After 700 ps, Mutant 4 had formed two helical hydrogen bonds in the same location as Mutant 6. The other mutants showed no regular structure in this region; neither did the wild-type, even after we extended the simulation for an additional 400 ps to give a total of 1100 ps. This suggests that the alanine mutations enhanced the helicity of Mutant 6 in the region 541–558.

Simulations at Elevated Temperatures

After 100 ps of simulation at 600 K, the helical structure of the wild-type, which initially spanned residues 541–554, was disrupted at residues ⁵⁴¹Ile–⁵⁴²Ser and ⁵⁴⁹Gly–⁵⁵⁰Thr; these residues have low helical propensities. The loss of α -helical hydrogen bonding is shown in Figure 7 for this simulation and other high-temperature simulations. At 600 K, the wild-type structure was more mobile, as expected. The atomic fluctuations increased to over 4 Å. The baseline fluctuations in Ψ rose from about 10° at 298 K to about 25° at 600 K and peaked at ⁵⁴⁸Asn and ⁵⁵⁶Ala, with fluctuations in Ψ of 90° and 130°, respectively. From the time evolution of the RMS of the entire structure, it was clear that the system was still undergoing structural changes.

To accelerate the conformational transition further, we performed an independent simulation at 800 K. After 100 ps of simulation at 800 K, the wild-type helix was completely unstructured (Fig. 7), with atomic fluctuations rising to over 6 Å. The dihedral angles fluctuated on the order of 100°, both inside and outside the region 541–558, indicating widespread disruption of the structure.

The structure of Mutant 6 was more stable at high temperature than the wild-type. After 100 ps of simulation at 600 K, the helical structure of Mutant 6, which also ran from residues 541 to 554, was disrupted at ⁵⁴¹Ile and from ⁵⁵¹Glu–⁵⁵⁴Ala. The alanine residues at 549 and 550, which were mutations from the wild-type sequence, retained their helical structure, as intended by the design. However, both ⁵⁵³Ala and ⁵⁵⁴Ala in Mutant 6 underwent transitions from helix to coil. The atomic fluctuations increased to over 3 Å but were notably less than the wild-type fluctuations. The dihedral fluctuations were enhanced. Large fluctuations in Ψ at residues 553, 554, and 556 of 75°, 60°, and 100°, respectively, correlated with helix loss at these positions. In both wild-type and Mutant 6, the number of helical hydrogen bonds in the region 541–558 decreased during the 100-ps simulations at 600 K (Fig. 7); in the wild-type, the drop was from 17 to 15, and in Mutant 6, from 17 to 13.

Even after 100 ps of dynamics at 800 K, Mutant 6 retains some of its helical structure in the region 541–558, specifically, between residues ⁵⁴²Ser–⁵⁴⁵Ala. There are two mutations present at ⁵⁴³Ala and ⁵⁴⁵Ala, which seem to stabilize this region compared to wild-type. Nevertheless, the conditions were severe enough to induce unfolding in the rest of the region, including the three mutated positions: ⁵⁴⁸Ala, ⁵⁴⁹Ala, and ⁵⁵⁰Ala. At 800 K, in the course of 100 ps, the number helical hydrogen bonds of the wild-type in the region 541–558 fell to one (Fig. 7), whereas Mutant 6 showed a drop from 15 to 8. This is another indication that the mutant helix is more stable, at least kinetically, than the wild-type.

CONCLUSIONS

In this study, we have employed SBMD to investigate the flexibility of a region of PcrA helicase, the dynamics of which are implicated in the protein function. This approach permits rather more extensive sampling of the conformational space of the loop region than would be possible in a simulation in which all atoms were treated with Newtonian dynamics. The challenge of adequately sampling the vast conformational spaces of biomolecules is formidable despite enormous advances in computer hardware, and SBMD is a (perhaps underutilized) technique that focuses resources on the region of interest.

Since the early application of SBMD to study active-site dynamics,²² the technique has been widely employed. Nevertheless, our study is one of the first to use SBMD to evaluate a series of mutants, with the goal of introducing increased rigidity within the protein. We have pursued this aim through successive mutation of helix-disfavoring residues to alanine, in an approach that is perhaps reminiscent of the computational alanine scanning method proposed by Massova and Kollman,²³ albeit in a quite different context. Because there are potentially very many possible mutations, a rapid protocol is desirable. High temperatures accelerate conformational dynamics, and in the case of the folding of ubiquitin, at least, appear not influence the underlying potential energy surface.²⁴ Such an approach has been previously applied to probe the stability of helical peptides.¹⁹ Our results suggest that relatively short simulations that use SBMD at several elevated temperatures will be an efficient procedure for evaluating the flexibility of a large number of mutants. This strategy could be useful in a range of problems in protein engineering and protein design, and has been exemplified in this article to study the domain swiveling of the PcrA helicase.

Domain swiveling that involves a rigid-body rotation of domain 2B in PcrA is the main conformational change associated with the activity of this helicase. Such a movement enables the enzyme to engage actively duplex DNA ahead of the ss-dsDNA junction and actively melt the duplex near the junction, in response to ATP binding/hydrolysis.⁶ This domain swiveling seems to be a characteristic of other homologous helicases, such as the *Escherichia coli* Rep helicase.²⁵ However, in the case of Rep helicase, the equivalent domain 2B was reported to be redundant for duplex unwinding,²⁶ in distinct contrast to PcrA helicase, in which domain 2B is important in this regard.⁶ In the latter protein, the 2B domain swiveling is mediated by a flexible loop between I⁵⁴¹ and A⁵⁵⁸. It is unexpected, albeit not exclusive, for such highly homologous proteins, such as PcrA and Rep helicases sharing 41% identity,⁷ to function by entirely different mechanisms. Therefore, the still unresolved issue of the functional significance of this domain swiveling in these helicases needs further investigation. A possible approach will be to create mutations in the flexible loop that mediate this rigid-body domain rotation and study the effects of these mutations on the helicase activity of PcrA. Our simulations offer a selective tool for identifying the most appropriate residues within this loop for site-directed mutagenesis.

The mutant that appears most clearly to be more helical and more rigid than the wild-type is Mutant 6. Evidence supporting this includes a variety of measures of flexibility computed for the simulation at 300 K, as well as the high-temperature simulations. For the other mutants, the difference in flexibility relative to the wild-type is less clear, and they seem to be less promising as candidates to take forward into experiments.

In the future, we intend to study the loop in the substrate and product forms, because it is known that the structure of the loop varies among the three. Different regions of the loop are “seen” in the crystallographic structures of each form. The introduction of DNA complicates the simulation, not least because DNA is highly charged and the electrostatics must be

treated with particular care. However, there are a number of examples of DNA-protein simulations,²⁷ and this is certainly an area of keen interest.

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Fig. 1. (Left) The native structure of PcrA helicase, with the subdomains 1A, 1B, 2A, and 2B colored green, yellow, red, and blue, respectively. (Right) AMPPNP-bound structure with DNA (ds-ss) also bound, clearly showing the rigid-body rotation of domain 2B. (Inset) The region and water molecules treated explicitly in the dynamics simulations.

Fig. 2.
Different regions in a stochastic boundary molecular dynamics simulation.

Fig. 3.
Root-mean-square deviation from initial helical structures for the wild-type and six mutants.

Fig. 4.
Root-mean-square deviation of the backbone dihedral angles for the wild-type and six mutants.

Fig. 5.
Generalized order parameters for the wild-type and six mutants.

Fig. 6. Time evolution of the main-chain hydrogen bond lengths of Mutant 6, color-coded by the carbonyl oxygen to amide nitrogen distance (Å).

Fig. 7.
Effect of high-temperature simulation on the number of α -helical hydrogen bonds in the region 541–558.

TABLE ISequence of the Loop Region of the Wild-Type and Mutants Considered in This Study^a

Mutant	Sequence
Wild type	⁵⁴¹ Ile-Ser-Asp-Leu-Asp-Glu-Leu-Asn-Gly-Thr-Glu-Gln-Ala-Ala-Glu-Gly-Asp-Ala ⁵⁵⁸
Mutant 1	⁵⁴¹ Ile-Ser-Asp-Leu-Asp-Glu-Leu-Asn-Gly-Thr-Glu-Gln-Ala-Ala-Glu- Ala -Asp-Ala ⁵⁵⁸
Mutant 2	⁵⁴¹ Ile-Ser-Asp-Leu-Asp-Glu-Leu-Asn- Ala -Thr-Glu-Gln-Ala-Ala-Glu- Ala -Asp-Ala ⁵⁵⁸
Mutant 3	⁵⁴¹ Ile-Ser-Asp-Leu-Asp-Glu-Leu-Asn- Ala-Ala -Glu-Gln-Ala-Ala-Glu- Ala -Asp-Ala ⁵⁵⁸
Mutant 4	⁵⁴¹ Ile-Ser-Asp-Leu-Asp-Glu-Leu- Ala-Ala-Ala -Glu-Gln-Ala-Ala-Glu- Ala -Asp-Ala ⁵⁵⁸
Mutant 5	⁵⁴¹ Ile-Ser-Asp-Leu- Ala -Glu-Leu- Ala-Ala-Ala -Glu-Gln-Ala-Ala-Glu- Ala -Asp-Ala ⁵⁵⁸
Mutant 6	⁵⁴¹ Ile-Ser- Ala -Leu- Ala -Glu-Leu- Ala-Ala-Ala -Glu-Gln-Ala-Ala-Glu- Ala -Asp-Ala ⁵⁵⁸

^aResidues that were mutated are indicated in bold, underscored text.

TABLE II

Maximum Backbone Atomic Fluctuations from Helical Starting Structures

Sequence	Maximum RMS backbone atomic fluctuation (Å)
Wild-type	2.96
Mutant 1	2.11
Mutant 2	1.98
Mutant 3	1.84
Mutant 4	1.40
Mutant 5	1.21
Mutant 6	1.48

TABLE III

Fluctuations of Dihedral Angles from Helical Starting Structures

Sequence	RMS fluctuation of $\psi(^{\circ})$		
	Residue 548	Residue 554	Residue 556
Wild-type	11	103	26
Mutant 1	34	19	23
Mutant 2	8	84	19
Mutant 3	9	47	23
Mutant 4	9	24	24
Mutant 5	10	24	27
Mutant 6	10	48	21

TABLE IV

Change in Helical Structure

Sequence	Number of helical hydrogen bonds	
	At 0 ps	After 700 ps
Wild Type	17	14
Mutant 1	17	13
Mutant 2	17	16
Mutant 3	17	15
Mutant 4	16	17
Mutant 5	17	17
Mutant 6	17	15