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Hot-spot residues at the E9/Im9 interface help binding via different mechanisms

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Abstract

Protein-protein association involves many interface interactions, but they do not contribute equally. Ala scanning experiments reveal that only a few mutations significantly lower binding affinity. These key residues, which appear to drive protein-protein association, are called hot-spot residues. Molecular dynamics simulations of the Colicin E9/Im9 complex show Im9 Glu41 and Im9 Ser50, both hot-spots, bind via different mechanisms. The results suggest Im9 Ser50 restricts Glu41 in a conformation auspicious for salt-bridge formation across the interface. This type of model may be helpful in engineering hot-spot clusters at protein-protein interfaces and, consequently, the design of specificity.

Keywords

molecular dynamics; protein-protein association; salt bridge

Introduction

Signal transduction¹, cell cycle progression^{2,3}, transcription⁴ and translation³, and nuclear transport⁵, among other essential biological processes, make use of transient protein complexes. Given that the human genome codes for approximately 25,000 proteins⁶, achieving the appropriate affinity and specificity in protein-protein interactions (PPI) is a colossally difficult, and yet necessary, biological feat. Consequently, molecular recognition and specificity in PPI's is an area of intense research because: 1) of the fundamental biological significance and 2) they hold promise as therapeutic drug targets⁷⁻¹⁰ e.g. in cancer therapy^{8,10,11}.

While the protein-protein interface consists of many residues, only a few make considerable contributions to the binding free energy. Clackson and Wells¹² made alanine mutants at the human growth hormone receptor/growth hormone interface; only a few mutations

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significantly lowered the binding affinity. These few critical residues are known as hot-spots and are a widespread phenomenon^{13,14}.

Exactly what makes hot-spot residues different is unclear. As a group, they are slightly enriched in aromatic side chains^{14,15}; however, they are not exclusively hydrophobic¹⁶. Keskin et al¹⁷ found a tighter packing around hot-spot residues in the bound state, but how that is achieved is unclear. Hot-spot residues can form clusters that bind in a cooperative manner^{18,19}, i.e. interactions of a given hot-spot can be enhanced by its neighboring residues. This effect may help to explain what sets hot-spot residues apart from other interface residues.

In our molecular dynamics (MD) simulations of the E9/Im9 complex, we find an example of this type of intra-molecular contact that enhances contacts across the protein-protein interface. This system, Colicin Dnase E9 and its immunity protein Im9, is the subject of many structural, kinetic and mutagenesis studies^{20–24}. In the present work, molecular dynamics (MD) simulations of the wild-type and a mutant (Im9 Ser50Ala) complex suggest Im9 Glu41 and Im9 Ser50 bind via different mechanisms. Glu41 makes direct salt bridge interactions across the interface; while Ser50 has an indirect effect. It, via a hydrogen bond, pulls the carboxylic group of Glu41 into a better position for salt bridge formation. The data supports this hypothesis which can be studied further in future work.

Methods

Molecular dynamics simulations

Three sets of MD simulations were performed using the Amber 925 program suite: two independent runs (i.e. initialized by different velocities, see below) of the wild-type Im9/E9 Dnase complex and a third simulation of the same complex with an Im9 Ser50Ala mutation. Coordinates of the Im9/E9 Dnase complex were obtained from the PDB26 (pdb code: 1BXI²⁷). All of the 104 crystallographic waters were left in place but nickel and phosphate ions were removed. No crystallographic structure of the complex with a Ser50Ala mutation in its Im9 monomer has been reported to date. Therefore, A model was generated by: 1) removing atoms beyond the C_β atom of Ser50 and 2) renaming the residue Ala. The latter species will be referred in the text as the Ser50Ala mutant complex.

Topology and coordinate files were generated using Tleap²⁵ and employing the parm99SB force field^{28–30}. The protein complexes were solvated in a pre-equilibrated box of TIP3P³¹ water (10 Å minimum solute-wall distance). Solvent molecules that overlapped with the protein, based on their van der Waals radii, were removed. Four Na⁺ ions, randomly placed using the “addions2” command, neutralized each system. For the wild-type and Ser50Ala, simulation boxes included 10397 or 10368 water molecules, respectively.

The three systems followed the same minimization, heating and equilibration protocol. Three successive steps of minimization were taken. All three of the minimization stages involved a 500 step, steepest-descent minimization with an 8 Å cut-off. A harmonic restraint (force constant of 0.5 kcal/mol/Å²) on all protein atoms during the first stage, C_α atoms during the second stage and absent in the final stage, was the only difference between the three consecutive minimizations. Crystallographic coordinates served as reference coordinates for the harmonic restraints.

The equilibration stage involved a similar scheme. During the first stage, a harmonic restraint (force constant of 0.5 kcal/mol/Å²) was applied on protein all atoms. During this stage, the system was heated from 0.1 to 300 K over 3·10⁴ time-steps and were followed by 2·10⁴ steps at 300 K (NVT). The second stage involved 50,000 time steps of NVT

simulation (300K) with a harmonic restraint on all atoms but using a weaker force constant (0.25 kcal/mol). The third stage involved 50,000 steps of NVT simulation at 300 K with no harmonic restraints. A fourth stage involved another 50,000 time steps of NPT simulation at 1 bar and 300 K. The production stage followed. A 1 fs time step was used throughout. A weak-coupling algorithm³² maintained temperature and pressure close to their reference values of 300 K and 1 bar, using 0.1 and 0.5 ps coupling constants, respectively. Periodic boundary conditions were in effect during all simulations and electrostatic interactions were evaluated using the particle mesh Ewald³³ (PME) approximation (8 Å cut-off for the direct space sum). The SHAKE³⁴ algorithm kept bonds involving hydrogens fixed.

The two wild-type simulations differed only in the seed used to assign initial velocities (labeled A and B). The production runs consisted of NPT simulations at 300 K and 1 bar. The two wild-type MD runs and the S50A mutant complex run were extended to 50 and 30 ns, respectively. Trajectory coordinates were saved every 5 ps for analysis and free-energy calculations.

Salt bridge analysis

A salt bridge was considered “formed” if the carboxylic oxygen – amino nitrogen distance fell below 3.5 Å. This threshold emerged from observation of the distance probability density for these species. Since there are 2 carboxylic oxygens, the shortest distance was used.

Fitting procedure

Structural fitting was performed by (i) superimposing molecular centers of mass (to remove overall translation) and (ii) performing an atom-positional least-square fitting procedure (to remove overall rotation) using all C $_{\alpha}$ atoms³⁵, but excluding the first and last five residues of each peptide chain unless otherwise specified.

Results

MD stability

The total energy histories for the wild-type complex simulations A and B and the Ser50Ala mutant are stable (Figure 1). The C $_{\alpha}$ Cartesian RMSD remains below 2 Å for wild-type simulations A, B and below 2.5 Å for the Ser50Ala mutant trajectory (Figure 2). Further analysis of the Im9/E9 complex simulation was reported elsewhere³⁶.

Ser50 holds Glu41 in place to form salt-bridge across the interface

During the wild-type MD simulation, Im9 Glu41 forms two salt bridges across the binding interface and an intra-molecular hydrogen bond to Im9 Ser50. The salt bridges involve E9 Lys97 and E9 Lys89 (Figure 3). This observation motivated the hypothesis that Im9 Ser50 helps position Im9 Glu41 for salt bridge formation.

A simulation of the complex mutant, Ser50Ala, is consistent with this hypothesis. In the absence of Im9 Ser50 (Ser50Ala mutant), Glu41 is less likely to form salt bridges with E9 Lys 97 (wild-type: 60 % or 87 %, Ser50Ala: 16.4 %) or E9 Lys 89 (wild-type: 56% or 76%, Ser50Ala: 24%). The Glu41 O^E – Lys89 N^Z distance distribution has a peak below 3.5 Å that is at least doubled in the presence of Im9 Ser50 (Figure 4). The same is true in the corresponding analysis with Lys97 (Figure 5).

Discrepancies between the two wild-type simulations are due to differences in sampling. Simulation B explores a conformations of a flexible loop, residues 50-65, absent from simulation's A trajectory³⁶. This underlines the advantage of multiple replicas when

analyzing MD results. Despite these differences, the two simulations qualitatively agree in forming the salt-bridges in question more frequently than the S50A mutant.

Analysis of Glu41 side-chain dihedral angle distributions did not show a uniform shift due to Ser50 (data not shown). Nonetheless, the carboxylic group at the end of the Glu41 side-chain contacts its salt bridge partners. So, this result may reflect coupling to: 1) backbone conformational changes, 2) the salt bridge partner's conformation, 3) fluctuations in the relative orientation of the protein bodies, or a combination of all of these.

It is noteworthy that E9 Lys97 also seems restrained by a hydrogen bond to Gln92, which also makes a hydrogen bond with the carbonyl oxygen of E9 Gly94. Other interactions with the E9 backbone (not shown) also seem to restrict the mobility of E9 Lys97. So, there may be similar interactions on the other side of the interface. Unfortunately, contributions of hot-spot residues at the E9 Dnase surface are not experimentally characterized.

Discussion

Ser50 and Glu41 are both “hot-spot” residues, but appear to contribute to binding via different mechanisms. The results show Im9 Ser50 increases the probability of Glu41 salt bridges across the Im9/E9 interface. Ser50 appears to contribute by stabilizing the salt-bridge interactions of Glu41 while Glu41 is, of course, necessary to form these key salt bridges.

Mutation of either residue may disrupt the interaction network of Figure 1. Experimental alanine mutation of Im9 Glu41 or Ser50 lowers the binding affinity to E9 by nearly the same amount (2.08 +/- 0.02 vs. 2.19 +/- 0.07 kcal/mol)²⁴. So, it is possible that when either residue is mutated, a similar decrease in binding energy is observed because of the weakening of the inter-protein salt bridge, either directly (Glu41Ala) or by loss of the conformational support (Ser50Ala).

This type of intra-molecular interaction, which aids binding, was postulated in other systems. Sohn and Rudolph¹⁹ performed double and triple mutant cycle experiments to study the energetics of a hot-spot cluster at the interface of Cdc25B phosphatase and Cdk2-pTpY/CycA. They proposed that Tyr497 restricts the conformation of Arg488 to form a favorable salt bridge across the interface. Another example is cooperativity within hot-spot clusters at the TEM1-BLIP interface. Albeck et al³⁷ concluded that the favorable binding contribution of a hot-spot lysine (TEM Lys234) stems nearly entirely from cooperative effects with a neighboring arginine residue (TEM Arg243).

The significance of this work is that it provides a hypothesis for how these intra-molecular interactions help binding. This hypothesis can be further studied in future work.

Conclusion

The data suggests Im9 Glu41 and Im9 Ser50, both hot-spot residues, contribute to binding via different mechanisms. A hydrogen bond between Im9 Glu41 and Im9 Ser50 appears better position Glu41 for salt bridge formation. Insight from these observations may be useful in the design of hot-spot clusters at PPI's, identification of hot-spot residues at protein surfaces and understanding the mutations via evolution at PPI's. They may also guide drug-design efforts aimed at hot-spot residues.

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Figure 1.
Total energy time series for simulations Wildtype A, Wildtype B and Ser50Ala.

Figure 2.
C α RMSD of simulations Wildtype A and B and the Ser50Ala mutant.

Figure 3.
Hydrogen-bond network involving hot-spots Im9 Glu41 and Im9 Ser50.

Figure 4. Normalized probability density of the Oxygen-Nitrogen distance for the salt bridge between Im9 Glu41 and E9 Lys89.

Figure 5. Normalized probability density of the Oxygen-Nitrogen distance for the salt bridge between Im9 Glu41 and E9 Lys97.