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Design of ribonuclease T1 mutants with tryptophan-59 in noncrystallographic conformations

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ABSTRACT

Minimum perturbation mapping reveals that five side chain conformations of tryptophan-59 can potentially pack in the hydrophobic core of ribonuclease T1. In the crystallographic structure tryptophan-59 is in the *trans* perpendicular conformation. The other four wells in the tryptophan-59 $\chi^1 \times \chi^2$ torsion space minimum perturbation mapping are *trans* antiperpendicular, *gauche*$^+$ antiperpendicular, *gauche*$^+$ perpendicular, and *gauche*$^-$ perpendicular. The point mutations V33I and V33L are predicted to stabilize the *trans* antiperpendicular over the perpendicular conformation by 1.3 and 0.6 kcal/mol, respectively. The three *gauche* conformations require the creation of a new pocket within the protein core. The Mutation F80G creates the pocket for the *gauche*$^+$ antiperpendicular tryptophan-59 conformation. The additional mutation V78A makes a more generous pocket for this conformation. The three mutations A19G, V78G and F80L create the pocket for the *gauche*$^+$ perpendicular conformation. A slightly more generous mutation at residue 80 may be required to fully stabilize this conformation. It does not appear possible to create a pocket that fits the *gauche*$^-$ perpendicular conformation better than the other two *gauche* conformations. The expression of ribonuclease-T1 mutants with tryptophan-59 in a *gauche* conformation probably requires filling in the crystallographic tryptophan-59 pocket. The mutations A22F, V33F, or V67F are all promising possibilities for this purpose.

1 DISCUSSION

Figure 1 shows the minimum perturbation mapping of the two *trans* and three *gauche* side chain conformations of ribonuclease T1 tryptophan-59.[1] The residues glutamine-20, tyrosine-24, lysine-25, leucine-26, valine-33, tyrosine-38, tyrosine-57, glutamate-58, valine-67, serine-69 and valine-79 are truncated at the C^β atom and residues alanine-19, valine-78 and phenylalanine-80 are truncated at the C^α atom. All atoms of these residues and threonine-18, alanine 21 and 22, glycine-23, proline-39, tryptophan-59, proline-60 and tyrosine-68 as well as interconnecting backbone carbonyl and amide groups are free. These simulations employed the CHARMM version 19 polar hydrogen parameters with charges on ionized side chains reduced by 80%.[2] Additional minimum perturbation mappings over tryptophan-59 $\chi^1 \times \chi^2$ torsion space with valine, isoleucine and leucine at position 33 indicate that the mutations V33I and V33L stabilize the *trans* antiperpendicular over the perpendicular conformation of tryptophan-59 by 1.3 and 0.6 kcal/mol, respectively. These mappings assumed that the valine and leucine at position 33 had their side chain χ^1 dihedral angles in the *gauche*$^-$ conformation and isoleucine had its in the *gauche*$^+$ conformation. The leucine χ^2 dihedral angle was in the *trans* conformation. This places valine in its crystallographic conformation and approximately superimposes the valine and isoleucine C^γ atoms.[3] The

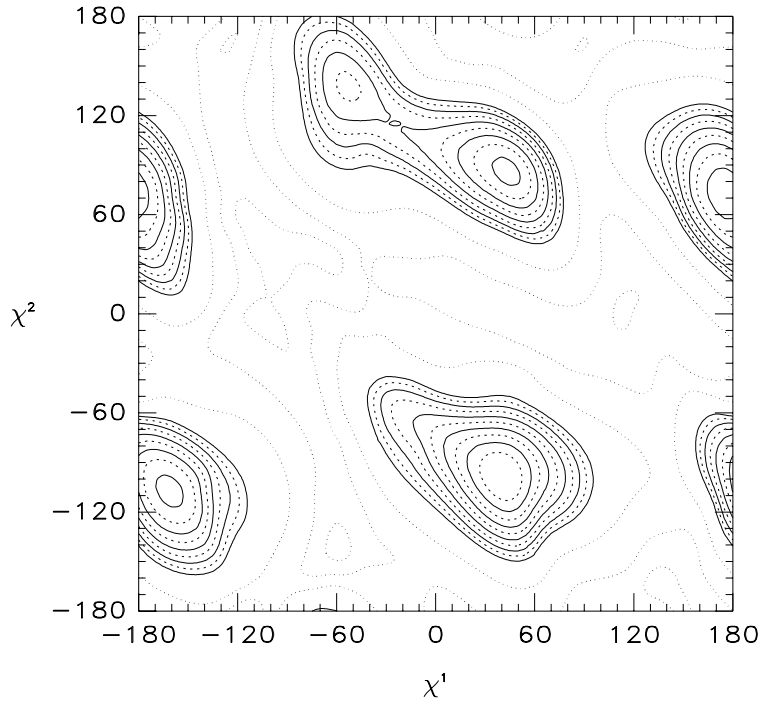

Figure 1: Minimum perturbation mapping of the five possible side chain conformations of ribonuclease T1 tryptophan-59. Contour levels are *dashed*, 1, 3, 5, 7, 9; *solid*, 2, 4, 6, 8, 10; *dotted*, 15.8, 25.1, 39.8, kcal/mol, where zero corresponds to −629.6 kcal/mol.

signs of the valine and isoleucine dihedral angles are opposite because the standard nomenclature interchanges the numbering of the C^{γ} atoms.[4] Unlike the *trans* conformations, the *gauche*$^+$ perpendicular and antiperpendicular conformations of tryptophan-59 simply flip the pyrrole ring over and project the benzol ring outwards in the opposite directions. The mutations suggested in the above abstract are thus highly effective in permitting only the *gauche*$^+$ perpendicular and antiperpendicular conformations. Minimum perturbation mapping of these mutations confirms they destabilize the unwanted conformations by many kcal/mol.

2 REFERENCES

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