Impact of Insertion and Deletion Mutations on Protein Thermodynamics

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Introduction
Insertion and deletion mutations are common events in protein evolution but occur much less frequently than point mutations. Due to this, the effects of inserting and deleting amino acids on the biophysical properties of proteins are much less studied and understood. Here, we use a coarse-grained protein model to assess the effects of these mutations on protein stability.

Coarse-Grained Model
This study used a coarse-grained model [1,4] for protein folding with 7 atoms per amino acid and a reduced alphabet of 3 amino acids: polar (p), hydrophobic (h), and glycine (g). The energy function is given by:

$$E(C) = E_{\text{local}} + E_{\text{inter}} + E_{\text{exvol}}$$

The stable native state for this model occurs at the minimum energy state. Model degrees of freedom are dihedral angles $\phi$ and $\psi$, while other angles and bond lengths are held fixed.

Computational Methods
To study the thermodynamic behaviour, simulated-tempering Monte Carlo (STMC) methods were used. Simulation parameters were as follows:

- 10^4 steps per simulation
- 8 model temperatures from $k_B T = 0.47$ to $k_B T = 0.70$
- MC updating change either $T$ or chain geometry by rotating $\phi$ or $\psi$

Stability of the Native State
A thermodynamic observable for stability $S$ was defined. $S$ can be considered the population of the native state and takes a value between 0 and 1. $S > 0.5$ can then be considered a change in stability between two sequences.

Stability changes resulting from insertion mutations to sequence $\text{II}_{\text{wp}}$

Criterion for the Native State
To be considered for a native fold, each protein must exhibit:

- 75% of residues exhibit appropriate native state configuration
- 4 or more inter-strand hydrogen bonds in the $\beta$-hairpin
- 5 or more hydrophobic contacts between $\alpha$-helix and $\beta$-hairpin

How do Indels Impact Stability?
From the initial sequence $\text{II}_{\text{wp}}$, 73 unique insertions and 25 unique deletions were found. Insertions were obtained by inserting each of the amino acids (p, h, and g) into each position in the sequence. Deletions were obtained by deleting each residue. Each sequence was simulated using STMC methods to find the stability of each sequence.

Sequences $\text{II}_{\text{wp}}$

This sequence was obtained using basic protein design principles:

- Glycine in turn regions
- $p$ and $h$ patterns such that the helix and sheet regions are amphipathic

$\text{II}_{\text{wp}}$ was designed to fold spontaneously into a protein exhibiting both an $\alpha$-helix and $\beta$-hairpin structural elements. The initial sequence used was: pphpphphpphpghpggphgpghphp

As seen in the melting curve for the protein, the stability $S$ of the native state decreases. For this reason, all $S > 0.5$ measures between sequence $\text{II}_{\text{wp}}$ and its mutants were made at $k_B T = 0.47$.

Future Directions
These results can serve as a baseline for research in the following directions:

- Isolation of the two secondary structure elements to assess the impact of indels in isolation in comparison to a protein with tertiary structure
- Exploring if and how indels can aid proteins in switching between stable folds in protein evolution
- Exploring similar proteins using more detailed all-atom protein models

Conclusions
From these results, the following observations can be made:

- Insertions are generally more tolerated than deletions
- Inserting glycine is often more deleterious than $p$ or $h$ due to its helix-breaking tendencies
- Indels are better tolerated in loop and terminal regions than in a helix or sheet
- Some hydrophobic insertions can help stabilize protein structure
- Indels (other than glycine insertions) in the $\alpha$-helix are less destructive than in the $\beta$-sheet

An important sequence pattern of “pphpp” was identified to be important in helix formation. Indels which disrupted this pattern were less stable. Hydrophobic insertions extending this pattern were found to promote stability.

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References

Further information
For further information, please visit: http://www.physics.mun.ca/~stefan/