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Original Research

In Silico Analysis of Non-synonymous Mutations in the *durA* Gene and Their Effects on the Stability and Physicochemical Properties of Duramycin

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Abstract

Duramycin is a lantibiotic peptide encoded by the *durA* gene, known for its antimicrobial activity against Gram-positive bacteria. This study aimed to evaluate the effects of single-point mutations on the stability and structural integrity of duramycin using a comprehensive in silico approach. Five variants—C1S, F7A, T11Y, D15E, and K19V—were designed and assessed using I-Mutant2.0 to predict their impact on protein stability. ProtParam analysis was conducted to determine molecular weight, isoelectric point (pI), net charge at pH 7, instability index, aliphatic index, and hydropathicity (GRAVY). In addition, PEP-FOLD3 was employed to model the 3D conformations of each mutant peptide. Results showed that K19V improved peptide stability and increased aliphatic index and GRAVY score, indicating enhanced hydrophobicity and potential thermal stability. In contrast, F7A led to a major structural shift marked by an α-helical conformation and reduced stability. C1S and T11Y induced minor destabilizing effects, while D15E offered a moderately stabilizing substitution with minimal structural deviation. Overall, this study highlights the functional relevance of C-terminal and hydrophobic residues in maintaining duramycin's structural compactness and provides a framework for future design of optimized antimicrobial peptides through rational mutation.

Introduction

Duramycin is a 19-amino-acid lantibiotic peptide produced by *Streptomyces cinnamoneus*, known for its potent antimicrobial activity and specificity toward phosphatidylethanolamine (PE) in bacterial membranes (Chatterjee et al., 2005; Huo et al., 2012). As a member of the ribosomally synthesized and post-translationally modified peptides (RiPPs), duramycin exhibits unique structural features, including multiple lanthionine bridges, a hydroxylated aspartic acid residue, and a lysinoalanine (Lal) crosslink. These modifications are essential for its conformational stability and biological function (You & van der Donk, 2007).

The core structure of duramycin is encoded by the *durA* gene, which directs the synthesis of a precursor peptide composed of a leader region and a core region. Post-translational modifications are carried out by enzymes such as DurM (dehydratase/cyclase), DurN (lysinoalanine-forming enzyme), and DurX (Fe(II)/2-oxoglutarate-dependent hydroxylase) (Li et al., 2010; Ortega et al., 2015).

Mutations in the *durA* gene, particularly within the core region, can affect the processing efficiency of these enzymes consequently alter and the final bioactivity of the peptide (Ge et al., 2019).

Peptide engineering through targeted non-synonymous mutations has emerged as a promising strategy for optimize antimicrobial peptides (AMPs) to improved activity, stability, solubility, or spectrum (Wang et al., 2016). In silico tools provide an efficient and cost-effective platform for predict the effects of such mutations on peptide properties prior to experimental validation. Computational approaches, including protein stability prediction, physicochemical analysis, and AMP classification, can help identify candidate mutations that enhance duramycin's properties without compromising its core functionality (Capecchi et al., 2021).

This research aims to analyze selected non-synonymous point mutations within the *durA* gene using a combination of in silico tools. The objective is to evaluate their effects on the stability and physicochemical characteristics of the resulting duramycin peptide, and to provide a theoretical basis for further peptide engineering and functional optimization.

Method

2.1 Peptide Sequence Retrieval and Mutation Design

The amino acid sequence of mature duramycin, consisting of 19 residues, was obtained from the UniProt database (UniProt ID: P36504) and supported by literature describing the biosynthesis of lantibiotics (Huo et al., 2012; Ortega et al., 2015). The selected sequence—CKQSCSFGPFTFVCDGNTK—represents the post-translationally modified product encoded by the *durA* gene in *Streptomyces cinnamoneus*. Based on functional residue importance reported in previous studies, five non-synonymous single-point mutations were rationally selected: C1S, F7A, T11Y, D15E, and K19V (Table 3.1). These were designed to explore structural flexibility, lanthionine bridge disruption, charge alteration, and effects on potential post-translational modification sites (You & van der Donk, 2007; Ge et al., 2019).

2.2 Stability Prediction Using I-Mutant2.0

To predict the impact of mutations on peptide stability, we used the I-Mutant2.0 webserver (https://folding.biofold.org/i-mutant/i-mutant2.0.html), a support vector machine–based tool that estimates changes in protein stability (ΔΔG) upon point mutation (Capriotti et al., 2005). The analysis was performed in sequence-based mode using the wild-type peptide sequence as input. Each mutation was tested under default parameters (pH 7.0, temperature 25°C). The output included the direction of predicted stability change (increase or decrease), the numerical ΔΔG value (kcal/mol), and a Reliability Index (RI) ranging from 0 to 10. Mutations with RI ≥ 6 were considered sufficiently confident for downstream analysis.

2.3 Physicochemical Property Analysis

The physicochemical characteristics of wild-type and mutant peptides were evaluated using the ProtParam tool provided by ExPASy (https://web.expasy.org/protparam/) (Gasteiger et al., 2005). For each peptide, we calculated the molecular weight, theoretical isoelectric point (pI), net charge at pH 7, instability index, aliphatic index, and GRAVY (grand average of hydropathicity) value. These properties are important for evaluating peptide solubility, potential bioavailability, and general structural behavior in aqueous environments (Wang et al., 2016).

2.4 Structural Modelling with PEP-FOLD3

Three-dimensional structural models of both wild-type and mutant duramycin peptides were generated using PEP-FOLD3 (http://bioserv.rpbs.univ-paris-diderot.fr/services/PEP-FOLD3), a tool for de novo structure prediction of short linear peptides (Lamiable et al., 2016). Each sequence was submitted individually, and the best-ranked model based on the sOPEP scoring function was selected

for qualitative assessment of structural integrity and residue-level conformation. The resulting PDB files were visualized using molecular modelling software for comparative inspection.

Results and Discussion

3.1. Stability Prediction Using I-Mutant2.0

To evaluate the impact of specific amino acid substitutions on duramycin's structural stability, five single-point mutations were introduced into the 19-residue mature peptide sequence derived from the *durA* gene (**Table 3.1**). These mutations—C1S, F7A, T11Y, D15E, and K19V—were selected based on their potential to interfere with key functional domains such as lanthionine bridge-forming cysteines, polar residues, and charged termini. Stability predictions were performed using the I-Mutant2.0 server (https://folding.biofold.org/i-mutant/i-mutant2.0.html), a machine learning–based predictor that estimates the change in free energy of folding (ΔΔG) as a result of point mutations (Capriotti et al., 2005). Each mutation was assessed at physiological pH 7.0 and temperature 25 °C, and results were reported in terms of predicted stability effect and reliability index (RI), which reflects model confidence on a scale from 0 to 10.

The detailed results are presented in Table 1. Among the tested mutations, F7A (phenylalanine to alanine) and K19V (lysine to valine) displayed high reliability indices (RI = 9 and 8, respectively). The F7A mutation resulted in a decrease in predicted stability, likely due to the loss of hydrophobic and aromatic interactions conferred by the phenylalanine side chain. Phenylalanine residues are often important for core packing in short peptides, and their replacement with smaller residues such as alanine may destabilize the structure (Zhou et al., 2020). In contrast, the K19V mutation was predicted to increase peptide stability, supported by a high RI of 8. The removal of the positively charged lysine residue and substitution with a hydrophobic valine may reduce electrostatic repulsion and improve hydrophobic packing at the peptide's C-terminal region.

Conversely, the C1S mutation (cysteine to serine at position 1), which targets a key lanthionine-bridging residue, was predicted to significantly reduce structural stability (RI = 2). Cysteine residues play a crucial role in forming thioether bridges that stabilize lantibiotic scaffolds, and their substitution with polar residues such as serine has been shown to disrupt bioactivity and folding (van Heel et al., 2016). Similarly, T11Y (threonine to tyrosine) exhibited a predicted decrease in stability with an RI of 2. Although both residues are polar, tyrosine introduces a bulky aromatic ring, which may cause steric hindrance or misfolding in tightly packed regions. An unexpected result was observed for the D15E mutation, where substitution of aspartic acid with glutamic acid at a hydroxylation site led to a predicted increase in stability (RI = 3). While the RI is low, the outcome suggests that elongation of the side chain does not necessarily destabilize the local environment. This mutation may warrant further investigation, particularly because Asp15 is a known post-translational modification target during duramycin maturation (Ortega et al., 2015). Overall, the analysis identified K19V as the most promising stabilizing mutation, while F7A represents a highly reliable destabilizing mutation. These findings provide a computational foundation for further in silico modelling and functional screening of duramycin variants.

Table 3.1. Predicted Effects of Single-Point Mutations on Duramycin Stability Using I-Mutant2.0

Mutation Code	Position	Mutated Sequence	Predicted Stability Effect	Reliability Index (RI)
C1S	1	SKQSCSFGPFTFVCDGNTK	Decrease	2
F7A	7	CKQSCSAGPFTFVCDGNTK	Decrease	9
T11Y	11	CKQSCSFGPFYFVCDGNTK	Decrease	2
D15E	15	CKQSCSFGPFTFVCEGNTK	Increase	3
K19V	19	CKQSCSFGPFTFVCDGNTV	Increase	8

3.2. Physicochemical Property Analysis

To assess how single-point mutations affect the biochemical characteristics of duramycin, the wild-type and five mutant peptide sequences (C1S, F7A, T11Y, D15E, and K19V) were analyzed using the ExPASy ProtParam tool (Gasteiger et al., 2005). Parameters evaluated included molecular weight, theoretical isoelectric point (pI), net charge at pH 7.0, instability index, aliphatic index, and the grand average of hydropathicity (GRAVY). The results are summarized in **Table 3.2.** The molecular weight (MW) of the wild-type duramycin was calculated to be 2069.35 Da, which is consistent with its 19-residue composition, including multiple cysteines involved in thioether crosslinking. Mutations such as F7A (1993.25 Da) and K19V (2040.31 Da) caused a reduction in molecular weight due to replacement of bulkier amino acids with smaller ones (phenylalanine to alanine, lysine to valine, respectively). In contrast, T11Y increased the molecular weight to 2131.42 Da, consistent with the substitution of threonine by the larger aromatic tyrosine residue.

The isoelectric point (pI) of the wild-type peptide was 7.95, reflecting the presence of a single positively charged lysine and a single negatively charged aspartic acid. Most of the mutants retained similar pI values (7.78–7.95), except for K19V, which significantly lowered the pI to 5.82 due to the loss of the only basic residue (lysine), thereby shifting the overall charge balance. Consistent with this, the net charge at pH 7 for the wild-type and all mutants except K19V was calculated as +1, whereas K19V showed a net charge of 0, indicating a neutral molecule at physiological pH. This shift may impact membrane interactions and antimicrobial activity, as many antimicrobial peptides (AMPs) rely on positive charge to target negatively charged bacterial membranes (Mahlapuu et al., 2016; Wang & Wang, 2022).

The instability index was used to estimate peptide stability in vitro. Values above 40 indicate potential instability. The wild-type duramycin had an index of 80.05, suggesting it may be unstable in solution without post-translational modifications. Interestingly, most mutants retained a similar instability index, but T11Y showed a notable increase to 90.72, likely due to the bulky tyrosine disrupting local structure. Conversely, D15E showed the lowest instability index (69.92), indicating a relative stabilization effect, which may be due to the conservative nature of substituting one acidic residue with another (Asp → Glu).

The aliphatic index, which reflects thermostability based on the volume of aliphatic side chains (Ile, Val, Leu, Ala), remained consistent (15.26) for most variants. However, K19V stood out with an aliphatic index of 30.53, indicating significantly improved thermostability due to the addition of valine, an aliphatic residue, at the peptide terminus. High aliphatic index values are correlated with increased thermal stability in globular proteins and short peptides (Ikai, 1980; Khondoker et al., 2023).

The Grand Average of Hydropathicity (GRAVY) values, which reflect the overall hydrophobic or hydrophilic character, ranged from −0.891 (D15E**)** to +0.237 (K19V). Wild-type duramycin had a moderately hydrophilic profile (GRAVY = −0.189), and most mutants remained similarly hydrophilic. However, the K19V mutation led to a shift toward hydrophobicity**,** suggesting potential alterations in membrane-binding affinity. Hydrophobicity is a critical determinant in AMP activity and can affect insertion into lipid bilayers (Torres et al., 2019).

Table 3.2. Physicochemical Properties of Wild-Type and Mutated Duramycin Peptides Predicted by ProtParam

Mutation	MW (Da)	pI	Net Charge	Instability Index	Aliphatic Index	GRAVY
WT	2069.35	7.95	+1	80.05	15.26	–0.189
C1S	2053.29	7.78	+1	80.05	15.26	–0.363
F7A	1993.25	7.95	+1	80.05	15.26	–0.242
T11Y	2131.42	7.94	+1	90.72	15.26	–0.221
D15E	2083.38	7.95	+1	69.92	15.26	–0.891
K19V	2040.31	5.82	0	80.05	30.53	+0.237

3.3. Structural Modeling with PEP-FOLD3

The three-dimensional (3D) structures of duramycin and its single-point mutants were predicted using PEP-FOLD3 **(Picture 3.1)**, a de novo structure prediction tool tailored for peptides ranging from 9 to 50 amino acids in length (Lamiable et al., 2016). The predicted models for the wild-type (WT) and five mutants (C1S, F7A, T11Y, D15E, and K19V) reveal substantial conformational differences that may influence peptide stability and function. The WT duramycin adopts a relatively compact conformation composed of two prominent β-strands connected by short loop regions. This structure is consistent with the known secondary elements of lantibiotics, which typically rely on constrained loops and β-sheets stabilized by lanthionine cross-links (Ortega et al., 2015).

In the C1S mutant, the replacement of the N-terminal cysteine—which likely participates in thioether bridge formation—with serine disrupts this organization, leading to a slightly more extended β-sheet and increased loop flexibility. This structural alteration supports the predicted reduction in stability from the I-Mutant2.0 analysis, as well as the expected loss of post-translational modification potential (Jumper et al., 2021). The F7A mutant shows the most drastic structural deviation, forming a distinct α-helix that is absent in the wild-type. This shift suggests that the substitution of the bulky aromatic phenylalanine with a smaller alanine residue may reduce steric hindrance and permit local helical formation. However, such a rearrangement likely compromises the native β-strand pairing and intramolecular hydrogen bonding, contributing to the substantial decrease in predicted structural stability (Veltri et al., 2018). Similar helix-inducing behavior upon alanine substitution has been documented in antimicrobial peptides such as LL-37 and indolicidin, where conformational changes affect both activity and target specificity (Mahlapuu et al., 2016; Agrawal & Bhattacharyya, 2021).

In contrast, T11Y maintains an overall similar topology to the WT, with two β-strands connected by a flexible loop, but with a slight elongation and curvature in the second strand. This subtle alteration suggests that tyrosine's larger side chain introduces localized steric strain without inducing complete conformational reorganization. The predicted reduction in stability aligns with this minor distortion and may reflect weakened β-sheet interactions. The D15E mutant displays an architecture similar to WT, with well-preserved β-strands and a slightly extended loop region. This finding is consistent with the conservative nature of the mutation, substituting one negatively charged residue (Asp) for another (Glu). Despite similar electrostatic properties, the additional methylene group in glutamic acid may impart increased local flexibility, potentially accounting for the slightly decreased instability index seen in ProtParam results. The K19V mutant shows a notably more compact and symmetrical β-sheet arrangement, with reduced loop length and improved strand alignment. The substitution of the positively charged lysine with a hydrophobic valine appears to promote β-sheet packing and reduce structural strain near the C-terminal region. This conformational tightening supports the I-Mutant2.0 prediction of increased stability and is further reflected by the elevated aliphatic index and GRAVY score in physicochemical analysis. Hydrophobic mutations at terminal regions have been previously shown to stabilize short antimicrobial peptides by enhancing hydrophobic collapse and β-strand interactions (Torres et al., 2019; Wang & Wang, 2022). The comparative structural modelling suggests that among all variants, F7A introduces the most disruptive conformational change, while K19V contributes to enhanced structural order and potential functional improvement. These insights underscore the importance of site-specific mutations in modulating peptide architecture, which in turn governs bioactivity, proteolytic stability, and membrane interaction in antimicrobial peptides (Waghu et al., 2016).

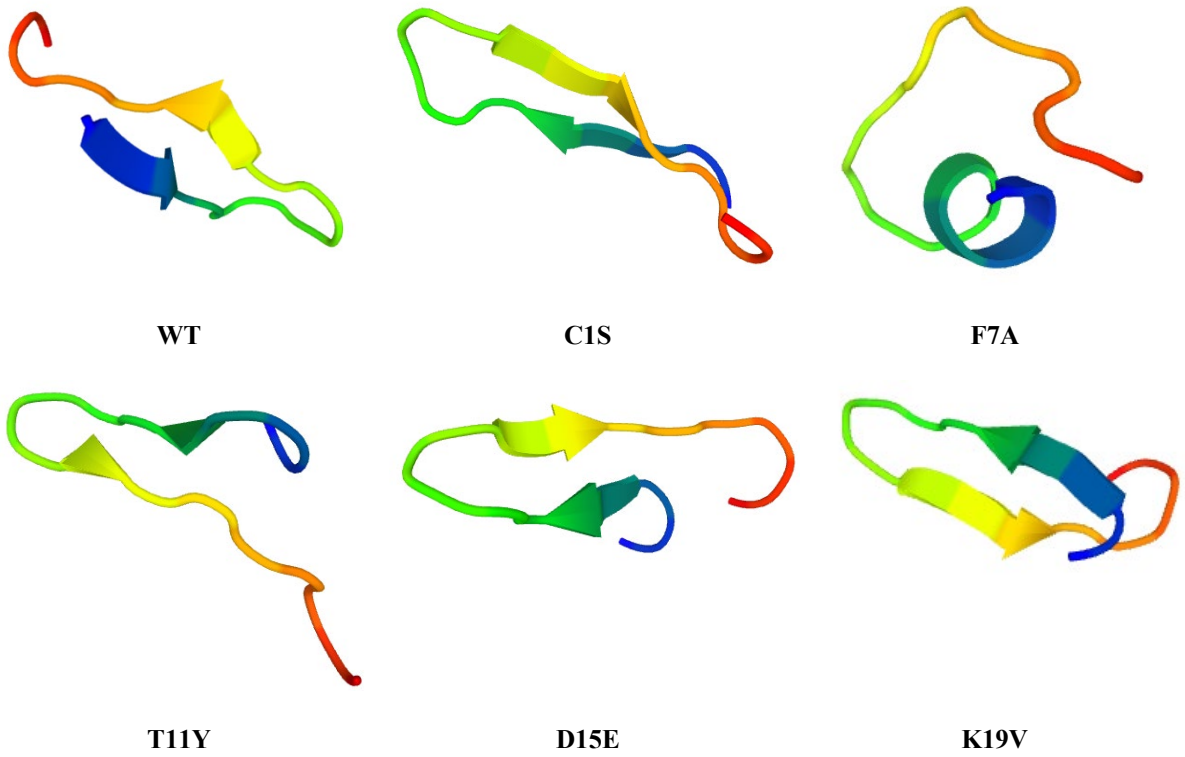

Figure 3.1. Predicted 3D Structures of Wild-Type (WT) and Mutant Duramycin Peptides Modelled Using PEP-FOLD3 (C1S, F7A, T11Y, D15E and K19V)

The *in silico* analyses indicate that point mutations can differentially influence the stability and structural organization of duramycin. The K19V variant demonstrated the strongest stabilizing effect, supported by enhanced hydrophobicity and more compact β-sheet packing. Conversely, mutations involving structurally critical residues, such as C1S and F7A, resulted in reduced stability and notable conformational disruption. The T11Y and D15E substitutions produced moderate effects consistent with their respective structural contexts. These findings highlight the importance of residue-specific interactions in maintaining duramycin's functional conformation and provide a rational basis for prioritizing K19V for further experimental validation.

Conclusion

The in silico analysis of duramycin and its single-residue mutants demonstrates that specific amino acid substitutions can significantly alter peptide stability, physicochemical properties, and 3D conformation. Among the five evaluated mutants, K19V was the most favorable, improving predicted stability and enhancing hydrophobic and aliphatic characteristics, which are essential for membrane interaction in antimicrobial peptides. In contrast, F7A caused the greatest structural disruption, forming an α-helical segment and reducing overall stability. Substitutions C1S and T11Y resulted in local conformational changes with slight destabilizing effects, while D15E provided a conservative change with minimal structural and biochemical impact. The findings underscore the utility of combining computational mutagenesis, biophysical parameter estimation, and structural modelling to guide peptide engineering. This integrative approach may accelerate the development of more stable and potent lantibiotic-based antimicrobial agents.

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