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RESEARCH ARTICLE

Coevolution of RuBisCO in bryophytes: Insights into structural adaptation and terrestrial transition

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Abstract

Research on the molecular evolution of the RuBisCO enzyme's major component in early-diverging terrestrial plants is inadequate. RuBisCO is a highly important enzyme in photosynthesis that catalyses carbon fixation and performs a central role in global carbon cycles. Bryophytes, which include liverworts, hornworts and mosses, provide a unique evolutionary role as some of the earliest earth plants. To find out better insight about the RuBisCO evolution, this study explores the coevolutionary dynamics in bryophytes using previously available sequence data from UniProt. The methodology pipeline includes the alignment of homologous sequences, filtering and data analysis using CoMap v1.5.2 to find out the coevolving residues. Results revealed that 35 coevolving groups were found in the result of CoMap and 35 coevolving residues i.e. amino acids come under category of secondary structural elements and a total 72 amino acids out total 475 amino acid protein was involved in coevolution. The findings revealed significant coevolution in RuBisCO, which primarily involves hydrophobic and uncharged polar residues, enhancing the structural integrity and catalytic efficiency. This coevolution highlights structural integrity and adaptive potential in response to environmental and functional constraints. This study not only advances our understanding of evolutionary history and functional mechanisms of RuBisCO but also opens new avenues for research and applications in plant biology, synthetic biology and environmental science.

Keywords: bryophytes; evolution; photosynthetic adaptation; RuBisCO coevolutionary dynamics; structural integrity

Introduction

RuBisCO (ribulose-1,5-bisphosphate carboxylase/oxygenase) is recognised as the most prevalent enzyme on our planet and plays a fundamental role in the process of photosynthesis by facilitating the carboxylation of ribulose-1,5-bisphosphate within the Calvin cycle (1). This enzyme is crucial for global carbon fixation, making a substantial contribution to the productivity of Earth's biosphere. Although it plays a crucial role, RuBisCO demonstrates limited catalytic efficiency and is susceptible to oxygenation reactions, leading to photorespiration, which diminishes the overall effectiveness of photosynthesis (2). The persistent inefficiencies have exerted ongoing evolutionary pressures on RuBisCO, enabling adaptations to fluctuating atmospheric CO_2 and O_2 levels throughout geological time (3).

Bryophytes, which encompass liverworts, hornworts and mosses, are a crucial group in the evolution of plants, serving as a transitional link between aquatic algae and vascular plants (4). Bryophytes, among the first to colonise land, faced distinct environmental challenges like desiccation, varying CO_2 levels and limited nutrients. These factors likely shaped the evolution of their photosynthetic systems, including RuBisCO (5). The distinctive evolutionary context of bryophytes positions them as an excellent model for exploring coevolutionary dynamics in RuBisCO.

A framework for comprehending how selection pressures have influenced RuBisCO's structural and functional characteristics is provided by coevolution, which is defined as the reciprocal evolutionary changes between interacting molecules or components (6). Under shifting environmental conditions, these changes frequently entail compensatory substitutions that maintain the enzyme's structural integrity and catalytic performance (7). Protein sequences from mosses (Q31795), liverwort (UniProt ID: B0YPN8) and hornwort (Q67FV0) were examined in this work to find coevolving residues using CoMap, a computer program that finds coevolution using clustering techniques and compensatory substitutions.

This study will map coevolving residues and analyse their biochemical characteristics to clarify the molecular modifications that have aided RuBisCO's development in bryophytes. These discoveries advance our knowledge of how early land plants adapted to terrestrial environments and emphasise the role that coevolution played in determining structural stability and enzymatic efficiency. This work further emphasises how crucial it is to combine biochemical and phylogenetic investigations to identify the evolutionary processes behind important enzymes like RuBisCO.

Materials and Methods

Sequence retrieval and filtering

Protein sequences for RuBisCO from bryophytes (liverwort: B0YPN8, hornwort: Q67FV0 and mosses: Q31795) were retrieved from the UniProt database. Additional homologous sequences were identified using the Basic Local Alignment Search Tool (BLAST). Sequences (410) from other organisms, fragmented or ambiguous sequences and those with uncharacterised annotations were excluded.

Structural and biochemical analysis

Amino acid residues have been masked and classified according to their metabolic features, facilitating the comprehension of residue conservation during coevolution. Additionally, many clusters have been examined depending on charge, Grantham, polarity and volume.

Coevolution detection using CoMap

Coevolutionary residues were identified using CoMap v1.5.2, which employs compensation and clustering approaches. Parameters included aligned sequences, a phylogenetic tree, a substitution model and a discrete rate distribution. Total 417 sequences were used in CoMap input. Coevolving residues were assessed for statistical significance (p-value ≤ 0.05) using R, with bootstrap replicates (n = 1000) and false discovery rate (FDR) evaluation.

Visualisation

Circular plots of coevolving residues were created using Circos, highlighting relationships among residues. Secondary structures (helices and sheets) were also mapped to analyze their involvement in coevolution.

Results

Coevolving groups structural and biochemical analysis

Table 1 summarises groups of residues examined for their chemical and structural properties using specific methods like charge, Grantham score, polarity and volume. FDR correction was applied differently across groups, depending on the analysis's statistical needs. This method ensures a balance between detecting significant results and controlling false-positive findings. The four groups were analysed based on the charge of amino acid residues and FDR correction was applied to ensure the results' reliability (Table 1). Fifteen groups underwent analysis using the Grantham score, which evaluates residue differences based on chemical properties. FDR correction was applied, ensuring statistical validity. Fifty-one groups were analysed for polarity (polar *vs*. nonpolar residues). The "Yes/No" indicates that FDR correction might have been applied to some analyses but not others, suggesting a mixed approach. Twenty-nine groups focused on volume, analysing the spatial size of amino acid side chains. Like polarity, the partial FDR application indicates selective or conditional adjustment during the analysis. More information is provided in the Supplementary file SF2. Table 2 provides a classification of amino acid residues based on their chemical properties, assigning each class a specific mask and listing the corresponding amino acids.

Table 1. Groups of residues analysis for chemical and structural properties

Group number	Method	FDR
4	Charge	Yes
15	Grantham	Yes
51	Polarity	Yes/No
29	Volume	Yes/No

Detection of coevolving groups

From the 417 homologous sequences analysed, 35 coevolving groups were identified in RuBisCO, with group sizes ranging from 2 to 10 residues. Method-wise, the total of 79 coevolving groups were given in Table 3. These groups accounted for 72 amino acids (15.16 %) involved in coevolution, highlighting their importance in maintaining structural and functional integrity. More information is provided in supplementary file SF3.

Secondary structure association

Of the 72 coevolving residues, 35 (48.61 %) were located within secondary structural elements, including 30 helices and five sheets. These findings align with previous studies (6, 7), which suggest that coevolutionary changes in secondary structures are critical for protein stability and functionality.

Biochemical properties of coevolving residues

Residue masks revealed that most coevolving positions were non-aromatic hydrophobic residues (40.12 %), followed by uncharged polar residues (16.83 %), basic residues (15.43 %), acidic residues (10.25 %), aromatic residues (10.13 %), proline (5.67 %) and glycine (1.57 %). The predominance of hydrophobic residues indicates their role in maintaining the chiral pool and ensuring proper folding and stability of RuBisCO (Fig. 1).

Functional insights from Group 17

Among the coevolving groups, Group 17 (Table 4) included a critical binding site at position 294, which interacts with substrates. This site exhibited coevolution with neighboring residues, enhancing its structural adaptability and binding efficiency.

Table 2. Amino acid residue mask based on residue class

Residue class	Mask	Amino acids
Acid	A	D, E
Base	B	R, K
Non-aromatic hydrophobic	N	A, L, I, V, M
Non-charged polar	Q	S, T, C, N, Q
Aromatic	R	F, Y, W, H
Proline	P	P
Glycine	G	G

Table 3. Coevolving groups methods wise

Method	Total coevolving group
Polarity	38
Charge	1
Grantham	13
Volume	27

Table 4. The coevolving group, their amino acid residues class (in bold) and secondary

S. No. in SF2[#]	Coevolving groups	Size	*p*-Value	weight^	FDR*
34	D/N293; H/Y301 **A/Q286; R294** Site 293(286)[$]; Helix; amino acid D Site 301(294)[$]; Sheet; amino acid H	2	0.002	Charge	yes

Abbreviations: [#]S. Nos. of coevolving groups as given in SF2; [$]Site-Relative position after MSA (Absolute position before MSA); [^]Biochemical property used for coevolution analysis; [*]FDR: False Discovery Rate.

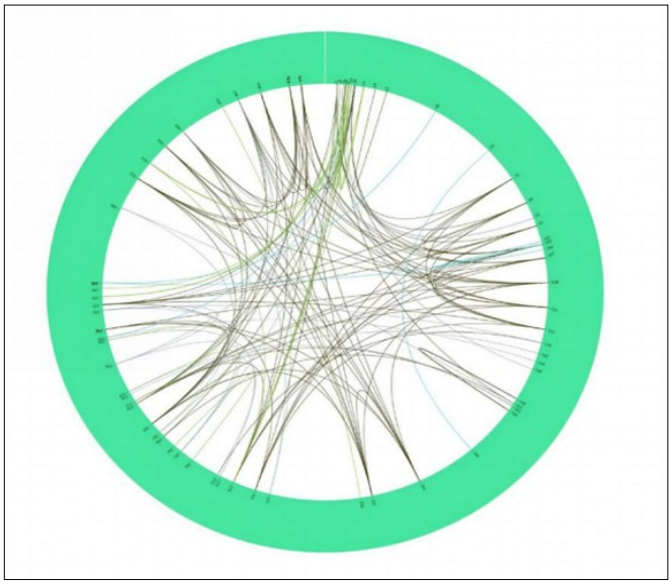

Fig. 1. The groups of coevolving amino acids detected in the RuBisCO. The outer green circle depicts the relative positions of amino acids detected as part of coevolving groups in the multiple sequence alignment. The colored links show the groups of coevolving amino acids detected by weighted substitutions namely Grantham (dark green), polarity (green), volume (blue) and charge (sky blue).

Discussion

Emphasising the need for compensatory replacements in secondary structures like helices and sheets to maintain catalytic effectiveness, our work shows that coevolutionary interactions in RuBisCO are strongly linked with its structural integrity. In line with other studies, these substitutions are highly significant in stabilising RuBisCO's folding and functional conformation especially under different environmental circumstances (6, 7). The prevalence of hydrophobic and polar residues among coevolving locations indicates RuBisCO's metabolic response to environmental restrictions including variations in CO_2 levels and water availability. These results complement prior research emphasising the adaptive relevance of hydrophobic residues in stabilising protein interfaces (7, 8). Early land plants, bryophytes had special selection pressures during their migration to terrestrial settings, which most certainly affected RuBisCO's evolutionary path as proposed in studies of early plant evolution (4, 5).

Finding binding site residues like position 294 emphasises how coevolution functions in RuBisCO. Interactions with substrates and inhibitors including 2-CABP depend on these residues. Previous studies have shown how such residues modify the enzymatic efficiency and specificity of RuBisCO, hence influencing its evolutionary relevance (1, 3). The coevolution of these residues with surrounding sites emphasises their involvement in the regulatory systems of the enzyme, therefore offering novel understanding of RuBisCO's functional evolution.

This work presented a strong framework for analysing coevolutionary dynamics, combining phylogenetic data, substitution models and biochemical attribute classifications by using CoMap and residue masks. Non-bryophyte sequences, however, may restrict more general evolutionary comparisons. Including extensive collections of algae and vascular plants might offer a more complete view of RuBisCO's coevolutionary past. Furthermore, structural modelling and *in vitro* mutational investigations would be useful in verifying the functional relevance of coevolving residues found in this work. The results highlight especially in bryophytes the relevance of coevolution in forming RuBisCO's structural and functional characteristics. The elevated frequency of hydrophobic and uncharged polar residues at coevolving sites indicates that these residues are essential in preserving protein stability and interaction networks. This fits the function of hydrophobic interactions in protein folding (1, 9) and the polar residues contribute to catalytic systems (10).

The coevolutionary adaptations of Group 17 underline the evolutionary strains operating on substrate-binding sites, guaranteeing catalytic performance under changing environmental circumstances. These results align up with past research connecting structural changes in RuBisCO to its functional performance (2, 11). The evolutionary adaptability of these residues emphasises their relevance in maximising RuBisCO's activity under many environmental conditions.

Future studies should seek to clarify how environmental elements such as CO_2 concentrations, temperature and light availability drive RuBisCO's coevolutionary adaptations. Comparative studies of bryophytes and vascular plants might reveal more about the evolutionary routes that influence RuBisCO's usefulness across plant lines. Such research would not only improve our knowledge of RuBisCO's development but also guide attempts to create more efficient RuBisCO variations for agricultural and environmental uses (12, 13).

Conclusion

This study revealed significant coevolutionary dynamics in RuBisCO of bryophytes, emphasizing the structural and functional adaptation of the RuBisCO enzyme. The dominance of hydrophobic and polar residues in coevolving positions highlights their role in maintaining the efficiency and stability of the RuBisCO enzyme. These findings contribute to understanding RuBisCO's evolutionary mechanisms and adaptation to terrestrial environments.

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Authors' contributions

KA conceptualized and designed the study, performed data analysis and interpreted the results. KA also developed the methodology, conducted structural and biochemical analyses and prepared the visual representations. AA reviewed and edited the final draft and provided critical revisions for intellectual content. AA approved the final version of the manuscript for submission.

Compliance with ethical standards

Conflict of interest: The authors do not have any conflict of interest to declare.

Ethical issues: None

Declaration of generative AI and AI-assisted technologies in the writing process

The authors acknowledge the use of AI-based tools (QuillBot & Grammarly) for language improvement including grammar, syntax and readability. Later, the authors thoroughly reviewed and revised the manuscript to ensure accuracy, originality and alignment with the intended scientific context.

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