

The Protonation and Deprotonation Along the Proton Translocation Pathway in *Vigna radiata* H⁺-Translocating Pyrophosphatase by Molecular Dynamics Simulation

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Abstract

Membrane-embedded pyrophosphatase (M-PPase) drives the transportation of H⁺ or Na⁺ between the plasma and vacuolar membranes by hydrolysis of pyrophosphate (PPi). In *Vr*H⁺-PPase, the proton transport along the translocation pathway R242-D294-K742-E301 has been established from our lab structurally and functionally. Meanwhile, we identified a continuous water flow which might reflect the path of proton transfer. However, the detailed molecular dynamic mechanism along the translocation pathway of *Vr*H⁺-PPase remains unclear. Thus, we applied molecular dynamics simulation method to study the detail molecular dynamics in the proton transport of *Vr*H⁺-PPase. The proton transport of *Vr*H⁺-PPase is identified as the Grotthuss-Mechanism. For R242, the previous study shown that when the arginine is partially buried in the protein structure or membrane, it will remain protonated. So, we defined that eight possible protonation and deprotonation states in D294, K742 and E301. From our results, we found that R242 may play the role of preventing the excess waters/protons from entering the proton translocation pathway when D294 had been protonated. Especially in the 0/0/0 state, K742 shown a higher flexibility in the proton transport pathway, it might increase the water interact with E301 by swinging between D294 and E301 then promote proton translocation, at the same time E301 reveals a unique conformation to penetrate the hydrophobic gate (L232-A305-L555-V746) and let protons passing through. Our results may illustrate how the proton translocation and describe the possible proton translocation mechanism in the *Vr*H⁺-PPase.

References

1. Lin, S. M., Tsai, J. Y., Hsiao, C. D., Huang, Y. T., Chiu, C. L., Liu, M. H., Tung, J. Y., Liu, T. H., Pan, R. L., & Sun, Y. J. (2012). Crystal structure of a membrane-embedded H⁺-translocating pyrophosphatase. *Nature*, 484(7394), 399.
2. Tsai, J. Y., Tang, K. Z., Li, K. M., Hsu, B. L., Chiang, Y. W., Goldman, A., & Sun, Y. J. (2019). Roles of the hydrophobic gate and exit channel in *Vigna radiata* pyrophosphatase ion translocation. *Journal of molecular biology*.