DATE: Day 12 Month 06 Year 2020

SUMMARY of
2019 RESEARCH RESULTS REPORT
For International Collaborative Research with IPR, Osaka University

Research Title		**Structural analysis of active site mutants of FK506-binding protein 35 (FKBP35) from Plasmodium Knowlesi**
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Summary

Plasmodium FK506-binding protein 35 (FKBP35) is a member of the peptidyl-prolyl *cis-trans* isomerase (PPIase) family proteins and was considered as a viable target for the development of the novel antimalarial drug. This protein exists in all malaria-causing parasites, including *Plasmodium knowlesi*. Structurally, this protein consists of an N-terminal FK506-binding domain (FKBD) and a C-terminal tetratricopeptide repeat domain (TPRD). While the atomic structure of FKBD of this protein was successfully elucidated using NMR, the structure of TPRD of P. knowlesi (Pk-TPRD) remains unknown. Amino acid sequence analysis predicted that Pk-TPRD might fold into a canonical structure of TPR consisting of three repeating motifs, each comprising two helices plus an additional α-helix. Earlier, it was reported that Pk-TPRD was found to play essential roles in the dimerization and predicted to serve as a binding site for protein substrates. The existence of FKBD and TPRD on Pk-FKBP35 lead to generating a dual function of the foldase and chaperone function of Pk-FKBP35 which might play vital roles in the pathogenicity of *P. knowlesi*. Despite extensive studies on FKBP35 of P. falciparum are available, it is worth noting that *P. knowlesi* is a unique malaria parasite which is transmitted to human through long-tailed macaque (*M. fascicularis*), structural studies on Pk-FKBP35 might lead us to discover drugs specifically targeting *P. knowlesi*. The current study aims to elucidate the atomic structure of Pk-TPRD using NMR spectroscopy to provide structural regulation of the functional properties of this domain. To address, $^{15}N/^{13}C$ -labeled Pk-TPRD was obtained by overexpression of this protein in Escherichia coli BL21(DE3) in M9 medium and purified with Ni-NTA chromatography followed by size-exclusion chromatography. Far-UV CD spectra indicated that the protein is a proper folded. Nevertheless, while the HSQC spectrum also indicated that Pk-TPRD is fully folded, the structural analysis of this domain remains challenging. The spectrum only covers a limited region of the peak which might not be feasible for further analysis. Further optimization on the NMR measurement remains needed.

***Deadline: May 15, 2020**
***Please submit it to E-mail: tanpakuken-kyoten@office.osaka-u.ac.jp.**
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