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ROLE OF THE H-NS FAMILY PROTEINS IN COOPERATIVE FUNCTION OF CARBAZOLE DEGRADATIVE PLASMID PCAR1 AND HOST CHROMOSOME

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Abstract

Degradation of environmental pollutants using bacteria is a valuable method in bioremediation. Although many degradative genes are carried by plasmids, behaviours of degradative plasmids and effects of plasmid carriage for host cells have not been well-investigated. In order to regulate bacterial degradative ability, it is important to clarify the effects of plasmid carriage. To clarify this, we used IncP-7 carbazole degradative plasmid pCAR1 and *Pseudomonas putida* KT2440 as a model plasmid and host strain. pCAR1 contains the gene encoding an H-NS family protein Pmr, which is one of the nucleoid-associated proteins. Pmr regulates the transcription of many genes on both pCAR1 and host chromosome, and simultaneously minimizes the effect of pCAR1 carriage for host cell. Because KT2440 chromosome contains two mainly-transcribed genes encoding H-NS family proteins, TurA and TurB, Pmr and these two proteins could function cooperatively when KT2440 receives pCAR1 through conjugation. Previously, our transcriptome analyses showed that disruption of each of these genes had different effect, suggesting that functions of the three proteins are non-equivalent. However, the distributions of the binding sites of these three proteins were shown to be almost identical. Because H-NS family proteins form homo- and hetero-oligomers, this discrepancy may be originated from their oligomerization manner.

In this study, we evaluated homo- and hetero-oligomerization functions of Pmr. We found that the first 61 residues (Pmr_nt_{61}) were enough to form homo-oligomers. Charged 22 residues of Pmr_nt_{61} were individually substituted with alanine, and their oligomerization capacity was evaluated. Seven of them formed less amount of homo-oligomers compared with wild type, suggesting that such residues were important for homo-oligomerization. These residues are well conserved in TurA, whereas only three are conserved in TurB, suggesting that Pmr/TurA and TurB have different oligomerization manner. To investigate hetero-oligomerization mechanism of the three proteins, we performed surface plasmon resonance analysis using TurA and TurB as ligand, and Pmr as analyte. Comparison of affinities and coupling ratio between TurA-Pmr and TurB-Pmr will be discussed.