Reconstruction of the original core of RNA polymerase from short peptide

*Sota Yagi[1], Aditya K. Padhi[2], Kam Y. J. Zhang[2], Shunsuke Tagami[1]

1. Laboratory for Advanced Biomolecular Engineering, RIKEN Center for Biosystems Dynamics Research, 2. Laboratory for Structural Bioinformatics, RIKEN Center for Biosystems Dynamics Research

The origin and evolution of the transcription system is a key to understanding how DNA-based life emerged on the earth. DNA-dependent RNA polymerase (DdRP) is the most fundamental molecular machine in transcription. Although DdRP is a huge multi-subunit enzyme with over 400 kDa molecular mass, its catalytic core is composed of a small domain with a simple beta fold called double-psi-beta-barrel (DPBB, ~80 a.a.). On the DPBB domain from DdRP three aspartates are highly conserved in its catalytic loop and coordinate the catalytic Mg^{2+}. Thus, it was considered that the DdRP originated from the Mg^{2+} coordinating DPBB. If so, how the ancestral DdRP with DPBB scaffold worked in early life? How DPBB emerged from ancient peptides? In order to answer these questions, we tried to reconstruct the ancestral DPBB experimentally.

We first tried to isolate the Mg^{2+} coordinating DPBB domain from modern DdRPs. However, the isolated DPBBs could not fold well. Then, we searched structurally homologous DPBB folds from PDB and found that the DPBB domain from putative thymidine phosphorylase (ptpDPBB) is well exposed on the protein surface. We successfully isolated ptpDPBB and solved its crystal structure to confirm it adopts the DPBB fold. Furthermore, we grafted the conserved catalytic loop from DdRP into the corresponding position of ptpDPBB and optimized its sequence by computational calculation to enhance structural stability. The engineered ptpDPBB seems to be folded as monomer and can bind Mg^{2+} as intended. Now, we are doing further structural analysis of the engineered ptpDPBB and trying to install a DdRP related function onto the engineered ptpDPBB scaffold.

Interestingly, the DPBB fold has an internal pseudo-twofold symmetry and it is thought to have evolved from a homo-dimeric peptide. To prove this evolutionary scenario, we designed symmetrical DPBBs in which two identical sequences of 45 a.a. were repeated. Three symmetrical DPBB designs could be purified as folded monomers. Then, crystal structures for two of them have been determined at 1.6Å resolution in both cases. Additionally, we have designed a 45 a.a. peptide derived from the N-terminal half sequence of symmetric DPBB and determined its crystal structure at 1.6Å resolution. Surprisingly, the short peptide formed DPBB fold through homo-dimerization. These results strongly support that the DPBB fold evolved from a short peptide with only ~40 a.a..

Furthermore, the designed short peptide showed high refolding capability. Even after thermal denaturation, the designed short peptide quickly refolded to the native structure. Also, when this sequence was chemically synthesized, the lyophilized peptide molecule can form DPBB structure by just being dissolved in physiological buffer solution. These results imply that the ancient short peptide, which formed DPBB fold by self-dimerization, encoded a safeguard against irreversible aggregation and unfolding for surviving in extreme conditions.

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