論文の内容の要旨

応用生命化学専攻

平成16年度博士課程　入学

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論　文　題　目

Structural analyses of Dim2p and Dim2p/eIF2alpha/rRNA complex from *Pyrococcus horikoshii* OT3

(超好熱菌 *Pyrococcus horikoshii* OT3 の Dim2p および Dim2p/eIF2alpha/rRNA 複合体の構造解析)

Eukaryotic small ribosomal subunit (SSU) synthesis involves eight major nonribosomal proteins, Dim2p, Rrp12p, Tsr1p, Enp1p, Hrr25p, Nob1p, Dim1p, and Rio2p, as well as SSU proteins and rRNAs. As a core constituent of SSU RRP complex (SSU ribosomal RNA processing factor complex), Dim2p in yeast follows the preribosomes from early nucleolar to late cytoplasmic stages and is required for the cleavage at processing sites A_1 and A_2 to generate the pre-20S rRNA.[1] Dim2p homologues have been found in archaea as well as in eukarya but not in eubacteria. Little is known about the exact processing and release mechanisms of eukaryotic Dim2p and other processing factors from 40S ribosomal subunit during 18S rRNA maturation thus far.

Recently the translational initiation in archaea has attracted more attention because of its remarkable similarity to that in eukaryotes but much simpler.[2] In archaea, there are about ten homologues to eukaryotic initiation factors.[3] Archaea seem to have two different

translational initiation mechanisms: 5' leaderless mRNA translation and Internal SD sequence dependent translation.[4] In this study, in order to study the roles of archaeal Dim2p and eIF2 (especially eIF2alpha) in the SSU synthesis process and translational initiation and the transition of these two sequent processes in archaea, Dim2p and Rio2p and eIF2alpha from *Pyrococcus horikoshii* OT3 were selected as targets to study.

1. Crystal structure of Dim2p from *Pyrococcus horikoshii* OT3 (PH-Dim2p)

Only one tertiary structure has been determined thus far for Dim2p-homologous proteins: the crystal structure of APE0754 from the aerobic hyperthermophilic archaeon *Aeropyrum pernix* (PDB code 1TUA, Zhang *et al.*, unpublished results). Here I determined the crystal structure of PH-Dim2p, the Dim2p from the hyperthermophilic archaeon *Pyrococcus horikoshii* OT3, at 2.30 Å (Figure 1).[5, 6]

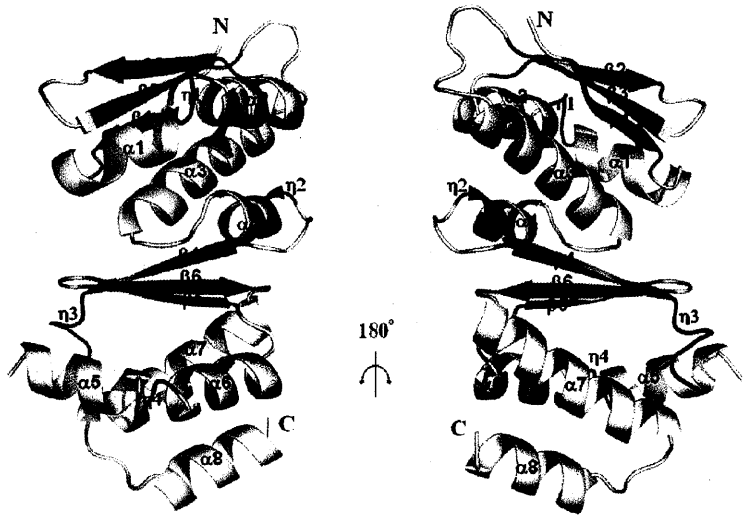

Figure 1. Overall structure of Dim2p from *Pyrococcus horikoshii* OT3

PH-Dim2p contains two KH domains: KH-1 (N-terminal) and KH-2 (C-terminal). The two KH domains are connected by a short linker, and the relative arrangements of the two KH domains are well-defined by a number of inter-domain interactions. The RNA-binding surfaces of KH-1 and KH-2, which are oriented in the same direction, involve many different amino acid residues.

2. Biological function studies of PH-Dim2p

Dim2p in yeast (with one KH-domain) was known to be required for the cleavage at processing sites A_1 and A_2 to generate the pre-20S rRNA, so it is expected to have rRNA binding activity and recognize specific sequence with other processing factors. Furthermore, as a core constituent of SSU RRP complex, the yeast Dim2p involves several physical and biological interactions with other components, Rio2p, Tsr1p, Enp1p, Nob1p, and Dim1p.[1]

We identified the homologue of Rio2p in *Pyrococcus horikoshii* OT3 (PH-Rio2p) and found that it physically interacts with PH-Dim2p. PH-Rio2p, as an RNA dependent protein kinase, phosphorylates the conserved phosphorylation site of eIF2alpha from *Pyrococcus horikoshii* OT3 (PH-eIF2alpha) in vitro. It was found that PH-Dim2p seems to regulate phosphorylase activity of PH-Rio2p in vitro. In addition, PH-eIF2α's phosphorylation depends upon the autophosphorylation of PH-Rio2p.

3. Crystal structure of Dim2p/eIF2α/rRNA complex from *Pyrococcus horikoshii* OT3

In order to study the roles of archaeal Dim2p and eIF2 (as a/Dim2p and a/eIF2) in the SSU synthesis process and translational initiation and the transition of these two sequent processes in archaea, the X-ray crystal structure of the complex of eIF2alpha and Dim2p from *Pyrococcus horikoshii* OT3 (PH-eIF2alpha and PH-Dim2p) together with a 16S rRNA 3'-terminal fragment derived from *E. coli* (almost identical with the one of *Pyrococcus horikoshii* OT3) was determined at 2.8 Å (Figure 2).[7]

The complex structure reveals that KH domain-1 and KH domain-2 of PH-Dim2p bind to a core anti-SD sequence (CCUCC) and a highly conserved rRNA sequence of target rRNA fragment respectively; the interaction between PH-eIF2alpha and PH-Dim2p is mainly electrostatic. The complex structure indicates that PH-Dim2p couples 16S rRNA's 3'-terminal maturation and translational initiation in *Pyrococcus horikoshii* OT3. Through aligning the structure of PH-eIF2alpha to those of eIF2alpha from *Pyrococcus abyssi* (PDB ID code 1YZ6) and eIF2alpha-gamma from *Sulfolobus solfataricus* (PDB ID code 2AHO),

we propose that a/eIF2 triggers the release of a/Dim2p from 16S rRNA in stead of forming a stable complex together, because apparent structural changes exist around a/Dim2p-binding region of a/eIF2alpha. Furthermore, it is suggested that a/Dim2p involves in translational type regulation with a/eIF2 and a/eIF2's initial orientation on 30S subunit in archaea.

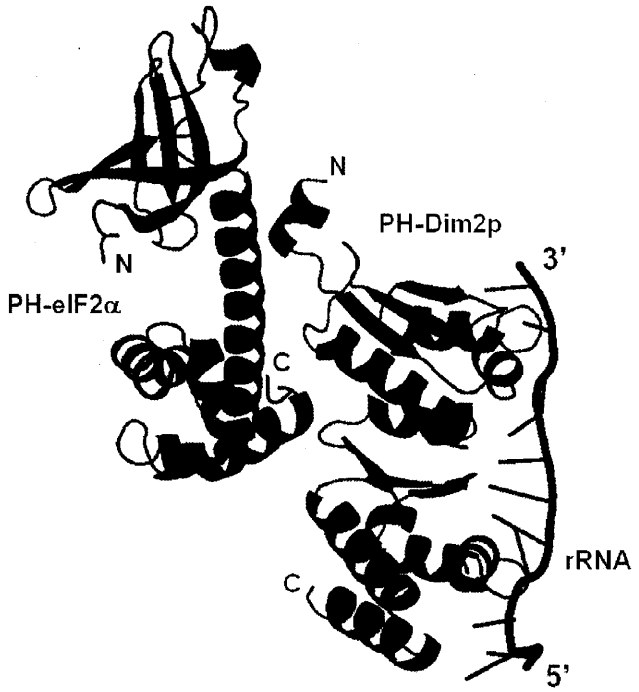

Figure 2. Overall structure of Dim2p/eIF2alpha /rRNA complex

References

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