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学位論文題目	Protein-protein contact surfaces on the β' subunit of *Escherichia coli* RNA polymerase
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論文内容の要旨

The core enzyme of *Escherichia coli* RNA polymerase is composed of four subunits, two α subunits, one β and one β' subunits. The β' subunit is a polypeptide containing 1407 amino-acid residues, and the core enzyme is formed by binding the subunit to the $\alpha_2\beta$ sub-complex. The resultant core enzyme has basic catalytic activity of RNA synthesis, and binds to a σ subunit to form a holoenzyme. In order to get insight into the structural organization of β' subunit in RNA polymerase, I mapped protein-protein contact regions with other subunits on β' subunit by using its recombinant partial fragments.

First, I searched for structural domains as regions relatively resistant to tryptic cleavage. The tryptic cleavage took place at sites initially between the residue 800 and 950, and then around the residue 350. The limited cleavage pattern of β' subunit was similar to those of core enzyme and its complex with σ^{70} subunit, that is holoenzyme. This means there are at least three structural domains in the subunit irrespective of its participation in core enzyme and holoenzyme.

Secondly, I designed several recombinant β' fragments from the results of the domain mapping and from a comparison of β' subunit of *E. coli* with that of other bacteria. Some of the fragments partially overlap with each other. I then overproduced these fragments with or without hexahistidine (His_6) tag and purified. The purified fragments were tested for the formation of complexes with the $\alpha_2\beta$ sub-complex or σ^{70} subunit by four assays; 1) retention of α and β subunits by His_6-tagged β' fragments, 2) inclusion of β' fragments in a complex containing His_6-tagged α and β after renaturation from a denatured state, 3) inhibition of renaturation of core enzyme in the presence of additional β' fragments, and 4) direct binding assay between His_6-tagged β' fragments and σ^{70}. For these assay, recombinant α and β subunits with or without His_6 tag were produced and core enzyme was reconstitutied with these tagged subunits.

According to the assays four of the β' fragments bounds to the $\alpha_2\beta$ sub-complex, and four sets of two different β' fragments simultaneously bound to the $\alpha_2\beta$ complex. The results consistently indicate that: the regions between residues 515

and 842 as well as downstream of 1141 are mainly involved in binding to the $\alpha_2\beta$ sub-complex. One of a minor binding region was involved in the 150 residues fron the N-terminus.

The major region contacting with σ^{70} subunit locates between residues 201 and 345, which overlaps with another minor $\alpha_2\beta$ binding region, and two minor regions identified are the same as the major $\alpha_2\beta$ binding regions. These results suggest the presence of three common contact regions on β' subunit for assembly of core enzyme and holoenzyme (binding to σ^{70} subunit).

Recently a three-dimensional model for the core enzyme of *T. aquatics* was obtained. On the assumption that the obtained structure is similar to that of E. coli, I confirmed these results by using the crystal structure of the core enzyme. Interestingly, the sites involved in the catalytic function of RNA synthesis are all located within two spacer regions sandwiched between these three subunit-subunit contact regions identified in this study.

In the form of holoenzyme β' may contact with some other proteins. He screened proteins associating with a glutathione S-transferase(GST)-fused β' subunit. I found five candidates by sequencing their N-termini. They are HepA, ATP: glycerol 3-phosphotransferase, ribosomal proteins S2 and L6, and YgfB whose function is unknown. The overproduced YgfB, however, seemed to bind to free β' in vivo rather than core enzyme.

論文の審査結果の要旨

提出された論文を元に、公開発表に続き、審査委員と片山君との間で質疑応答がなされた。その議論と博士論文及び関連分野での知識、実験結果の解釈とその有効性について審査委員で審議し、学位にふさわしい知識と考察力を有していると判断した。本論文は英語で書かれており、既に受理された国際誌から、水準以上の英語による表現力を有していることが明らかであった。以上、学位の要件が全て満たされたと判断した。