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学位論文題目	Structure-Function Relationship of the Alpha Subunit of Escherichia coli RNA polymerase.
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The RNA polymerase of *Escherichia coli* plays a key role in transctiption of all species of cellular RNA. The control of the activity and specificity is now recognized as a major mechanism of the global regulation of transcription.

The RNA polymerase consists of four core subunits (2α, β and β′) and one of the multiple molecular species of σ subunit. The assembly of RNA polymerase proceeds sequentially under the order: $2\alpha \rightarrow \alpha_2 \rightarrow \alpha_2\beta \rightarrow \alpha_2\beta\beta'$ (core enzyme) $\rightarrow E\sigma$ (holoenzyme). The α subunit consists of 329 amino acid residues and plays essential roles in protein-protein contacts not only for RNA polymerase assembly but also for transcription activation by class-I factors and DNA UP elements. To reveal the structure-function relationship of α subunit, I attempted to reveal the organization of structural domains by analysis of the pattern of limited proteolysis with two endoproteases, V8 protease and trypsin. Results indicate that one region, Arg235–Glu244, is highly accessible to both endoproteases. I propose that the α subunit consists of two major structural domains, amino (N) terminal-proximal domain upstream from Arg235 and carboxy (C) terminal-proximal domain downstream from Glu 245, each being connected by an inter-domain linker formed by the spacer between these two amino acid residues. The structural organization is in good agreement of its functional map. The N-terminal domain corresponds to the assembly domain of core enzyme and the C-terminal domain corresponds to the transcription activation domain including the contact sites with class-I transcription factors and DNA UP element. Based on detailed analysis of the secondary proteolytic cleavage sites, intra-domain structures are also proposed. The N-terminal domain was cleaved into two subdomain fragments (Na and Nb) between Arg45 and Glu68. The subdomain structure is discussed in relation to the location of subunit-subunit contact sites. On the other hand, the C-terminal proximal domain of 85 amino acids in length (aa residues 245-329) was highly resistant to endoproteases. This domain was expected to form a compact structure.

Next, I tried to determine the conformation of this C-terminal domain (αCTD) by NMR analysis in collabolation with Prof. Y. Kyogoku and colleagues (Institute for Protein Research, Osaka University). The PCR-amplified fragment of the *rpoA,* including the coding region for αCTD, was inserted into an expression vector pET-3a to make pETαCTD. The plasmid was transformed into BL21(λDE3) and αCTD was expressed in the transformants. αCTD was purified by ion exchange column chromatography (DEAE-TOYOPEAL) and gel filtration column chromatography (TOYOPEAL-HW55F). The purity of αCTD for ^{1}H NMR analysis was higher than 99% as juged by SDS-PAGE followed by stainning with Coomassie brilliant blue. ^{15}N-labeled and $^{13}C/^{15}N$-double labeled

αCTD were expressed in M9 medium containg $^{15}NH_4Cl$ (0.05%) and ^{13}C-D-glucose (0.1%) as nitrogen and carbon sources. The purification of labeled sample was performed by the column procedures employed for purification of the unlabeled sample. Purity of the sample was more than 99%. The structure of αCTD was determined by multidimentional heteronuclear magnetic resonance spectroscopy in Prof. Kyogoku's laboratory.

The NMR analysis revealed that αCTD consists of four helices and two long loops at both termini, together forming a compact and rigid structure. The four helices, helix1 (Val264 to Leu273), helix 2 (Ile278 to Gln283), helix 3 (Glu286 to Thr292) and helix 4 (Lys297 to Ser309) are considered to form the hydrophobic core. The helix 1 is perpendicular to the largest helix 4 and thus the N-termini of helix 1 and helix 4 are very close to each other on the tertiary structure. The locations of the contact sites for class-I transcription factors, mapped based on mutant studies, are discussed in relation to the tertiary structure of αCTD. The tertiary structure of αCTD indicated that CRP contact site is located on the helx 1.

In order to determine the residue of αCTD to interact with *rrnB*P1 UP element, the chemical shift perturbation experiments were carried out in Prof. Kyogoku's laboratory by using ^{15}N-labeled αCTD and the *rrnB*P1 promoter UP element duplex DNA. Results indicated that the helix 1 and the N-terminal region of helix 4 interact with *rrnB*P1 promoter UP element. It is worthwhile to note that the tertiary structure of αCTD shows that the relative configuration between helix 1 and helix 4 is similar to the helix-turn-helix motif which is widely observed in DNA-binding proteins.

Finally, I tried to confirm the CRP contact site by inhibition assay of *lac* transcription using four synthetic peptides (A, B, C and D) as inhibitors. These peptides were synthesized with an automated peptide synthesizer by the standard tert-butoxycarbonyl (*t*-Boc) method using phenyl-acetamidometyl (PAM) resin and purified by μ BONDASPHERE column (reverse phase HPLC). These peptides were designed as to include parts or whole of the CRP contact site (Arg265 to Leu270) and helix 1. Peptide C containing both the CRP contact site and the entire helix 1 showed the highest activity of inhibition than any of the test peptides. In the case of lack a half of the helix 1 (peptide B) or substitution of Ala for Arg265 (peptide D), the level of *lac* transcription inhibition was half the level of peptide C. Peptide A (tetrapeptide including Arg265) did not inhibit *lac* transcription. The *lac* transcription inhibition experiment showed that a peptide including the whole sequence of helix 1 significantly inhibited CRP-RNA polymerase interaction, suggesting that the intact conformation of helix 1 is necessary for effective interaction with CRP. The inhibition test of transcription by

synthetic peptides will be used for mapping contact sites with other class-I factors.

論文の審査結果の要旨

根岸智史君は、転写の活性化において、活性化因子とＲＮＡポリメラーゼとの相互作用を分子生物学的および物理的に解析することを目的として、大腸菌ＲＮＡポリメラーゼのαサブユニットのＣ末ドメインの構造と機能を研究した。

まず、αサブユニットの２種のプロテアーゼに対する限定分解の経時変化から、ペプチド配列を決定することにより、分解されやすい部分と、分解に対する耐性を持ったN末ドメインとＣ末ドメインを決定した。また、蛋白質やＤＮＡの転写活性化因子と遺伝学的に相互作用するアミノ酸残基は、すべて決定されたＣ末ドメインに含まれていた。この結果、転写の活性化は、ＲＮＡポリメラーゼのなかの、立体的にまとまりのある単一の構造体をとおして起こることが解明された。

次に、このＣ末ドメイン（Ｅ２４５－Ｅ３２９）を含む９８残基の部分の高収率量産・高純度精製法を確立し、安定同位体核導入による多次元NMRディスタンスジオメトリーによる構造決定（京極研究室との共同研究）を可能にした。決定された３次元構造は、ＲＮＡポリメラーゼ上の転写活性化部位として初めてのものである。その構造は、４つのヘリックスとループ部分とから構成され、転写活性化に重要な部位として、２つのヘリックスとその付近のループが同定された。

これらの情報をもとに、大腸菌cyclic AMP receptor protein（ＣＲＰ）による*lac*プロモーターの活性化を利用して、Ｃ末ドメインのより高分解能の機能マッピングを、オリゴペプチドによる転写活性化の阻害を指標に行った。その結果、ＣＲＰの相互作用部位として、Ｌ２６０－Ｉ２７５の領域が意味を持つ可能性を示した。

提出された論文（英文）は、以上の研究に用いられた手法、得られた結果、それに基づく議論を明瞭に記述し、その内容は、学位の要件を満たすものであった。