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研 究 成 果 報 告 書

（国立情報学研究所の民間助成研究成果概要データベース・登録原稿）

研究テーマ（和文） AB		Redβ-Exo-DNA リコンビナーゼ DNA-タンパク質複合体の結晶構造解析			
研究テーマ（欧文） AZ		Solving the Crystal Structure of the Redβ-Exo-DNA Recombinase-DNA-Protein Complex			
研究氏代表名者	カタカナ CC	姓)ヘドル	名)ジョナサン	研究期間 B	2009 ～ 2010 年
	漢字 CB			報告年度 YR	2009 年
	ローマ字 CZ	Heddle	Jonathan	研究機関名	理化学研究所
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概要 EA （600 字～800 字程度にまとめてください。）

The aim of this project was to solve the high resolution structure of Beta protein from bacteriophage lambda through combination of approaches, including (1) determination of stable well-folded domains of Beta protein, (2) elucidation of binding conditions of Beta towards single-stranded and double-stranded oligonucleotides, and (3) studying the binding of Beta to its functional partner, Exo. The knowledge gained in these studies should greatly enhance the ability to produce diffracting crystals of Beta.

Full-length Beta protein was expressed in E. coli and purified. Crystallization trials yielded pseudo-crystals that failed to diffract under X-rays. Truncated versions of Beta protein were engineered with the aid of domain prediction software and molecular cloning techniques. A total of eight different truncated Beta proteins were expressed and purified, and solubility and stability studies were purified. One such truncated Beta protein, termed "core domain" displayed high solubility and binding to DNA oligonucleotides. Large crystals of the Beta core domain protein were obtained with extensive optimization of conditions, but unfortunately displayed very poor diffraction. Wild-type Exo protein was expressed, purified, and analyzed with respect to stability and solubility. Binding studies between Beta and Exo were carried out using size-exclusion chromatography, which failed to yield positive results. Co-crystallization of Beta and Exo proteins also failed to yield promising results.

On another front, experiments were carried out to probe the mode of binding of Beta to single-stranded and double-stranded DNA oligonucleotides of varying lengths. Visualization by transmission electron microscopy (TEM) revealed that DNA binding caused large-scale changes in Beta quaternary structure, in particular forming extended, flexible helices in the presence of double stranded DNA, as long as the DNA is added sequentially. It was established that the length of the length of the DNA strands determines the length of the helical nucleoprotein. Experiments using cryo-EM tomography, done in collaboration with Osaka University, has produced a low-resolution 3D model of the Beta nucleoprotein helix structure. Furthermore, it was also shown that the helical nucleoprotein is resistant to digestion by a diverse array of nucleases, which may shed light on the way Beta protein interacts with the DNA binding partner. Using the information garnered from these DNA-binding studies, crystallization trials were carried out using the Beta-DNA nucleoprotein. However, these have to date not yielded crystals of sufficient quality for diffraction.

キーワード FA	Recombinase	Protein structure		

（以下は記入しないでください。）

助成財団コード TA					研究課題番号 AA									
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発表文献（この研究を発表した雑誌・図書について記入してください。）

雑誌	論文標題GB									
	著者名 GA		雑誌名 GC							
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