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Crystallization and preliminary X-ray study of *Pk*-REC from a hyperthermophilic archaeon, *Pyrococcus kodakaraensis* KOD1

Kazuaki Harata,[a]* Noriyuki Ishii,[a] Naeem Rashid,[b] Masaaki Morikawa[c] and Tadayuki Imanaka[b]

[a]Biomolecules Department, National Institute of Bioscience and Human Technology, 1-1 Higashi, Tsukuba, Ibaraki 305-8566, Japan, [b]Department of Synthetic Chemistry and Biological Chemistry, Graduate School of Engineering, Kyoto University, Yoshida-Honmachi, Sakyo-ku, Kyoto 606-8501, Japan, and [c]Department of Material and Life Science, Graduate School of Engineering, Osaka University, 2-1 Yamadaoka, Suita, Osaka 565-0871, Japan

Correspondence e-mail: harata@nibh.go.jp

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Pk-REC is a protein which binds to DNA and catalyzes the central step of recombination and repair. The protein was crystallized using the hanging-drop vapour-diffusion method with PEG as a precipitant. Two orthorhombic crystal forms I and II with the same space group $P2_12_12_1$ were obtained at pH 8.0 using PEG 3000 and PEG 550 monomethylether, respectively. The unit-cell parameters were $a = 151$, $b = 174$, $c = 241$ Å for form I and $a = 151$, $b = 176$, $c = 300$ Å for form II, indicating that the asymmetric unit contains more than 20 molecules.

1. Introduction

Proteins such as bacterial RecA and eukaryotic RAD51 play several key roles in genetic recombination and repair (Cox & Lehman, 1987; Shinohara *et al.*, 1992). These proteins bind to both single-stranded and double-stranded DNAs and catalyze the central step of recombination: the pairing and strand exchange of homologous DNA molecules. X-ray analysis of *Escherichia coli* RecA has revealed a helically extended polymeric structure suggesting the formation of a helical filament of RecA on DNA (Story *et al.*, 1992), which has been observed by electron microscopy (Yu & Egelman, 1997).

A protein (*Pk*-REC) homologous to the essential domain of RecA and RAD51 has been found in a hyperthermophilic archaeon, *Pyrococcus kodakaraensis* KOD1, which grows at 368 K (Rashid *et al.*, 1996). The molecular weight of the protein, 23 kDa, is considerably smaller than that of RecA and RAD51. Even though the protein comprises only the main central domain of the RecA protein with N-terminal and C-terminal truncations, *Pk*-REC is a multifunctional protein and shows an unusual DNAase activity on both single-stranded and double-stranded DNAs, along with a DNA-independent ATPase activity (Rashid *et al.*, 1997). In addition, the reaction product of ATPase activity is AMP instead of ADP. A recent mutagenesis approach has strongly suggested that the active sites of the ATPase and DNAase activities are common (Rashid *et al.*, 1999). In order to understand these unique characteristics of *Pk*-REC, we require its three-dimensional structure and have therefore initiated its X-ray crystallographic study.

2. Experimental

Pk-REC was overexpressed in *E. coli* and purified according to the previously reported method (Rashid *et al.*, 1999). Crystals were prepared by the hanging-drop vapour-diffusion method. Crystallization conditions were first screened at room temperature using the sparse-matrix screen (Crystal Screen and Crystal Screen II, Hampton Research). The protein solution contained 1.6% protein, 10 m*M* Tris buffer pH 8.0 and 100 m*M* NaCl. To a droplet of protein solution, an equal amount of reservoir solution was added and the droplet was equilibrated over 1 ml reservoir solution. The protein crystallized in two forms at pH 8–9 in the presence of PEG. The crystallization conditions of each crystal form

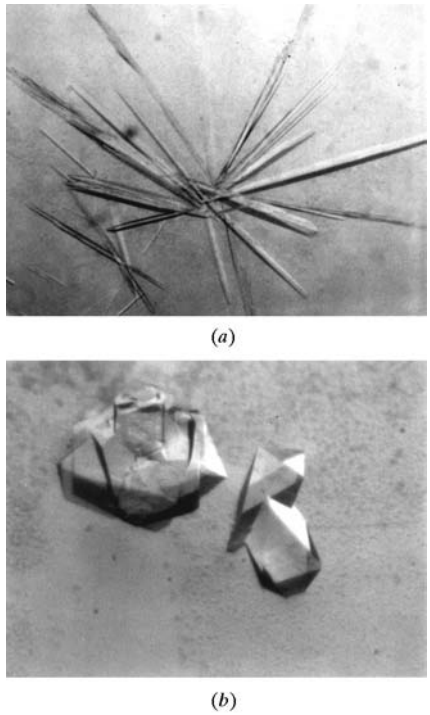

Figure 1
Crystals of *Pk*-REC. (*a*) Form I crystals are ~1.5 mm in the longest dimension. (*b*) Form II crystals are ~0.4 mm in the longest dimension.

were optimized for pH, the concentration and molecular weight of PEG and the concentration of NaCl.

X-ray diffraction experiments were carried out on a Rigaku R-AXIS IIc image-plate detector equipped with a Rotaflex FR rotating-anode generator (45 kV, 50 mA, focal spot size 0.1 mm). The incident Cu $K\alpha$ radiation was passed through Franks-type mirror–mirror optics (Charles Supper). Collected images were processed using a program incorporated in the *R-AXIS IIc* software package.

2.1. Results and discussion

Pk-REC was crystallized at pH 8.0 in two orthorhombic forms, I and II (Fig. 1), with the same space group $P2_12_12_1$. The optimum conditions for form I were obtained at pH 8.0 with a reservoir solution containing 10% PEG 1000 and 200 m*M* NaCl, while a reservoir solution containing 10% PEG 550 monomethylether and 150 m*M* NaCl was used for the crystallization of form II. The form I crystal was a thin needle and diffracted to 3.5 Å resolution. The unit-cell parameters were $a = 151$, $b = 174$, $c = 241$ Å. The asymmetric unit contained between 20 and 40 molecules, estimated from the normal V_m range of 1.7–3.5 Å^3 Da^{-1} (Matthews, 1968). The form II crystal had a prismatic shape and diffracted to 5 Å resolution. The unit cell, with dimensions $a = 151$, $b = 176$, $c = 300$ Å, contained between 25 and 51 molecules in the asymmetric unit. Intensity data were collected to 5 Å resolution for the form I crystal, with an $R_{\rm merge}$ value of 0.147 and a completeness of 58%, and to 6.5 Å resolution for the form II crystal, with an $R_{\rm merge}$ and completeness of 0.165 and 77%, respectively. Both crystals have similar *a* and *b* dimensions, but their Patterson maps showed no significant similarities. We are planning to collect higher resolution data using a synchrotron X-ray source.

It is known that RecA forms oligomeric structures with or without DNA. A hexameric ring structure of RecA has been observed in the absence of DNA by electron microscopy (Yu & Egelman, 1997). The *Pk*-REC protein lacks the N-terminal and C-terminal domains of RecA which contribute to its self-assembly and the regulation of its binding affinity to DNAs, respectively (Story *et al.*, 1992). The dimer formation in solution has been suggested by the molecular weight determined by gel-filtration chromatography (Rashid *et al.*, 1997). The large number of molecules in the asymmetric unit of the current crystals suggest the possibility of the formation of a highly aggregated structure. We are trying to find local symmetry by means of the Patterson rotation function. The form I crystal is suitable for structure determination and we are currently screening heavy-atom derivatives.

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