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Crystal structure of the nucleosome containing ultraviolet light-induced cyclobutane pyrimidine dimer

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ABSTRACT

The cyclobutane pyrimidine dimer (CPD) is induced in genomic DNA by ultraviolet (UV) light. In mammals, this photolesion is primarily induced within nucleosomal DNA, and repaired exclusively by the nucleotide excision repair (NER) pathway. However, the mechanism by which the CPD is accommodated within the nucleosome has remained unknown. We now report the crystal structure of a nucleosome containing CPDs. In the nucleosome, the CPD induces only limited local backbone distortion, and the affected bases are accommodated within the duplex. Interestingly, one of the affected thymine bases is located within 3.0 Å from the undamaged complementary adenine base, suggesting the formation of complementary hydrogen bonds in the nucleosome. We also found that UV-DDB, which binds the CPD at the initial stage of the NER pathway, also efficiently binds to the nucleosomal CPD. These results provide important structural and biochemical information for understanding how the CPD is accommodated and recognized in chromatin.

1. Introduction

The integrity of genetic information is constantly threatened by assaults from various endogenous and environmental factors. Within the broad spectrum of DNA damage, cyclobutane pyrimidine dimers (CPDs) and pyrimidine-pyrimidone (6-4) photoproducts (6-4PPs) are detrimental DNA cross-links of neighboring pyrimidine bases, and are specifically induced by ultraviolet (UV) light. If left unrepaired, these damaged bases interfere with replication and transcription, thereby evoking diverse deleterious effects, including mutations, chromosomal aberrations, and cell death. Nucleotide excision repair (NER) is the main pathway that is responsible for removing these UV-induced photolesions from the genome [1]. Hereditary defects in the NER pathway are associated with several rare autosomal recessive disorders in humans, including xeroderma pigmentosum (XP), which is clinically characterized by a marked predisposition to skin cancer [2].

In the mammalian NER subpathway operating throughout the genome, two XP-related damage surveillance protein complexes have been implicated in the recognition of UV-induced photolesions [3]. One is the UV-damaged DNA-binding protein (UV-DDB), the DDB1-DDB2 (XPE) heterodimer, which is capable of binding to both CPD and 6-4PP [4–9]. The other is the XPC complex (XPC-RAD23-centrin2 heterotrimer), which recognizes a wide range of helix-distorting DNA lesions including 6-4PP, but not CPD [10–12]. Unlike XPC, which functions as an essential and versatile NER damage sensor, UV-DDB specifically recognizes UV photolesions and recruits XPC to the damaged sites *in vivo* [3].

Notably, in cells, these photolesions occur in DNA organized

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within chromatin, in which most of the DNA is wrapped around nucleosome core particles. The nucleosomal 6-4PP structure has been determined by X-ray crystallography [13]. In the nucleosome, the 6-4PP bases are flexibly disordered, and UV-DDB specifically binds to the flexible DNA backbone around the damaged bases [13]. However, it is currently unclear how UV-DDB recognizes the CPD in the context of chromatin, because the structure of a CPD in the nucleosome has not been solved.

In the present study, we determined the crystal structure of the nucleosome containing two CPDs at symmetric sites. A biochemical analysis revealed that the CPDs in the nucleosome are specifically recognized by UV-DDB. Therefore, the observed structure provides the framework for lesion recognition by UV-DDB in chromatin.

2. Materials and methods

2.1. Preparation of DNA containing CPD for crystallization

The single-stranded DNA 75-mer containing the CPD lesion (CPD ssDNA) and the complementary single-stranded DNA 71-mer were synthesized at Tsukuba Oligo Service, Japan, and purified by HPLC. The CPD ssDNA contained a 5′ phosphate. The complementary ssDNA was mixed with the CPD ssDNA in a 1:1 ratio, and the double-stranded DNA (dsDNA) 71-mer containing a 4-base overhang, 5′-AATT-3′, at one end for self-ligation was prepared by annealing. The dsDNA 71-mer containing the CPD lesion was self-ligated, and the dsDNA 146-mers (CPD dsDNA) were formed. In the CPD dsDNA, the CPD lesions were positioned symmetrically (Fig. 1A).

2.2. Preparation of the CPD nucleosome

Human histones H2A, H2B, H3.1, and H4 were produced in *Escherichia coli* cells, as described previously [15,24,25]. The purified H2A-H2B–H3.1-H4 octamer (0.9 mg) and the 146 base-pair DNA (1 mg), in which the CPDs are located at two symmetric sites, were mixed in a solution containing 2 M KCl, and the samples were dialyzed against dialysis buffer (10 mM Tris–HCl (pH 7.5), 2 M KCl, 1 mM dithiothreitol, and 1 mM EDTA), at 4 °C for 3 h. The dialysis buffer was exchanged with a peristaltic pump (0.8 ml/min flow rate), and the KCl concentration was gradually decreased to 250 mM. The sample was further dialyzed against 10 mM Tris–HCl buffer (pH 7.5), containing 250 mM KCl, 1 mM dithiothreitol, and 1 mM EDTA, at 4 °C for 3 h, and then incubated at 55 °C for 2 h. The CPD nucleosome was purified by non-denaturing polyacrylamide gel electrophoresis, using a Prepcell apparatus (Bio-Rad) according to the published method [26]. The purified CPD nucleosome was concentrated, and then dialyzed against 20 mM potassium cacodylate buffer (pH 6.0) containing 1 mM EDTA.

2.3. Crystallization and structure determination

The purified CPD nucleosome was crystallized by the hanging drop method, after mixing equal volumes of the CPD nucleosome solution and 20 mM potassium cacodylate buffer (pH 6.0), containing 90–115 mM $MnCl_2$ and 50 mM KCl. The CPD nucleosome sample was equilibrated against a reservoir solution of 20 mM potassium cacodylate buffer (pH 6.0), 35–40 mM KCl, and 70–85 mM $MnCl_2$. Crystals of the CPD nucleosome were soaked in a cryo-protectant solution, containing 20 mM potassium cacodylate buffer (pH 6.0), 40 mM KCl, 75 mM $MnCl_2$, 30% (+/−)-2-methyl-2,4-pentanediol, and 2% trehalose. The crystals were flash-cooled in a stream of N_2 gas (−173 °C). The CPD nucleosome crystals belonged to the orthorhombic space group $P2_12_12_1$, and contained one nucleosome per asymmetric unit. Diffraction data were collected using the synchrotron radiation source at the beamline BL41XU station of SPring-8, Harima, Japan.

The diffraction data of the CPD nucleosome were integrated and scaled with the HKL2000 program [27]. The data processing was performed with the CCP4 program suite [28]. The molecular replacement method was employed to solve the structure using the Phaser program [29], with the human nucleosome structure (PDB ID: 3AFA) as the search model. Using the Phenix program [30], rigid body refinement of the obtained solution, further structural refinement consisting of iterative rounds of energy minimization, and B factor refinement were performed. Model building was conducted using the COOT program [31]. The Ramachandran plot of the final CPD nucleosome structure showed 99.7% of the residues in the most favorable and additional allowed regions, and no residues in the disallowed region. Summaries of the data collection and refinement statistics are provided in Table S1. All structure figures were created using the PyMOL program (http://pymol.org). The atomic coordinates of the CPD nucleosome have been deposited in the RCSB, with the PDB ID 5B24.

2.4. Preparation of the fluorescently labeled nucleosome

For the UV-DDB-nucleosome binding analysis by EMSA, the fluorescently labeled histone H2B was prepared by the method described previously [13]. The nucleosome was reconstituted with dsDNA, the H3.1-H4 complex, and the Alexa488-labeled H2B T122C–H2A complex by the salt-dialysis method, and then purified as described previously [13].

2.5. Purification of the human UV-DDB protein

Recombinant human UV-DDB was produced with an insect cell expression system, and was purified to near homogeneity by the methods described previously [9].

2.6. Gel electrophoretic mobility shift assay

EMSA was performed by the same method described previously [13]. Briefly, for the nucleosome binding analysis by EMSA, UV-DDB (0, 2.5, 5, 10, and 20 nM) was incubated with each nucleosome (5 nM, containing a fluorescently labeled histone) in the presence of a 19-fold excess of undamaged nucleosome as an unlabeled competitor and 91.4 pM of ϕX174 supercoiled DNA (New England BioLabs). The reactions were performed in 28 mM sodium phosphate buffer (pH 7.5), containing 150 mM NaCl, 3.4 mM $MgCl_2$, 1.4 mM EDTA, 2% glycerol, 0.014% Triton X-100, 0.1 mg/ml BSA, and 1 mM DTT, at 30 °C for 30 min. The samples were then fractionated by electrophoresis on a 6% non-denaturing polyacrylamide gel (acrylamide:bis = 37.5:1) in 0.5 × TGE buffer (12.5 mM Tris base, 96 mM glycine, and 0.5 mM EDTA), and the bands were visualized with a Typhoon 9410 imager (GE Healthcare).

3. Results

3.1. Overall structure of the CPD nucleosome

We reconstituted the nucleosome core particle by the salt-dialysis method with a palindromic 146 base-pair DNA sequence, in which two CPDs were introduced at symmetric sites (Fig. 1). The CPD nucleosome was crystallized, and its structure was determined at 3.6 Å resolution (Fig. 2A, Supplementary Table S1). The overall structure of the CPD nucleosome is essentially the same as those of nucleosomes with undamaged DNA [14–16]. In the present nucleosome structure, we found that the DNA regions around the CPD sites are bent along with the nucleosomal DNA curvature (Fig. 2A).

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