X-Ray analysis of a DNA dodecamer containing 2' -deoxy-N^4- methoxycytidine

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ABSTRACT
The crystal structure of DNA dodecamer with the sequence of d(CGCAAATTXGCG), where X is 2'-deoxy-N^4-methoxycytidine, has been determined by X-ray analysis. The dodecamers form a double helix with B-form conformation. The electron density indicates that the two modified cytosine bases respectively make a pair with the adenine bases on the opposite strand in a manner of Watson-Crick geometry and that the methoxy groups are in anti conformation to the N3 atom.

INTRODUCTION
Methoxamine is known as a mutagen which causes nucleotide transition during DNA replication. Amino groups of 2'-deoxycytidine residues in DNA are attacked with this chemical and the resulting 2'-deoxy-N^4-methoxycytidine (mo^4C) has been identified to be the origin of such mutation. Reeves and Beattie demonstrated that N^4-methoxylated dCTP was incorporated at both templates, G and A, the latter template being rather preferred. To establish the structural basis for nucleotide transition, many investigations have been published so far. Brown's group solved the crystal structure of DNA hexamer with the sequence of d(CGCGXG). However, the hexamer from a duplex with Z-form conformation, and the methoxy group of the mo^4C residue is in syn conformation around the N4-C4 bond to form a wobble pair with the guanosine moiety on the opposite strand. These structural features might be unacceptable to the DNA polymerase. In the case of 2'-deoxy-N^4-methoxyadenosine residue (mo^4A), we have found as the first example that the adenine moiety takes the imino form to form a Watson-Crick pair with cytosine base in the B-form DNA dodecamer. In the present study, we have synthesized the DNA dodecamer containing mo^4C as the reversed case in which mo^4C interacts with A on duplex formation, and its crystal structure has been investigated.

EXPERIMENTAL
mo^4C was derived from 2'-deoxycytidine and incorporated into the DNA dodecamer with a DNA synthesizer. Crystallization condition was surveyed by the hanging drop vapor diffusion method at 4°C. Crystals suitable for X-ray experiment were obtained in a 4μl droplet of 5mM sodium cacodylate buffer (pH 7) containing 0.5mM DNA dodecamer, 2.3mM spermine tetrahydrochloride, 6mM magnesium acetate, 20mM sodium acetate and 10% 2-methyl-2,4-pentanediol (MPD), which was equilibrated against a reservoir solution containing 20mM sodium cacodylate, 12mM spermine tetrahydrochloride, 36mM magnesium acetate, 80mM sodium acetate and 25% MPD for 3 weeks. Several crystals were soaked in the same reservoir solution containing 40% MPD for flash freezing. X-Ray data were collected at 110K on the Sakabe-Weissenberg camera with synchrotron radiation (λ=1.0Å) at the Photon Factory (BL-18B) in Tsukuba. Two data sets were collected to compensate the over loaded reflections.

STRUCTURE DETERMINATION
The crystal belongs to the space group of P2_1_2_1, with cell dimensions of a=24.8, b=40.7 and c=66.2Å. Reflection
spots recorded on imaging plates were indexed and their intensities were integrated with the program DENZO\textsuperscript{7}. The intensity data in the range of 100-1.8\AA~resolution were scaled on a common scale and merged into the independent reflections with the programs SCALA and AGROVATA in CCP4\textsuperscript{8}. Initial phase was derived by the molecular replacement method with the program AMoRe\textsuperscript{9} using the structure of DNA dodecamer \textit{d}(CGCGAATTCGCG)\textsuperscript{10} as a probe. The structure was constructed and modified on a graphic workstation by referring omit maps at every nucleotides with the program QUANTA (Molecular Simulations Inc.). The atomic parameters were refined with the program X-PLOR\textsuperscript{11}.

RESULTS AND DISCUSSION

Figure 1 shows that the modified DNA dodecamer form a double helix with B-form conformation similar to the unmodified dodecamer\textsuperscript{10}. The methoxy groups of mo\textsuperscript{4}C are \textit{anti} to the N3 atoms and projected into the major groove of the duplex, indicating that methoxylation has no significant influence on the DNA conformation. The 2F\textsubscript{o}-F\textsubscript{c} electron density (Fig. 2) shows clearly that each mo\textsuperscript{4}C in the duplex forms a Watson-Crick type pair with adenine base on the opposite strand. To form the hydrogen bonds between N4(mo\textsuperscript{4}C) and N6(A) and between N3(mo\textsuperscript{4}C) and N1(A), mo\textsuperscript{4}C must have the imino form, which is consistent with the facts that mo\textsuperscript{4}C prefers the imino form in solution and in crystalline state. It is concluded that mo\textsuperscript{4}C can mimic thymidine by taking the imino form.

ACKNOWLEDGMENT

This work was partially supported by a grant for the RFTF(97L00503) (Research For The Future) from the Japanese Society for the Promotion of Science.

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