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MODEL FOR A RECOGNITION OF 3-METHYLADENINE BY 3-METHYLADENINE DNA GLYCOSYLASE: THE STACKING INTERACTION BETWEEN 3-METHYLADENINE AND INDOLE RINGS

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X-Ray crystallographic study shows that 3-methyladenine, the product of methylation of DNA by carcinogenic and/or mutagenic methylating agents, stacks strongly with the indole ring, the aromatic side group of tryptophan.

KEYWORD 3-methyladenine-indole-3-acetic acid complex; stacking interaction; X-ray analysis; enzyme recognition model

Cellular DNA is easily modified by carcinogenic and/or mutagenic alkylating agents when cells are treated with them. Of the modified DNA constituents, 3-methyladenine seems to be the major lethal lesion.[1)] For the release of the 3-methyladenine residue from alkylated DNA, cells have DNA repair enzymes called 3-methyladenine-DNA glycosylase.[2,3)] *Escherichia coli* 3-methyladenine DNA glycosylase II liberates 3-methylguanine, 7-methylguanine, O^2-methylpyrimidine, N^2,3-ethanoguanine and N^2,3-ethenoguanine in addition to 3-methyladenine.[4)] However, the recognition mechanism of alkylated DNA by any DNA glycosylase is not known at all. It has been reported that *N*-methylpurines such as 1-methyladenine and 7-methylguanine strongly interact with the aromatic rings by the stacking.[5)] The predominant stacking interaction may play an important role in the interaction between alkylated DNA and the DNA repair enzyme. In this work, we investigated the interaction between 3-methyladenine and indole rings by means of the X-ray crystallographic technique. The result provides the model for a recognition of 3-methyladenine in DNA by 3-methyladenine DNA glycosylase.

The colorless crystals of 3-methyladenine indole-3-acetic acid (1:1) complex were grown as pentahydrate in 40% ethanol solution containing equimolar amounts (40mM) of 3-methyladenine and indole-3-acetic acid by slow evaporation at 7°C. Crystal data are as follows: Chemical formula $C_6H_8N_5^+ \cdot C_{10}H_8NO_2^- \cdot 5H_2O$, $M = 414.42$, triclinic, space group $P1$, $a = 13.534(2)$, $b = 11.269(3)$, $c = 7.008(1)$ Å, $\alpha = 78.61(2)$, $\beta = 94.54(2)$, $\gamma = 77.82(2)°$, $V = 1016.0(4)$ Å^3, $Z = 2$, $D_c = 1.355$ g cm^{-3}, μ(Cu Kα) = 0.869 mm^{-1}, 3301 reflections (3013 unique, $R_{int} = 0.033$) were measured with $2\theta_{max} = 120°$ on a Rigaku AFC-5R diffractometer with graphite-monochromated Cu Kα radiation, of which 1665 with $|F_o| \geq 2\sigma(F_o)$ were used in the calculations. The structure was solved by direct methods[6)] and refined by full matrix least-squares.[7)] All hydrogen atoms were located on the difference Fourier map, and the final R factor is 0.057 (unit weight). Atomic coordinates, bond lengths and angles, and thermal parameters will be deposited at the Cambridge Crystallographic Data Centre. All numerical calculations for X-ray analysis were carried out on an ACOS 930 computer at the Research Center for Protein Engineering, Institute for Protein Research, Osaka University. The molecular orbital calculations were performed on a personal IRIS 4D/25G using the program SPARTAN.[8)]

Figure 1 shows the molecular arrangement in the crystal with the stacking and hydrogen bonded interactions between 3-methyladenine and indole-3-acetic acid. A 3-methyladenine molecule takes a cationic form with N7 protonated[9)],

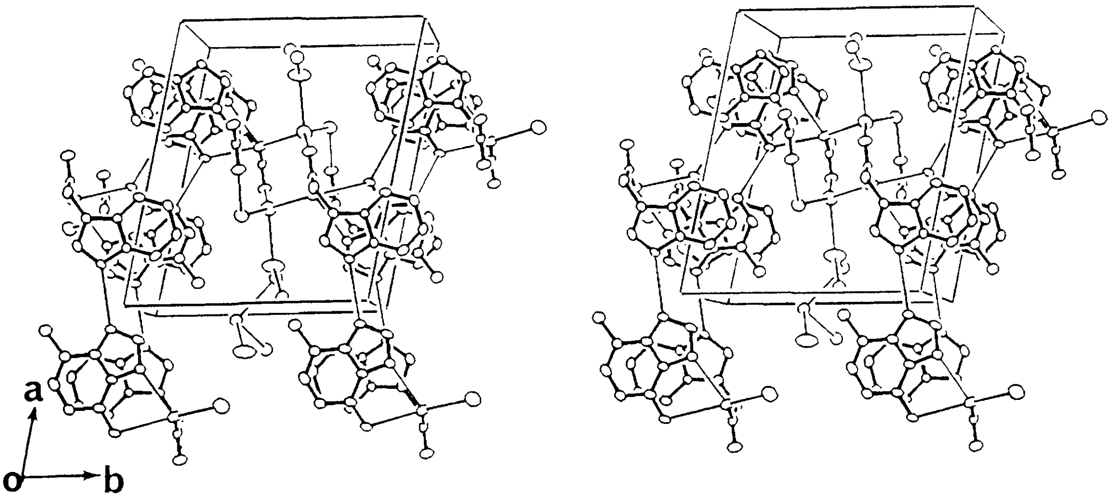

Fig. 1. Stereoview of the Molecular Arrangement in the Crystal
The thin lines indicate hydrogen bonds.

and the carboxyl group of indole-3-acetic acid is anionic. The geometry and dimensions of 3-methyladenine and indole 3-acetic acid are in good agreement with those found in 3-methyladenine hydrochloride[9] and indole-3-acetic acid nicotinamide (1:1) complex.[10] The most prominent feature of the molecular arrangement is the alternating stacking of the 3-methyladenine and indole rings. The distances of interplanar separation are 3.38 Å and 3.44 Å for the upper and lower sets, respectively (Figure 2). The dihedral angle between adjacent 3-methyladenine and indole ring planes is 6.1(2)°. Both pairs show an extensive overlap of adjacent rings, which suggests that the stacking interactions between the 3-methyladenine and indole rings are strong and specific. There are also hydrogen bonds in the stacked upper pair of the 3-methyladenine and indole rings, and they may increase the stabilizing of the interaction between 3-methyladenine and indole rings. The other hydrogen bonds, between 3-methyladenine and indole rings and between 3-methyladenine rings, occur between the neighboring columns. The stacking interaction between 3-methyladenine and aromatic rings was also observed in the crystal of 3-methyladenosine *p*-toluenesulfonate.[11]

Although many model compounds including both nucleic acid bases and protein components have been investigated by X-ray analysis,[12] stacking interactions have rarely been observed, mainly because of the weakness of the interaction

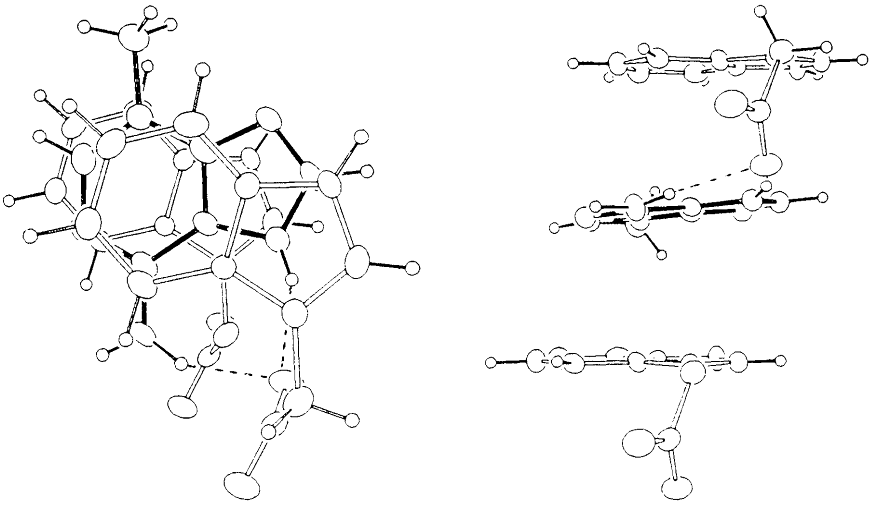

Fig. 2. Stacking Mode between 3-Methyladenine and Indole Rings
The indole-3-acetic acid molecules are shown as open lines.

force and partly because of disturbance by the crystal packing force.[13] As an exceptional case, adenine, 1-methyladenine and 7-methylguanine bases having a positive charge exhibit stacking interactions with aromatic rings such as indole ring of tryptophan and phenyl ring of tyrosine and phenylalanine in all the crystals reported.[5] This means that the positively charged purine bases specifically bind to the aromatic side chains in amino acid residues by the stacking interaction. In general, dipoles, π-electron systems, dipole-induced dipolemoments and London dispersion forces appear to be important in the stacking interaction.[14] In particular, the specific stacking interaction arises from the interaction between the highest occupied molecular orbital (HOMO) of the donor ring and the lowest unoccupied molecular orbital (LUMO) of the acceptor ring. Ishida and co-workers have shown that in an indole-adenine system the protonation of adenine lowers the LUMO energy, leading to reinforcement of the HOMO-LUMO interactions.[5a] We calculated the LUMO energies of positively charged and uncharged 3-methyladenines and adenine by the PM3 method using the program SPARTAN. The LUMO energy of the positively charged 3-methyladenine (-5.276 eV) is lower by 4.3-5.0 eV than that of the uncharged one (-0.968 eV) or of adenine (-0.243 eV); i.e., the HUMO-LUMO interaction between positively charged 3-methyladenine and indole rings gains the energy of 4.3-5.0 eV as compared with cases of uncharged 3-methyladenine-indole rings and adenine-indole rings. As a 3-methyl-adenine residue usually adopts a positive charge in DNA, it may be able to interact with a tryptophan side chain easily.

The crystal structure of *E. coli* DNA repair enzyme, 3-methyladenine DNA glycosylase II, is close at hand.[15] As the preliminary result, a tryptophan residue is found near the surface area which is suggested to be in the active site. Therefore, the tryptophan residue may be one of the residues yielding clues to the recognition of 3-methyladenine in DNA by 3-methyladenine DNA glycosylase II in the manner of the specific stacking interaction as found in this crystal. Site-directed mutagenesis experiments, including the mutation of the tryptophan, are currently under way.

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