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THE STACKING INTERACTION IN 9-(INDOLE-3-PROPYL)-1-METHYL ADENINIUM IODIDE CRYSTAL, A MODEL STUDY ON THE INTERACTION BETWEEN TRYPTOPHAN RESIDUE AND ADENINE BASE IN PROTEIN–NUCLEIC ACID INTERACTIONS

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The crystal structure of 9-[indole-3-propyl]-1-methyl adeninium iodide has been determined by X-ray diffraction method as a model for possible interaction between the tryptophan residue of peptide and the N(1)-protonated or methylated adenine base of nucleic acid. The indole ring stacks intermolecularly with the adeninium ring, which might be caused by their dipole-dipole and/or charge-transfer interaction between both the aromatic rings as suggested from the absorption spectra.

INTRODUCTION

The specific recognition of nucleic acid by protein requires direct interactions between the chemical groups constituting each of the two macromolecules. These interactions involve electrostatic, hydrogen bonding and stacking interactions(1-4), all of which may contribute to the proper fitting of protein active site with respect to nucleic acid.

Tryptophan residue may play an important role in the π-π stacking and/or charge-transfer interaction with nucleic acid bases(5-8), because the indole ring virtually possesses the

Abbreviations: I^3-C_3-MA^9, 9-(Indole-3-propyl)-1-methyladeninium
I^3-C_3-A^9, 9-(Indole-3-propyl)adenine
HOMO, Highest occupied molecular orbital
LUMO, Lowest unoccupied molecular orbital
CNDO/2, Complete neglect of differential overlap

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marked tendency to devote an electron compared with the other amino acids(9).

In order to understand the physiological role of tryptophan, it is especially of interest to elucidate the detailed interacting mode with adenine base, which in addition to the component of nucleic acid, also constitutes the biologically important molecules such as pyridine and flavin coenzymes and furthermore binds more preferentially to the indole ring than the other bases(10).

However, no such stacking interaction has yet been confirmed by X-ray crystal analyses for the model compounds: 3-(9-adenyl)-propyltryptamine(11,12), I^3-C_3-A^9 (13), and 9-ethyladenine:indole complex(14). The lack of the stacking interaction would be partly responsible for the weakness of this interaction force and partly for the unfavorable crystal packing force. When the adenine base is protonated in acidic condition(pK_a=4.1), it could be expected to form the prominent stacking even in crystalline state because the value of LUMO of base is decreased from 0.1367 a.u.(adenine) to 0.0185 a.u.(N(1)-protonated adeninium cation) with respect to the value of HOMO of indole ring(-0.3996 a.u.). Indeed the stacking interaction of both aromatic rings has been observed in acidic solution(15-17).

According to these idea(18), we synthesized I^3-C_3-MA^9 iodide as a intramolecular base-indole stacking model compound and now report the stacking mode actually observed in its crystal structure, which could gain an insight into the unique interaction between the tryptophan residue of protein and the N(1)-protonated or methylated adenine base of nucleic acid in living cell.

MATERIALS AND METHODS

I^3-C_3-MA^9 iodide was synthesized by the reaction of methyl iodide with I^3-C_3-A^9, which was prepared from indole-3-propionic acid and adenine in the same way as described in the literature (10). An aqueous solution of the sample was slowly evaporated at room temperature and a single crystal(0.3×0.1×0.5 mm), a pale

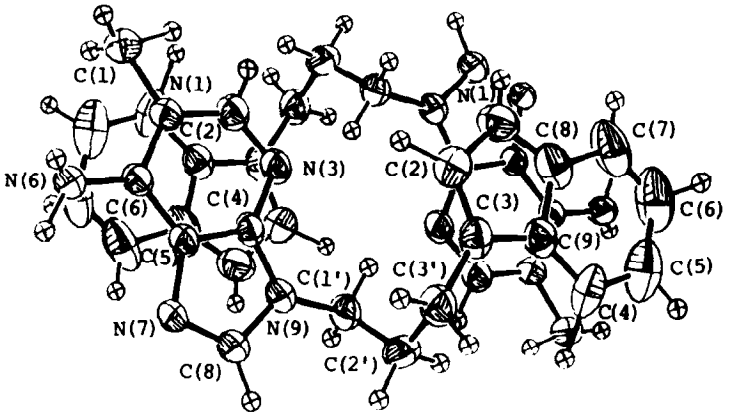

Fig.1 The ORTEP drawing of I^3-C_3-NM^9 dimer formation by the stacking interaction between the indole and adeninium rings, in which the respective molecules are related to each other by a center of symmetry.

brown platelet(mp. 268-270°C), was used for X-ray analysis; it is triclinic with the space group $P\bar{1}$, and two units of the chemical formula, $C_{17}H_{19}N_6I\cdot 2H_2O$, in the unit cell: a=12.199(5), b=11.923(6), c=7.449(3) Å, α=95.88(5), β=97.88(5), γ=67.31(3)° and V=988.7(8) Å^3. Intensity data for 3367 independent reflections were collected on a Rigaku automatic diffractometer with graphite-monochromated Cu Kα radiation, using the ω-scan mode. The structure was solved by the heavy-atom method and refined by the least-squares method up to the present R-factor of 0.065.

The energy values(in atomic unit, a.u.) of HOMO and LUMO and dipole moments(in Debye,D) of respective molecules were calculated by using CNDO/2 method(19).

Absorption spectra in the range 220-360 nm at 25°C were measured three times with Hitachi 624 spectrometer using 10-mm quartz cell, and were averaged. Experiments were performed with buffer solutions(0.025M phosphate, 10% ethanol, pH=7.1) containing samples(all adjusted to final concentration of 5.9×10^{-5}M).

RESULTS AND DISCUSSION

In this crystal, the prominent stacking layers consisting of alternate adeninium and indole rings were formed parallel to the c-axis. The overlapping mode between two molecules related by a center of symmetry is shown in Fig.1, in which the I^3-C_3-MA^9 molecule exhibits somewhat a folded conformation with approximately parallel alignment of the indole and adeninium rings(the dihedral angle=2.1(1)°), and the indole ring intermolecularly stacks with the neighboring adeninium ring related by a center of symmetry. These molecular conformation and associatien are largely in contrast

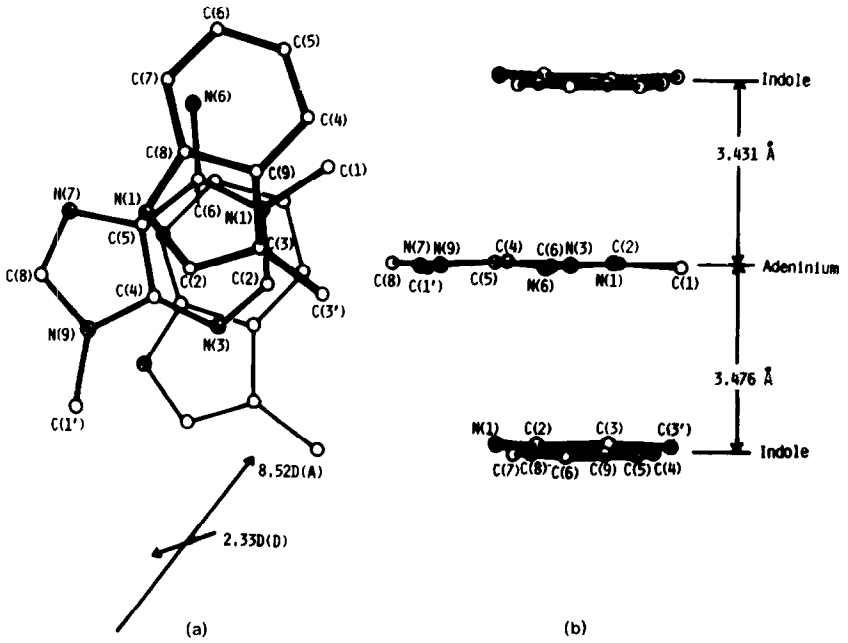

Fig.2 Stacking between the adeninium and its two nearest neighboring indole rings, projected perpendicular to the central adeninium ring(Fig.2(a)) and parallel to the ring(Fig.2(b)). The vectors of the permanent dipole moments are represented by arrows; these were calculated by the CNDO/2 method using the coordinates of 3-methylindole(D) and 1,9-dimethyladeninium cation(A), respectively.

with those of I^3-C_3-A^9(13), a compound without the protonation at N(1) position of adenine base: the molecule takes a extended conformation with dihedral angle of 83.5° between the indole and adenine rings, and the hydrogen-bondings between the neighboring adenine bases.

The stacking between the adeninium and indole rings is illustrated in Fig.2. Both the upper and lower indole rings are well stacked on the central adeninium ring, in particular on the pyrimidine portion. The average interplanar spacing in the overlapping region is 3.431 Å for the upper pair and 3.476 Å for the lower pair. These values are both in the normal van der Waals separation.

We propose that this association is partly ascribable to the dipole-dipole interaction between the indole and adeninium rings,

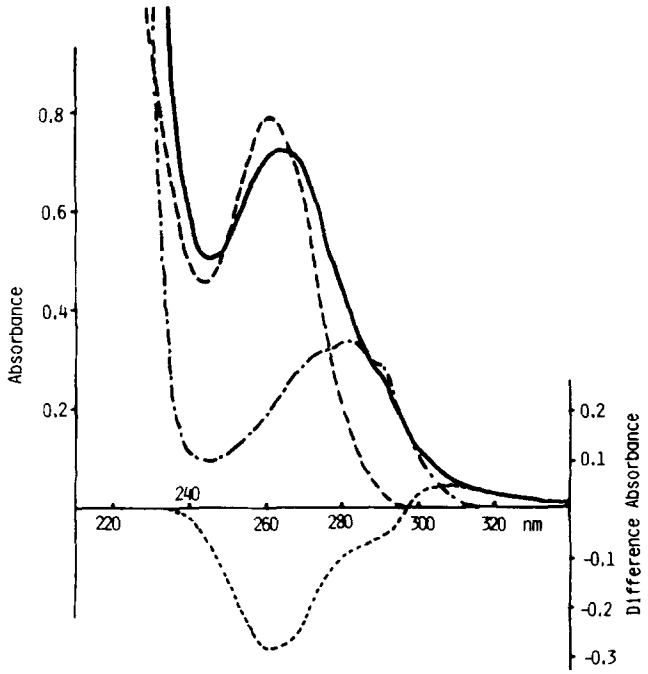

Fig.3 Absorption spectra of I^3-C_3-MA^9 iodide(——), 3-methylindole (-·-·-) and 1,9-dimethyladeninium iodide(-----), and their difference spectrum[(I^3-C_3-MA^9) - (3-methylindole + 1,9-dimethyladeninium)](·····).

because the directions of respective dipole moments in each aromatic ring being almost antiparallel may strengthen the dipole-dipole interaction. Furthermore, the difference absorption spectrum of I^3-C_3-MA^9 and its reference molecules could be partly ascribable to the π-π charge-transfer interaction between the indole and adeninium rings, *i.e.*, as shown in Fig.3, the negative band from 238 to 297 nm is due to the hypochromic effect of the stacking interaction between both aromatic rings, and the positive broad band above 297 nm(λ_{max}=307 nm) could be assigned to a charge-transfer band resulting from electron transfer from the HOMO of indole ring to the LUMO of adeninium ring(20).

Thus this model compound shows a possibility that the indole ring in the tryptophan residue of peptide can selectively interact with the N(1)-protonated or methylated adenine base in single-stranded nucleic acid system.

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