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X-RAY CRYSTAL STRUCTURE OF 7,8-DIMETHYLISOALLOXAZINE-10-ACETIC ACID:TYRAMINE (1:1) TETRAHYDRATE COMPLEX. A MODEL FOR FLAVIN COENZYME–TYROSINE RESIDUE CHARGE–TRANSFER COMPLEXES IN FLAVOPROTEINS.

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SUMMARY

The structure of the 1:1 molecular complex of 7,8-dimethylisoalloxazine-10-acetic acid and tyramine tetrahydrate has been determined by X-ray crystallography. The molecules are associated in a pair by what is clearly a charge-transfer interaction. X-ray results suggest that tyrosine residues may be significant in flavin coenzyme-apoprotein binding in flavoproteins.

INTRODUCTION

To understand the mechanism of action of flavin enzymes, it is important to elucidate details of the interaction between flavin coenzymes and apoproteins. Although the importance of tyrosine and tryptophan residues in the binding of flavin coenzymes to flavoproteins has been suggested from various spectral studies(1-9), little is known about the mode of interaction between these molecules. X-ray study using model compounds is a useful method to elucidate the mode of their interaction at the molecular level.

To increase our knowledge of flavin coenzyme-tyrosine residue interaction in flavoproteins, we have determined the crystal structure of 7,8-dimethylisoalloxazine-10-acetic acid (DIA)*:tyramine(TRA)* (1:1) tetrahydrate complex.

*Abbreviations: DIA, 7,8-dimethylisoalloxazine-10-acetic acid
TRA, tyramine

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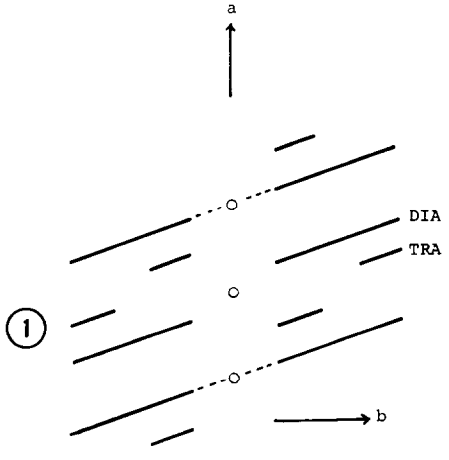
MATERIALS AND METHODS

DIA was synthesized according to the previously described method(10). Addition of an equimolar amount of TRA to the aqueous suspension of DIA increased the solubility of DIA and produced colour change from yellow to red, with the appearance of a charge-transfer band at 440 nm-600nm. Unstable crystals obtained from the aqueous solution consisted of a 1:1 complex and contained four water molecules per complex. Crystals are monoclinic, the space group is $P2_1/c$ with four units of the chemical formula, $(C_{14}H_{12}N_4O_4)\cdot(C_8H_{11}NO)\cdot 4H_2O$, in the unit cell: a=10.724(3)Å, b=11.647(4)Å, c=19.728(6)Å and β=96.67(3)°. The intensity data for 4286 independent reflections were collected by a computer-controlled four-circle diffractometer (Rigaku Denki Co.) with Cu-Kα radiation using the ω-2θ scan technique. The structure was solved by the direct method with the "MULTAN" program(11), and refined by a block-diagonal least-squares method. Present refinement stands at R=0.13 excluding F_o=0.0.

RESULTS AND DISCUSSION

DIA molecule forms a dimer with two hydrogen bonds[N3---O2, 2.855(6)Å] around the centre of symmetry. The layers consisting of DIA and TRA molecules are piled up in the a-direction as shown in Fig.1. Four water molecules are presented among these layers and stabilized the molecular packing



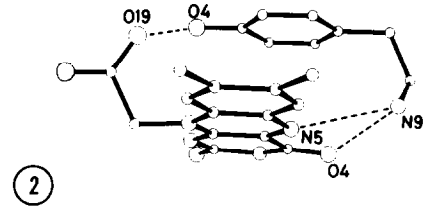


Fig.1. Diagrammatic view of crystal packing of DIA:TRA complex. The dashed lines represent the hydrogen bonds between N3 and O2 atoms of DIA. Open circles represent the centre of symmetry.

Fig.2. Intermolecular complex formation by three hydrogen bonds between donor atoms(O4 and N9) of TRA and acceptor atoms (O4, N5 and O19) of DIA.

by hydrogen bond formations. The hydroxyl group of TRA is hydrogen bonded to the carboxyl oxygen atom of DIA[O4---O19, 2.675Å], and the amino group of TRA to 'chelate site' of DIA, O4 and N5 atoms[N9---O4, 3.050(7), N9---N5, 3.070(7)Å]. These hydrogen bonds form an intermolecular complex as shown in Fig.2. DIA and TRA molecules are associated in a pair by what is clearly π_D-π_A charge-transfer interaction.

The mode of overlapping of isoalloxazine and phenol rings is presented in Fig.3, and the interatomic distances between these rings less than 3.5 Å are listed in Table 1. The stacked rings are almost parallel (dihedral angle, 0.1°), and the separation between mean planes is 3.18 Å, suggesting partial charge-transfer from the phenol ring to the lowest unoccupied orbital of the isoalloxazine ring in the ground state.

It is interesting that the phenol ring lies above the uracil and pyrazine rings of the isoalloxazine ring. Similar overlapping is also found in DIA:tryptamine complex(12), but not in riboflavin:hydroquinone complex(13). It may be that the mutual orientations of the stacked rings depend mainly on the

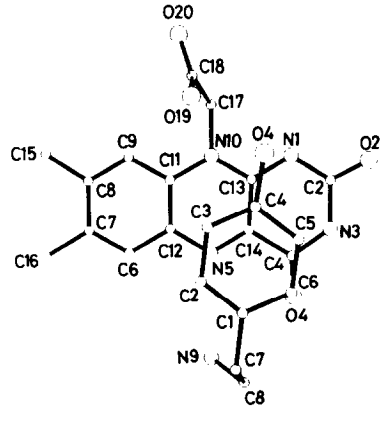


Fig.3. The observed mode of overlapping and orientation of the isoalloxazine and phenol rings, viewed perpendicular to the isoalloxazine plane.

Table 1. The interatomic distances(Å) of a stacked pair between DIA and TRA molecules.

DIA		TRA		DIA		TRA	
N 1	–	O 4	3.289(6)	N 3	–	C 5	3.374(8)
C 4	–	C 5	3.339(8)	C 4	–	C 6	3.322(8)
O 4	–	C 6	3.375(8)	O 4	–	C 8	3.252(8)
N 5	–	C 2	3.256(8)	N 5	–	C 3	3.356(7)
C12	–	C 3	3.432(8)	C13	–	C 4	3.274(7)
C13	–	O 4	3.311(7)	C14	–	C 3	3.381(8)
C14	–	C 4	3.316(8)	C14	–	C 5	3.464(8)
O19	–	C 4	3.493(7)				

interaction between N1 and O4, N5 and C2, and N5 and C3 atoms, because the values of their free valences are larger than the others(14). On the other hand, it is noteworthy that the phenol ring is strongly stacked from the upper site, not from the lower site, of the isoalloxazine ring having a specified orientation as shown in Fig.3, and this mode of stacking is also observed in isoalloxazine-indole interaction(12).

The stacking interaction between tyrosine residue and isoalloxazine ring of flavin mononucleotide has been observed in the structures of flavodoxin from *Desulfovibrio vulgaris*(15, 16) and *Clostridium MP*(17). Therefore, the charge-transfer interaction between phenol ring of tyrosine residue and isoalloxazine ring is important for the binding between flavin coenzymes and apoenzymes in flavoproteins.

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