The Specific Hydrogen Bonding of Riboflavin Derivatives to an Adenine Compound

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Riboflavin-2',3',4',5'-tetraacetate was prepared by the reaction of riboflavin with acetic anhydride in pyridine. Yellow flaky crystals were obtained (mp 246°C). In the infrared spectrum of the 0.02 M solution (in $CDCl_3$) of riboflavin tetraacetate (R), a strong band due to the free imino group of the isoalloxazine ring is observed at 3380 cm^{-1} (Fig. 1 R), indicating that most of the riboflavin tetraacetate molecules are present as monomers. The infrared spectrum of 9-ethyladenine (A) shows two strong bands at 3527 and 3416 cm^{-1} which are due to the non-bonded amino group[1] (Fig. 1 A). When both solutions are mixed together, however, the association bands of the R-A complex appear at 3485, 3330, 3255 and 3200 cm^{-1} (Fig. 1 R+A). Using procedures described previously[1], the association constant for the formation of the R-A complex at 25°C was determined to be 130 (*l*/mol of dimer), as compared with the self dimerization constant of riboflavin tetraacetate (4.5) and of 9-ethyladenine (3.1).

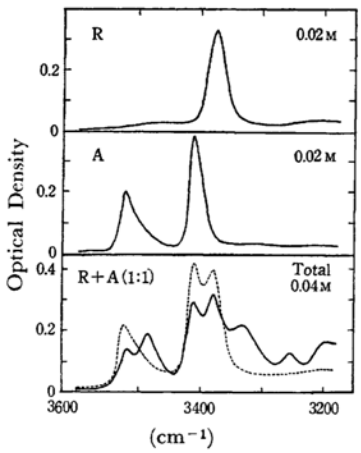

Fig. 1. Infrared spectra of the deuterochloroform solutions in a 1 mm quartz cell. Solid line: observed spectra. Dotted line: calculated sum of the upper two spectra.

From the mixing curve (the intensity of the association bands *vs.* mole fraction) and also from an analysis of the spectra in the carbonyl stretching region, the R-A complex was found to be the 1 : 1 cyclic dimer in which two hydrogen bonds are formed between the amino group of the ethyladenine molecule and one of the carbonyl oxygen atoms of the isoalloxazine ring and between the imino group of the riboflavin molecule and the nitrogen atom, probably, of the adenine ring. It has also been found that riboflavin tetraacetate does not associate with 1-cyclohexyluracil, 2',3'-benzylidine-5'-trityl-guanosine and -cytidine to any observable extent at 0.008 M. A similar experiment has been made with riboflavin tetrabutyrate.*1 The results were quite similar to those obtained for riboflavin tetraacetate.

Present results suggest the formation of the coplanar complex of the adenine and isoalloxazine rings in the flavin adenine dinucleotide (FAD) molecule, in contrast with the ring-stacking model proposed by Tsibris *et al.*[2]

Recently it has been shown that barbiturates form strong specific hydrogen bonds with the adenine residue.[3] The association constant for the formation of the phenobarbital-9-ethyladenine complex is 1200. Barbiturates are known to be inhibitors in the respiratory chain in mitochondria, where FAD serves as an electron carrier. It is suggested, therefore, that barbiturates inhibit respiration by disrupting the structure of FAD: it takes the adenine moiety from the intramolecular adenine-isolloxazine complex.

1) Y. Kyogoku, R. C. Lord and A. Rich, *J. Am. Chem. Soc.*, **89**, 496 (1967).

*1 Riboflavin tetrabutyrate was kindly provided to us by Prof. K. Yagi, Nagoya University and by Prof. J. Okuda, Meijo University.

2) J. C. M. Tsibris, D. E. McCormick and L. D. Wright, *Biochemistry*, **4**, 504 (1965).

3) Y. Kyogoku, R. C. Lord and A. Rich, *Nature*, **218**, 69 (1968).