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Charge Transfer Complexes and Self-association of Flavin Adenine Dinucleotide

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Synopsis. Spectrophotometric studies on the formation of FAD-L-tryptophane and FAD-D-tryptophane complexes, and the optical rotatory and polarographic studies of FAD have been carried out. The steric influence of ribosyl and ribityl groups on FAD for the complex formation is discussed. The dimerization of FAD in solution is suggested.

Many papers have appeared on the charge transfer complexes of flavins with tryptophane.[1] Flavin adenine dinucleotide (FAD) is known to form the charge transfer complex as an electron acceptor with caffeine,[2] while the intramolecular interaction between the flavin and adenine moieties in FAD has been established.[3] The ribosyl and ribityl groups in FAD are so bulky that they may sterically influence the complex formation with an electron donor. It was expected that FAD complex formation with L-tryptophane and D-tryptophane causes a difference in equilibrium constants and the thermodynamic parameters. If we suppose that ribosyl and ribityl groups interact with a side chain of indole, the molecular rotations of FAD-tryptophane complexes might differ from the sum of the individual molecular rotations of pure samples of FAD and tryptophanes. For optical rotatory dispersion studies, the molecular of FAD has been measured at very low concentration (10^{-4} mol/l) in the region of shorter wavelength than 450 nm.[4] In our studies, it was necessary to make the concentrations of FAD and tryptophanes higher (10^{-3}~10^{-2} mol/l) for the facilitation of complex formation. For comparison the molecular rotation of FAD was determined at the same concentration. It was found that the molecular rotation of FAD at 590 nm changes with concentration. This paper presents spectrophotometric studies on the formation of FAD-L-tryptophane and FAD-D-tryptophane complexes, and the optical rotatory and the polarographic studies of FAD.

TABLE 1. ANALYTICAL DATA OF FAD AND TRYPTOPHANES

Compound	UV, visible molar extinction coefficient	$[\alpha]_D^{28}$	Paper chromatograph, R_f
FAD	1.11×10^4	—	0.15[a]
	1.13×10^4		
	(450 nm)		
L-Tryptophane	5.9×10^3	−31.4	0.69[b]
D-Tryptophane	5.9×10^3	+32.4	0.70[b]
	5.6×10^3	±31.5	
	(280 nm)		

a) *n*-PrOH : *n*-BuOH : 5%Na_2HPO_4 aq. = 50 : 15 : 30,
b) *n*-BuOH : AcOH : H_2O = 4 : 1 : 1.

Experimental

The analytical data of FAD and tryptophane are given in Table 1. Requisite amounts of FAD and/or tryptophane were dissolved in 0.1 mol/l of phosphate buffer (pH 7.0) for the measurements. The adsorption spectra and the optical activities were determined with a Shimadzu spectrophotometer SV50AS and a JASCO automatic spectropolarimeter Model J-5, respectively. The polarograms were determined with a dropping mercury electrode.

Results and Discussion

From the differential absorption spectrum, the absorption maximum was found to appear at 505 nm and the absorbance of FAD-trypotphane systems was thus determined at 505 nm. The results are plotted according to Benesi-Hildebrand's equation[5] in Fig. 1. The equilibrium constant K for each temperature T was obtained from the values of slopes and intercepts. From the plots of log K vs. $1/T$, the enthalpy changes and entropy changes were calculated. The results are summarized in Table 2. It can be expected that FAD, in which flavin and adenine groups form an intramolecular complex, can form a complex weaker than flavin mononucleotide (FMN). Thus it is reasonable that the ΔH for FAD-tryptophane complexes is smaller than that for FMN-tryptophane complex (−7.4 kcal/mol).[1] The values of ΔH and ΔS for FAD–L–tryptophane and FAD–D–tryptophane systems,

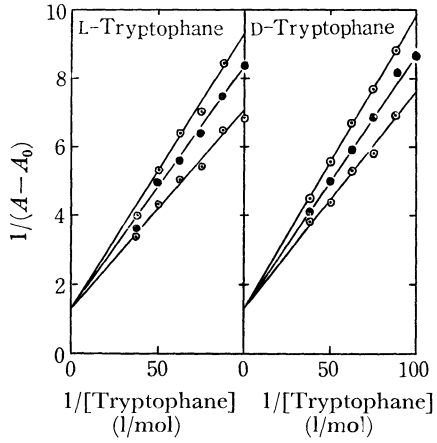

Fig. 1. Plots of $1/(A-A_0)$ at 505 nm *vs.* 1/(Tryptophane)
FAD: 4×10^{-4} mol/l } in 0.1 mol/l phosphate
Tryptophane: 1—2.7×10^{-2} mol/l } buffer (pH 7.0)
A: The absorbance of FAD-tryptophane solution
A_0: The absorbance of FAD solution
---- $1/(A-A_0)$ *vs.* 1/(Tryptophane) (l/mol)

Table 2. Thermodynamic data for the complex formation

System	K (l/mol) 25 °C	35 °C	45 °C	ΔH (kcal/mol)	ΔS (eu)
FAD-L-Tryptophane	$22._3$	$18._3$	$16._2$	−3.0	−3.9
FAD-D-Tryptophane	$20._5$	$17._6$	$15._2$	−2.8	−3.5

respectively, actually show no difference. It seems that ribosyl and ribityl groups can not sterically influence the complex formation. The side chain of indole might be located on the site opposite the ribosyl and ribityl groups in the FAD-tryptophane complex. Equimolar concentrations of FAD and tryptophane solutions were mixed for the determination of the molecular rotation of the complex. In the solution of 0.015 mol/l FAD and 0.015 mol/l L-tyrptophane or D-tryptophane, 20.3% or 19.2% FAD forms the complex at 28 °C as calculated from the values in Table 2. The molecular rotation of the mixture of FAD and tryptophane was compared with the sum of the individual molecular rotations of pure samples at 28 °C, and found to be almost the same. We see that the ribosyl and ribityl groups might not influence the molecular rotation for complex formation.

The plots of the molecular rotation *vs.* the concentration of FAD are given in Fig. 2. The molecular rotation of FAD was estimated to be $[M]^{28}_{590}$ -3.5×10^2 by the extrapolation to the zero concentration of FAD. In contrast to the extrapolation to higher concentration, the molecular rotation was found to be in the direction of positive value. Wave heights of polarograms are plotted against the concentration of FAD (Fig. 2). It seems that the reduction velocity of FAD decreases in higher concentration. The half-wave potential of FAD was found to be −0.55 V *vs.* SCE. The wave height is proportional to concentration in the region $10^{-3}\sim10^{-2}$ mol/l. Dimerization of FAD was suggested as a result of the nuclear magnetic resonance study.[6] The concentration dependence of the molecular rotation might confirm the dimerization of FAD. The dimer of FAD might hardly be reduced at the electrode, if the two flavin moieties associate with each other back to back and are enclosed by the two adenine moieties in FAD. If this is the case, the dimer can not form FAD-tryptophane complex. This can be justified because the plots in Fig. 1 fall on a line according to Benesi-Hildebrand's equation, which is derived by assuming the formation of 1 : 1 complex.

Dimerization constant K_2 could be calculated if we assume the observed molecular rotation R of FAD to be the weight average of monomer R_1 and dimer R_2:

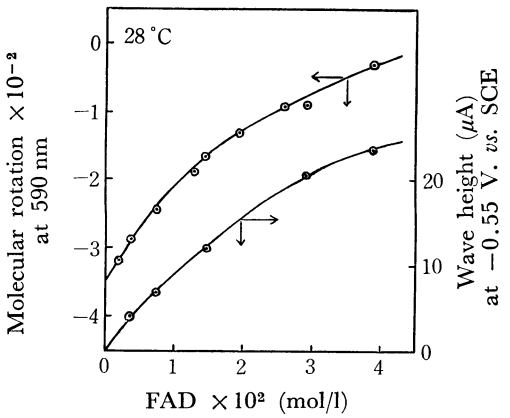

Fig. 2. The optical activities and the polarographic data of FAD 0.1 mol/l phosphate buffer (pH 7.0)

$$R = (M/C)R_1 + (2D/C)R_2, \quad C = M + 2D \tag{1}$$

where C, M, and D are FAD concentrations of total, monomer and dimer, respectively.

$$K_2 = D/M^2 \tag{2}$$

From Eqs. (1) and (2), we obtain,

$$R = -\left\{(R_2-R_1)2K_2\right\}^{1/2}\left\{(R-R_1)/C\right\}^{1/2} + R_2. \tag{3}$$

Plots of R *vs.* $\{(R-R_1)/C\}^{1/2}$ by means of this equation with data in Fig. 2 gives a straight line, from which the values of K_2 and R_2 are determined to be 22±5 l/mol and $+6.5\times10^2$ (28 °C), respectively. In the systems in Fig. 1, less than 3.3% FAD dimerizes and more than 17% FAD forms the charge transfer complex with tryptophane, which are calculated from the values of K_2 and the data in Table 2. The dimer concentration is not very high in the systems. The values of data in Table 2 might be reliable within 5% error.

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