Studies on Donor-Acceptor Complexes Relating to the Intramolecular Association of the Riboflavin and Adenosine Moieties of Flavin–Adenine Dinucleotide*

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ABSTRACT: The complexing of flavins has been examined in detail by light absorption and fluorescence emission to characterize more exactly the nature of the inter- and intramolecular forces involved. Donor-acceptor complexes are formed from derivatives of 6,7-dimethylisoalloxazine with derivatives of purines and pyrimidines. Mutual polarizability effects between nucleoside bases as apparent electron donors and flavins as apparent electron acceptors can be ascribed by molecular orbital theory to the complementarity of charge in a region from C_6 to N_9 of purines or, less extensively, C_6 to C_4 of pyrimidines to a region from N_1 to N_{10} of flavins. The additional avidity of binding of 6-aminopurines, such as adenine, probably reflects hydrogen bonding of the 6-amino group with the 2-keto function of flavin. Both types of forces lead to fluorescence quenching in flavin–adenine dinucleotide wherein the adenine and flavin moieties are compactly overlaid. Secondary effects caused by the nature and molarity of buffer suggest enhancement of complex stability through dispersion of coulombic forces between complexing halves in ionic media. Solvation and anionic charge on the N_9 substituents of the reactants impede complex formation. Elevation of temperature increases dissociation of these complexes. Neutral to slightly acid conditions allow complexing, whereas strong acid decreases association by protonating both the 6-amino group of adenine and the flavin.

The close intramolecular association of the riboflavin and adenosine portions of flavin–adenine dinucleotide (FAD) has been indicated by numerous investigations. Studies on light absorption of flavins (Warburg and Christian, 1938a,b) showed that the molar extinction coefficient of FAD at 260 mμ is lower than the sum of molar extinction coefficients of riboflavin and adenosine at this wavelength. This was confirmed (Whitby, 1953) by demonstrating that after hydrolysis of FAD the absorption rose to a level almost identical to the sum of the individual spectra of flavin mononucleotide (FMN) and 5′-adenylic acid (AMP). Studies on fluorescent properties of flavins closely paralleled the light absorption studies. Purine derivatives have been shown to quench efficiently the fluorescence of flavins (Weil-Malherbe, 1946; Burton, 1951). An internal quenching takes place within the FAD molecule that reflects an association between the adenine and fluorescent isoalloxazine moieties to form a nonfluorescent form which is in an equilibrium influenced by solvent, salt, and hydrogen ion concentrations (Bessey *et al.*, 1949; Walaas and Walaas, 1956; Cerletti and Siliprandi, 1958). The quenching of fluorescence in near-neutral solutions of FAD relative to riboflavin (Weber, 1950) is analogous to the diminution of absorption which occurs on combination of FMN and AMP to form FAD. In general, those substances which quench the fluorescence of riboflavin also produce changes in its absorption spectrum which are similar to that of FAD (Sakai, 1956; Yagi and Matsuoka, 1956; Harbury and Foley, 1958). There is in both cases a decrease of intensity and a slight band shift toward longer wavelengths. Other corroborative evidence for the internal complex of FAD has been supplied by observations on the greater light stability of FAD relative to riboflavin or FMN. Photolysis of the ribityl chain in the latter flavins occurs at a greater rate. Moreover, compounds which complex with riboflavin protect it from photodecomposition (Wada and Sakurai, 1953; Yagi and Ishibashi, 1954). The action of flavins as photosensitizers also is decreased by complexing. The internal quenching in FAD makes the dinucleotide less suitable as a transmitter of light energy, as had been substantiated by its decreased photodynamic action (Frisell *et al.*, 1959).

The general nature of the intramolecular association in FAD has become more apparent in recent years. The interactions among several isoalloxazine derivatives with purines and pyrimidines have been studied (Harbury and Foley, 1958). Comparisons of the apparent dissociation constants of the complexes formed suggest that partial electron transfer may be a dominant factor with complementary enhancement by hydrogen

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‡ This paper is the continuation of investigations concerned with flavins by D. B. M., to whom requests for reprints and inquiries should be addressed.

bonding. That purines form complexes with riboflavin more readily than do pyrimidines (Weber, 1950, 1958) favors this conclusion. The overall findings have led to considering such interaction, including the intramolecular case of FAD, as representing a situation somewhat similar to charge transfer (Szent-Györgyi, 1960). Studies on so-called charge-transfer complexes of FMN with indole compounds demonstrate an intense interaction between such pairs (Isenberg and Szent-Györgyi, 1958, 1959). Even though indole is only a fair donor as judged by the k value of its highest filled molecular orbital, the findings have been explained reasonably by the essential planarity of both indole and isoalloxazine rings together with opposite net electronic charges of the carbons and nitrogens along an overlapping region of mutual polarization between both molecules (Karreman, 1961, 1962). The ubiquity of biochemically reactive compounds, including flavins which undergo at least a partial charge transfer during complex formation, has been demonstrated recently (Wright and McCormick, 1964).

The present investigation was undertaken to quantitate more completely the effects that concentration and nature of reactants, solvent, temperature, and *p*H have on both inter- and intramolecular association of flavins. The results, largely obtained through the sensitive technique of measuring fluorescence quenching, lead to a more detailed understanding of the specific role and localization of interactive forces, ultimately as related to the intramolecular donor-acceptor complex of FAD.

Experimental

Materials. Purines, pyrimidines, and their derivatives were the purest available from Sigma Chemical Co. St. Louis, Mo. To obtain the free bases of those compounds available only as their salts, 2,6-diaminopurine sulfate, 4-amino-5-aminomethyl-2-methylpyrimidine dihydrochloride, and deoxycytidine hydrochloride were passed through an AG-1-X2 anion-exchange column. The purity of all free nucleosides was assessed by paper chromatography in two dimensions with 86% (v/v) aqueous *n*-butyl alcohol and the aqueous alcohol with 5% by volume of concentrated NH_4OH as descending solvents (Markham and Smith, 1949). Only deoxyguanosine and deoxycytidine contained detectable impurities as slower-moving components, presumably the corresponding nucleotides. As these amounts were small, no additional purification was done. Tetraacetylriboflavin was prepared by acetylation of D-riboflavin at the 2′, 3′, 4′, and 5′ positions (Kuhn and Wagner-Jauregg, 1933). The melting point of this compound was reported as 240° with decomposition; the melting point of the product was determined as 242° (uncorr) with decomposition. Ultraviolet and infrared spectra support the identification and purity of the product. Analogs of FAD were obtained chromatographically pure after synthesis by reaction of the pyridinium salt of FMN in pyridine with the appropriate 4-morpholine *N,N′*-dicyclohexylcarboxamidinium nucleoside-5′-phosphoromorpholidate (McCormick *et al.*, 1964).[1] All other chemicals used were of the highest purity from commercial sources.

Methods. Generally solutions contained 3.75×10^{-5} M riboflavin for light-absorption studies or 6.25×10^{-6} M riboflavin or its tetraacetyl derivative for fluorescence-emission studies with varying concentration of quencher up to 5×10^{-3} M in buffer (usually 0.01 M sodium phosphate buffer, *p*H 7) or spectral grade *N,N*-dimethylformamide at 30°. Initial solutions of FAD analogs were made to give an absorbancy reading of 0.2 at 445 mμ and room temperature (approximately 25°); these solutions were then diluted in 0.2 M buffers of varying *p*H to give working solutions of approximately 1.44×10^{-6} M. Absorbancy at 445 mμ was measured in a Cary Model 14 spectrophotometer at room temperature. Measurements of fluorescence were done with an Aminco-Bowman spectrophotofluorometer (Cat. No. 4-8106) which was equipped with a xenon lamp (Cat. No. 416-992) and a photomultiplier tube (1P 21) and slit arrangement No. 2. The activating wavelength was set at an optimum of 468–469 mμ and fluorescence read at 510–520 mμ. The cell compartment was specially thermostated by fitted brass blocks which were bored to allow passage of liquid at a desired temperature maintained within ±0.5° by a Haake Model F constant temperature circulator. The temperature of solution within the cell was measured through a copper-constantan thermocouple with a Leeds and Northrup millivolt potentiometer (No. 8691). For more rapid attainment of constant temperature, continuous stirring of the cell contents was accomplished by a glass-coated iron bar with an external magnetic rotor.

Results

The decrease in 445-mμ absorbancy of riboflavin caused by complexing with adenine in aqueous solution at room temperature is illustrated in Figure 1 by a double-reciprocal plot. The apparent dissociation constant (K_{app}) for the 1:1 complex formed is calculated from the slope (Harbury and Foley, 1958) as 8×10^{-3} mole/liter. This is in good agreement with the values of 7.4×10^{-3} (5°) and 8.3×10^{-3} (17°) found previously by measuring the quenching of riboflavin fluorescence by adenosine (Weber, 1950).

The ratio of fluorescence intensities (I_0/I where I_0 = fluorescence of flavin alone and I = fluorescence of flavin with quencher) affords a more sensitive measure of the combining abilities of nonfluorescent compounds with flavins. As shown in Figure 2, the change in this ratio is influenced considerably by the nature of the

[1] Abbreviations for FAD analogs indicate the purine or pyrimidine which has replaced adenine by the following capital letters: H, hypoxanthine; G, guanine; X, xanthine; C, cytosine; U, uracil; T, thymine. Thus: FHD, flavin–hypoxanthine dinucleotide; FGD, flavin–guanine dinucleotide; FXD, flavin–xanthine dinucleotide; FCD, flavin–cytosine dinucleotide; FTD, flavin–thymine dinucleotide; FdeAD, FdeHD, FdeGD, FdeXD, and FdeCD, the deoxy derivatives of flavin–adenine, flavin–hypoxanthine, flavin–guanine, flavin–xanthine, and flavin–cytosine dinucleotides.

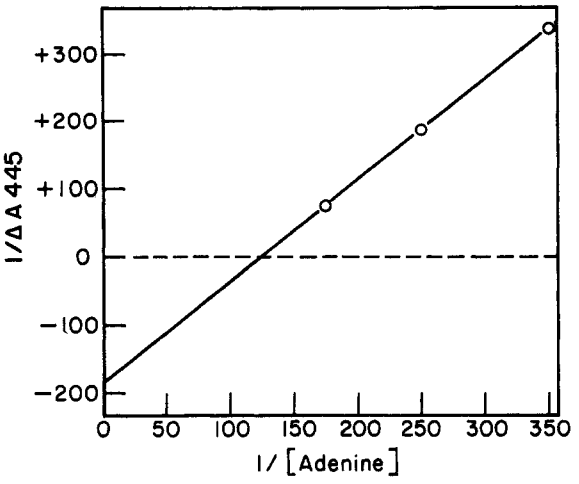


FIGURE 1: Double-reciprocal plot of change in riboflavin absorbancy at 445 mμ caused by varying concentration of adenine. Riboflavin was 3.75 × 10^{-5} M in 0.01 M sodium phosphate buffer, *p*H 7, at room temperature.

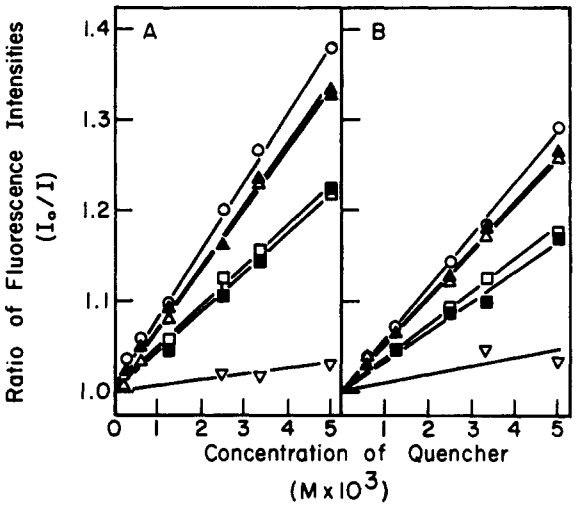


FIGURE 2: Change in the ratio of fluorescence intensities of flavin as influenced by the nature of N_9 substituents on both flavin and adenine. Data are given in (A) for riboflavin and in (B) for tetraacetylriboflavin. Flavins were 6.25 × 10^{-6} M in 0.01 M sodium phosphate buffer, *p*H 7, with adenine (○), adenosine (△), deoxyadenosine (▲), AMP (□), and deoxy-AMP (■), and in dimethylformamide with adenosine (▽), all at 30°.

side groupings on both isoalloxazine and adenine rings at *p*H 7 and 30°. The slopes of these lines, from which the apparent association constants are calculated (Weber, 1950), indicate that flavin complex formation follows the order adenine > adenosine > AMP and is considerably diminished in dimethylformamide. Only insignificant change is caused by absence of the 2'-

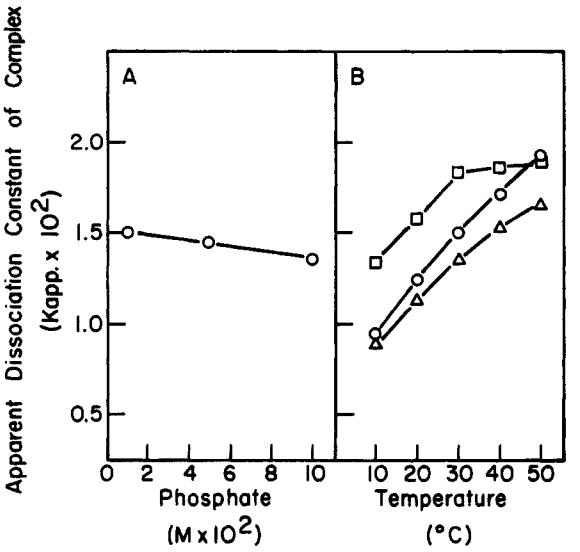


FIGURE 3: Effects of concentration of phosphate and temperature on apparent dissociation constants of flavin complexes. In (A), sodium phosphate buffers, *p*H 7, and 30° were used with riboflavin plus adenosine. In (B), 0.01 M sodium phosphate buffer, *p*H 7, was used with riboflavin plus adenosine (○), riboflavin plus adenine (△), and tetraacetylriboflavin plus adenine (□). Flavins were 6.25 × 10^{-6} M with quenchers at 5 × 10^{-3} M.

hydroxy function from the N_9 substituent of the adenine moiety as evidenced by similar behavior of adenosine and deoxyadenosine and of AMP and deoxy-AMP. Moreover, the complexing affinity of riboflavin with these compounds (part A) is decreased by acetylation of the N_9-ribityl chain (part B). The lesser ability of tetraacetylriboflavin to form complexes is also apparent from the more intense fluorescence of the pure compound in dimethylformamide when compared with riboflavin. This may be attributed in part to more extensive self-quenching of riboflavin which tends to dimerize, especially in concentrated solutions. Both increased size and polarity of the N_9 substituents, especially the anionic phosphate ester group of AMP, are detrimental to complex formation; however, greater complexing is seen in aqueous media than in dimethylformamide, a less heteropolar solvent.

Increasing the ionic strength of the medium has a slight enhancing effect on formation of the riboflavin and adenosine complex as shown in Figure 3 (part A) where the K_{app} for dissociation decreases with increasing phosphate concentration at *p*H 7 and 30°. Higher temperatures cause a shift in equilibria for dissociation of complexes of riboflavin and tetraacetylriboflavin with adenine and adenosine (part B). With these temperature studies, the effect of the N_9 substituents of both flavin and adenine again is observed. Relatively greater dissociation with increasing temperature is exhibited by the riboflavin complex with adenosine than with adenine; also the weaker complex of tetraacetylribo-

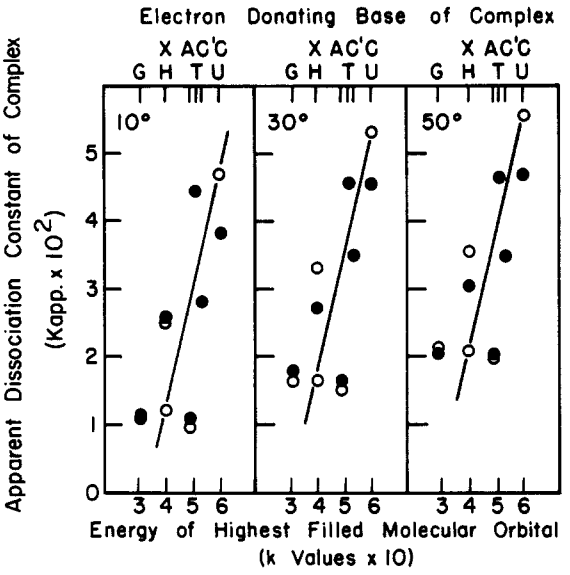


FIGURE 4: Relationship of electron-donating abilities of nucleosides as reflected by energies of their highest filled molecular orbitals to apparent dissociation constants of the riboflavin complexes at different temperatures. Riboflavin was 6.25 × 10^{-6} M in 0.01 M sodium phosphate buffer, *p*H 7, with 5 × 10^{-3} M ribofuranosides (○) and deoxyribofuranosides (●) of guanine (G), xanthine (X), hypoxanthine (H), adenine (A), thymine (T), 5-methyldeoxycytosine (C′), cytosine (C), and uracil (U).

TABLE I: Dissociation Constants of Complexes of Riboflavin with Purine and Pyrimidine Analogs.[a]

Analog	K_{app} × 10^2
Purines	
2-Amino-6-methylaminopurine	5.8
Guanine	7.1
Xanthine	7.7
2-Amino-6-methylpurine	11.4
8-Azaadenine	11.5
8-Azaxanthine	12.7
Adenine	13.1
2,6-Diaminopurine	15.3
2-Aminopurine mononitrate	15.7
Hypoxanthine	25.4
6-Methylpurine	200
Purine	550
Pyrimidines	
2,4,6-Triaminopyrimidine	17.8
4,5-Diaminopyrimidine	20.6
2-Aminopyrimidine	36.2
Thymine	41.0
4-Amino-5-aminomethyl-2-methylpyrimidine	50.0
Cytosine	55.0
Uracil	190
6-Azathymine	200
6-Azauracil	700

[a] Determined by fluorescence quenching of 6.25 × 10^{-6} M riboflavin with approximately 5 × 10^{-3} M analog in 0.01 M sodium phosphate buffer, *p*H 7.0, at 30°.

flavin and adenine is less sensitive at elevated temperature. Thus solvation effects appear to exert a significant secondary role on complex formation of reactants having polyhydroxy chains appended.

A general correlation of electron-donating ability of nucleosides to the stability of complexes formed with riboflavin at *p*H 7 and different temperatures is shown by the data in Figure 4. Uridine and deoxyuridine have large values of K_{app} for dissociation which do not fall within the plot for the other nucleosides. Also, 6-methylaminopurine forms a relatively stable riboflavin complex whose K_{app} was determined as 6.6 × 10^{-3}, 10.9 × 10^{-3}, and 15.1 × 10^{-3} mole/liter at 10°, 30°, and 50°, respectively. The donor abilities of the bases are indicated by the *k* values for their highest filled molecular orbitals as calculated by Hückel's linear combinations of atomic orbital approximations by molecular orbital theory (Pullman and Pullman, 1963). Again the similarity in behavior of ribo- and deoxyribofuranosides of the bases is in agreement with their nearly equal abilities to form complexes with riboflavin. The stability of all such complexes is decreased with increase in temperature as seen by the shifts of apparent dissociation constants to higher values at higher temperatures.

More extensive comparisons of the apparent dissociation constants of riboflavin with analogs of purines and pyrimidines at *p*H 7 and 30° are given in Table I. These data extend the foregoing observations that purines are better complexers than pyrimidines; molecular variations which increase electron-donating propensities enhance the complex stability; and hydrogen bonding, particularly by amino substituents at the 6 position of the base, facilitates the complex formation.

Formation of a riboflavin-adenosine complex is influenced by change in *p*H and concentration of reactants in 0.1 M buffers at *p*H 7 as shown by the data in Figure 5. In the low acid range, I_0/I approaches the same value as the protonation of riboflavin produces a nonfluorescent species, and the fluorescence is quenched independently of the riboflavin-to-adenosine ratio. Moreover, the tendency toward confluence of the lines in the acid region reflects protonation of the adenosine amino group, which has a pK_a of 3.3 (Long, 1961), and complexing is impeded. The *p*H range of 5–7 appears optimal for complex formation where a greater ratio of adenosine to flavin increases the quenching most markedly.

The effect of *p*H change at 30° on the intensity of fluorescence of several analogs of FAD, in which the adenosine moiety was replaced by other purine and pyrimidine ribo- and deoxyribofuranosides, is shown in

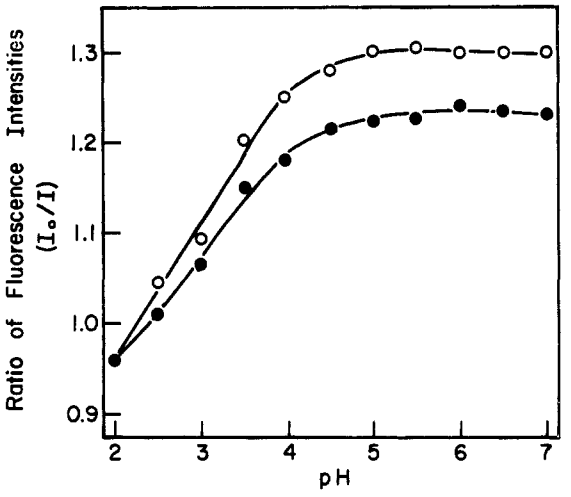


FIGURE 5: Change in the ratio of fluorescence intensities of riboflavin as influenced by *p*H and reactant concentration. Riboflavin at 6.25×10^{-6} M with 3.75×10^{-3} M adenosine (○) and at 1.87×10^{-6} M with 5×10^{-3} M adenosine (●) was buffered in 0.1 M solutions at 30° covering the following *p*H range: KCl-HCl, 1–2; glycine-HCl, 2.5–3.5; sodium acetate, 4–5; sodium phosphate, 5.5–7.

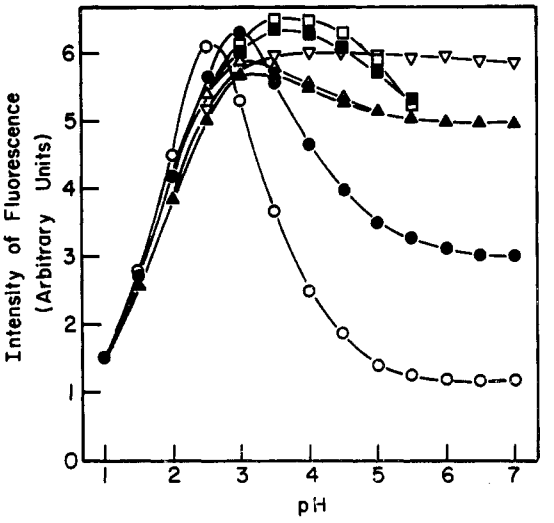


FIGURE 6: Effect of *p*H change on the intensity of fluorescence of FAD analogs. FAD (○), FdeAD (●), FHD (△), FdeHD (▲), FCD (□), FdeCD (■), and FTD (▽) were approximately 1.44×10^{-6} M at 30° in 0.2 M buffers as described under Figure 1.

Figure 6. The intramolecular complexing, which is observed best above *p*H 4, is stronger with analogs containing the purine bases and weaker with pyrimidines, e.g., FAD and FHD versus FCD and FTD. In addition, greater quenching is seen with those analogs which bear a 6-amino group when compared with similar keto compounds, e.g., FAD versus FHD and FCD versus FTD. Both ribo- and deoxyribo-compounds are similar as expected, e.g., FAD versus FdeAD, FHD versus FdeHD, and FCD versus FdeCD. Similar results were obtained with other analogs, e.g., FGD, FdeGD, FXD, and FdeXD, which expand these observations. All of these data substantiates the foregoing results and extends the phenomena observed to the intramolecular situation.

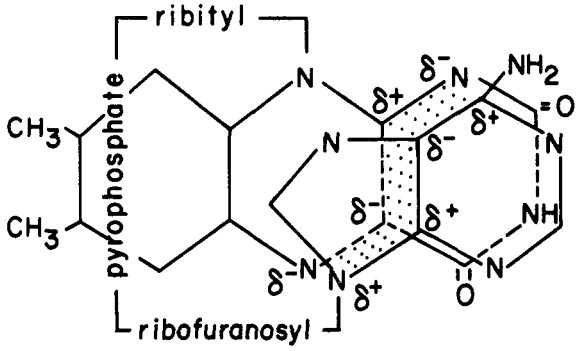
Discussion

The preceding results allow a more detailed insight into the molecular association of flavins with bases containing purines and pyrimidines than has been previously possible. The complexes which result show only small changes in the flavin absorption spectrum and therefore are probably not of the classic charge-transfer type wherein electronic perturbation between complexing halves is sufficient to exhibit an additional absorption band absent in either separate partner. However, the flavin complexes studied herein may be characterized as of the donor-acceptor type as broadly defined (Mulliken and Person, 1962). Such a classification includes mutual polarizability or "partial" charge-transfer effects which may occur within these complexes and the additional role of hydrogen bonding which may become especially important with 6-amino bases. Actually both types of forces show close parallelism in their infrared spectra, as the intensity of a vibration which changes the mixing of resonance structures contributing to the ground-state wave function is similarly determined by quantum chemical consideration for hydrogen-bonded and partial or total charge-transfer complexes.

In general, the lower the k value for the highest occupied orbital the lower the ionization potential of the compound and the easier an electron or fraction of an electronic charge can be donated, with other factors being equal. The electron-accepting property of riboflavin is constant within the series of compounds studied and has a k value for its lowest empty molecular orbital of -0.344, which indicates a good electron affinity. The decrease in apparent dissociation constants of riboflavin-nucleoside complexes with increasing donor ability of the nucleoside may be consistent with the idea of a partial molecular charge-transfer nature of the association. The lower k values for the purines as compared with pyrimidines agree with the greater complexing of the former as nucleosides. Furthermore, the relatively large magnitude of the difference in reactivity between these two classes of bases, as observed by their quenching abilities, points to aggrandizement of complexing conferred by extension of the interaction between flavin and purine as including both five- and six-membered rings in the latter.

An examination of calculated electronic charges approximated by linear combinations of atomic orbitals–molecular orbital theory for a purine, e.g., adenine, and for the oxidized 6,7-dimethylisoalloxazine system, e.g., riboflavin (Pullman and Pullman, 1963),

suggests regions of potential charge complementarity within these molecules which is quite similar to the so-called charge-transfer overlap recently recognized between flavin and indole rings (Karreman, 1961, 1962). Net charges can be calculated to show that atoms[2] including and immediately between N_1 and N_{10} of the flavin and including and between C_6 and N_9 of adenine are oppositely charged, as indicated for the intramolecular case of FAD illustrated as follows:



The relative magnitude of the net charges in both overlapping portions of the FAD molecule may be considered. Prior to receiving a fraction of an electron, the net charge belt for the flavin in the ground state is: $N_1 = -0.470$, $C_\alpha = +0.167$, $C_\beta = +0.021$, and $N_{10} = -0.035$. Following acceptance of a fraction of an electron with additional contribution to the net charges of these centers, the net charges become more negative with the greatest contribution made at N_{10}. Prior to donating an electron, the net charge belt for adenine is: $C_6 = +0.133$, $C_5 = -0.087$, $C_4 = +0.037$, and $N_9 = +0.407$. With loss of a fraction of an electron during polarization with the flavin, the net charges at these centers of adenine become more positive with greatest differences shown at C_6 and N_9. Hence the net charge differences between these regions of flavin and adenine in the ground state already are nearly all oppositely polarized and sizable. In the ground state, but where an electron has been partially exchanged, the differences in net charges become greater and stabilization of the complex would be expected. The greatest charge disproportion between flavin and purine occurs at N_1 and C_6 and N_{10} to N_9. Also donation of a fraction of an electron to the π system of the flavin elicits the largest increase at N_{10}, the "free-radical "region. The essential coplanarity of the entire overlapping regions is well established and easily recognized from the close fit seen with molecular models. As expected from the foregoing data, extended regions of complexing and the sizable contribution from the N_{10} of flavin with N_9 of adenine may explain why a pyrimidine which lacks the N_9 position complexes more weakly. The greater association of flavin-base complexes found in solvents with increasing ionic strength may be explained on the basis of a strong polarization reaction (Briegleb, 1961).

[2] Conventional numbering of positions in the heterocycles is followed. Here C_α and C_β designate the adjoining carbons bonded to N_1 and N_{10}, respectively, of the isoalloxazine system.

Whereas weak charge transfer is favored by homopolar solvents, strongly polar solvents generally promote the spontaneous transfer by depolarizing the strong coulombic attractions generated between the donor and acceptor portions. The extreme case of separation of a flavin-free radical does not occur to any significant extent, as electron spin resonance signals are not observed (Massey and Gibson, 1964).

The contribution of hydrogen bonding to formation and stability of the flavin-adenine complex, both inter- and intramolecular, is appreciable as was noted in the comparisons of results with 6-amino versus 6-keto analogs of purines and pyrimidines and the corresponding FAD analogs. Adenosine and cytidine are more effective complexers than is indicated by their k values in relation to those of inosine and uracil, respectively. The behavior of the 6-amino compounds indicates additional forces which enhance formation and stability of the flavin complexes. Hydrogen bonding through the 6-amino groups of adenyl and cytosyl portions is the likely explanation when the proximity of these amino functions to the 2-keto function of the isoalloxazine is visualized in a molecular model of the complex. The net enhancement is difficult to distinguish quantitatively from mutual polarizability per se, especially since secondary effects of solvation and side groupings are present. For these reasons, calculations of the Van't Hoff isochores for heats of dissociation of the complexes give varying values at extremes of temperature. However, approximately +1.5 to +2.5 kcal/mole may be in the range of ΔH for the riboflavin-adenine complex near room temperature.

Finally, the biological significance of the intramolecularly complexed form of FAD is not yet obvious. Under physiological conditions of pH, temperature, and perhaps ionic strength, the equilibrium is near 80% for the complexed species. This value can be calculated from the present results. FdeAD approaches this equilibrium ratio and is also coenzymatically active; other FAD analogs exist in lesser degrees of complexing and are essentially inactive (McCormick *et al.*, 1964). A dependence of complex form on coenzymatic activity may yet be directly demonstrated.

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Corrinoid Compounds of *Methanobacillus omelianskii*. I. Fractionation of the Corrinoid Compounds and Identification of Factor III and Factor III Coenzyme*

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ABSTRACT: The corrinoid compounds from an extract of *M. omelianskii* were separated into ten components by chromatographic methods. 5-Hydroxybenzimidazolylcobamide and its Co-5′-deoxyadenosyl derivative (Factor III coenzyme) are the most abundant corrinoid compounds in this organism, accounting for 31 and 23%, respectively, of the total vitamin B_{12} activity in the bioassay with *Escherichia coli* 113-3.

The coenzyme was synthesized from Factor III and found to be identical with the natural product in spectrum, paper chromatography, paper ionophoresis, and in the glutamate mutase assay. The nature of four other yellow coenzymes is discussed.

The possible participation of a vitamin B_{12} coenzyme in the metabolism of one-carbon compounds of *Methanococcus vannielii* and *Methanobacillus omelianskii* was first suggested by Stadtman (1960) because of the high level of cobamide coenzymes in these organisms. The recent finding of Blaylock and Stadtman (1963) and Wolin *et al.* (1963) that the methyl group of synthetic Co-methyl cobalamin can be converted into methane by cell-free extracts of *Methanosarcina barkeri* and *M. omelianskii* indicates a possible role of the vitamin as a methyl-carrying coenzyme in the methane fermentation. The presence of methyl cobalamin or one of its analogs in bacteria or other organism has not been demonstrated so far, although substantial indirect evidence for the participation of an enzyme-bound methyl cobalamin intermediate in the biosynthesis of methionine has been reported by Foster *et al.* (1964). The present investigation was started with the objective of determining whether a Co-methyl cobamide is present in cells of *M. omelianskii*. We soon found, in agreement with previous reports (Neujahr and Callieri, 1958, 1959), that this organism contains a variety of corrinoid compounds which could not be positively identified by chromato-

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