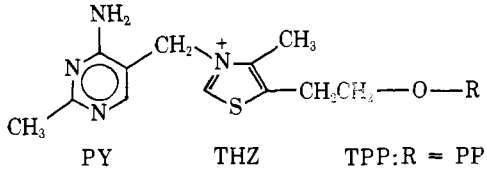
Solvent Effects on Thiamin–Enzyme Model Interactions. I. Interactions with Tryptophan†

Bijan Farzami, Yitbarek H. Mariam, and Frank Jordan*

ABSTRACT: The solvent polarity dependence of the interaction between thiamin and tryptophan was studied by spectrophotometric methods. The ultraviolet (UV) data clearly indicate that the interaction is weakened when the complex is transferred from water to aqueous ethanol or aqueous dioxane. The interaction of thiamin and tryptophan could also be detected by fluorescence-quenching studies (excitation of tryptophan at 287 nm, maximum emission at 348 nm). Appropriate treatment of the quenching data allowed dissection into static and dynamic contributions. A pyrimidine derivative related to thiamin, both in its neutral and protonated states, was shown to interact with tryptophan by fluorescence techniques, but not by UV. A thiazolium model was shown to interact with tryptophan by UV but was an inefficient quencher of the tryptophan fluorescence. Theoretical models are presented to explain the solvent dielectric constant dependence of the association constant between tryptophan and thiamin. Both electrostatic and dispersion forces are found to contribute to the stability of the complex.

Thiamin pyrophosphate (TPP[1]) is a coenzyme whose thiazolium ring forms a covalent intermediate with the substrates of its enzymatic reactions (Krampitz 1969).



PY THZ TPP: R = PP
THC: R = H

It is important to elucidate the role of all components of this cofactor in the catalytic processes. Sable and Biaglow (1965) reported that thiamin forms weak molecular complexes with the indole nucleus of tryptophan and they suggested that such complexation may be of importance in binding the coenzyme to the enzyme. Crosby and Lienhard (1970) and Crosby et al. (1970) studied model reactions related to the decarboxylation of pyruvate by TPP and the enzyme pyruvate decarboxylase (PDCase) and found that the model (THZ-bound pyruvate) decarboxylated considerably (about 10^4 times) faster in ethanol than in water. All other kinetically significant steps in the mechanism were also found to occur faster in ethanol than in water. They suggested that one of the contributions of PDCase to the overall rate enhancement would be creation of a low-dielectric medium (hydrophobic, resembling ethanol more than water).

Based largely on the above findings, we have undertaken a study of the solvent dielectric constant dependence of the interaction in enzyme–coenzyme models. Elsewhere, we shall describe our findings on the 2,3,4-trimethylthiazolium iodide system, as model for the interaction of the THZ ring of thiamin with a negative charge.

In this communication, we report the results of UV and fluorescence studies on the interaction between thiamin and indole derivatives in solvent mixtures with different dielectric constants. The results also allow us to probe the theoretical nature of the interaction between the two partners in the complex.

Experimental Section

D,L-Tryptophan was purchased from Fisher, and THC and 3-indoleacetic acid from Eastman. All inorganic reagents used were high-purity chemicals employed as received. Distilled, deionized water was employed, as well as absolute alcohol. pH was measured and adjusted on a Radiometer pH, Model 26 instrument equipped with autoburette and titrator assembly. The ionic strength was maintained at 1.0 in all experiments.

UV measurements were performed in 1-cm 3-mL quartz cuvettes on a Cary 14 or Beckman Acta III spectrophotometer equipped with thermostatted cell compartments, in most experiments maintained at 30 ± 0.1 °C. The spectra for the K_{assoc} determination were collected over the 300–400-nm wavelength range (Cary 14) or at specific single wavelengths (Beckman Acta III digital readout).

Fluorescence measurements were performed on a Farrand MK1 spectrofluorometer equipped with a thermostatted (30 ± 0.1 °C) cell compartment. Doubly distilled, deionized, degassed, and oxygen-free water (purged with argon or nitrogen) was used. One-centimeter 3-mL glass cuvettes were used, with an excitation wavelength of 287 nm and a maximum emission (tryptophan) occurring at 348 nm.

Solvent mixtures were made up by volume % (v/v).

Treatment of UV Data. Preparation of solutions for measurement of the association constant, as well as the Benesi–Hildebrand equation, in a form suitable to treat the data, followed closely the prescription of Mieyal et al. (1969) for a 1:1 complex.

$$THC + TRP \rightleftarrows THC \cdot TRP \quad (1)$$

† From the Carl A. Olson Chemical Laboratory of Rutgers, the State University, Newark, New Jersey 07102. *Received April 26, 1976.* This work was supported by Grant AM-17495 from the National Institute of Arthritis, Metabolic and Digestive Diseases, United States Public Health Service; by the Rutgers University Research Council; and by the Biomedical Research Support (to Rutgers University). Part of this work was submitted to the Graduate School, Rutgers University, in partial fulfillment of the requirements for the Ph.D. (B.F.), October 1975. Presented, in part, at the 7th Northeast Regional ACS Meeting, Burlington, Vermont, August 1974.

[1] Abbreviations used are: TPP, thiamin pyrophosphate; THC, thiamin chloride; PY, 4-amino-2-methylpyrimidine; TRP, tryptophan; 3IA, 3-indoleacetic acid; THZ, thiazolium; BH, Benesi–Hildebrand plot; Sc, Scatchard plot; PDCase, pyruvate decarboxylase; UV, ultraviolet spectroscopy.

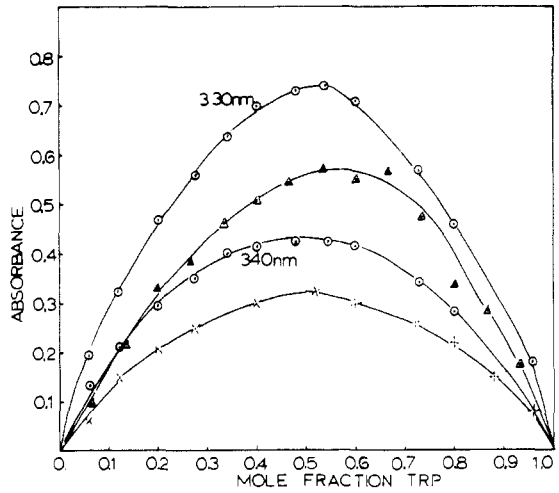


FIGURE 1: Job's plot for THC–TRP interactions: (⊙) THC in excess over TRP at pH 5 in H_2O; (x) THC in excess over TRP pH 5 in 40% (v/v) ethanol in H_2O; (△) 3IA in excess over THC pH 6.5 in H_2O.

$$\Delta = \frac{A_0 K \Delta_0}{1 + A_0 K} \tag{2}$$

In eq 2, Δ is the change in absorbance due only to the absorbance of the complex; A_0 is the initial concentration of component A, always in excess of component B ($A \geq 10 \times B$); Δ_0 is the maximum change in the spectroscopic property obtained by extrapolation of A to infinite concentration. This equation is valid providing $A_0 \geq 10 \times B_0$ and that Δ is clearly distinguishable from the spectra of the components. Biaglow et al. (1969) showed that in the wavelength range 320–380 nm this indeed is the case (indicated by a "tail" on the long-wavelength side of the principal absorption bands of the components). K was determined from a double-reciprocal version of eq 2

$$\frac{1}{\Delta} = \frac{1}{A_0 K \Delta_0} + \frac{1}{\Delta_0} \tag{3}$$

where A_0, in most cases, stood for 0.05–0.25 M THC, B_0 for 0.005 M TRP (in some cases, 3-indoleacetic acid was in excess), the ionic strength being maintained with KCl or NaCl. A plot of $1/\Delta$ vs. $1/A_0$ was linear. K was determined from the intercept on the negative abscissa axis ($1/\Delta = 0$).

The K_{assoc} was also determined from a Scatchard-type plot (1949), as elaborated for UV data by Derenleau (1969), by plotting Δ/A_0B_0 vs. Δ/B_0. Δ is the spectroscopic change due to complex; B_0 is the concentration of the species present at constant limiting concentration ($A_0 \geq 10 \times B_0$). Derenleau (1969) presented cogent arguments indicating that the Scatchard plot is more sensitive to multiple equilibria involving weak molecular complexes than is the B–H plot. In particular, linearity of a plot of Δ/A_0B_0 vs. Δ/B_0 for the process here investigated, e.g.

$$\text{THC} + \text{TRP} \overset{K_1}{\rightleftharpoons} \text{THC} \cdot \text{TRP} \overset{K_2}{\rightleftharpoons} \text{THC}_2 \cdot \text{TRP} \tag{4}$$

could indicate either that $K_2 \ll K_1$ or $K_1 \simeq K_2$ (i.e., identical microscopic association constants). Observation of two intersecting lines would allow determination of both K_1 and K_2 and of the extinction coefficients corresponding to THC·TRP($\epsilon 1$) and THC_2·TRP($\epsilon 2$), providing accurate data are available in the entire range of saturation function, s, where $s = \Delta/\epsilon B_0$. We employed this treatment to confirm the quantitative trend suggested by the Benesi–Hildebrand plot and to see if multiple equilibria existed under our experimental conditions.

Under strictly linear conditions, the slope of Scatchard plot is related to $-K_{assoc}$, the intercept at the Δ/A_0B_0 axis to $K\epsilon$, and the intercept along the ϵ/B_0 axis to ϵ. All data (both Benesi–Hildebrand and Scatchard treatments) were processed through linear least-squares, and the results reflect such computer-generated numbers.

Results

Studies on the Stoichiometry of the Complex. Several spectroscopic tests were performed to test the stoichiometry of the complex formed between THC and TRP.

(a) First, absorbances at 330 nm were plotted against absorbances at 340 nm of the same solution containing THC or TRP in excess in the concentration range of 0.0045 to 0.075 M. Linear behavior was observed extrapolating to the origin and implying a single 1:1 complex. A more severe test consists of plotting ratios of absorbances, e.g., A_{350nm}/A_{340nm} vs. A_{360nm}/A_{340nm} (five points in the above concentration range). Within experimental error, a single point was observed (0.575, 0.575, 0.570, 0.570, 0.578 for A_{350nm}/A_{340nm} and 0.302, 0.320, 0.325, 0.306, and 0.308 the corresponding values for the A_{360nm}/A_{340nm} ratio). This suggests the existence of a single 1:1 complex *or* (the unlikely possibility) of two complexes, providing the ratios of their extinction coefficients at the three wavelengths are nearly identical (Coleman et al., 1970; Wallace, 1960; Ainsworth, 1961; Wallace and Katz, 1964; Katakis, 1965; Varga and Weatch, 1967).

(b) Secondly, Job's plot (Job, 1928) was constructed keeping the sum of TRP and THC concentrations constant at 0.05 M and varying the mole fraction TRP from 0 to 1. The plots at 330 and 340 nm in H_2O and in 40% (v/v) ethanol gave symmetrical (maxima at 0.5 mol fraction) profiles under all conditions (Figure 1), suggesting a 1:1 complex. With 3IA in excess, the unsymmetrical Job's plot suggested the existence of a 2:1 complex of the type, $(3IA)_2$·THC. As was argued by Derenleau (1969), this test does not necessarily rule out two types of complexes coexisting.

(c) Thirdly, a computer-fitting test was designed, as outlined below, for a theoretical 1:1 complex:

$$K = (A \cdot B)_{eq}/(A)_{eq}(B)_{eq} = \frac{\text{OD}/\epsilon}{(A_0 - \text{OD}/\epsilon)(B_0 - \text{OD}/\epsilon)} \tag{5}$$

Assuming ϵ values of 20–1000, ϵ was incremented by two units, and a K was calculated for each concentration employed in any one series. The true K is independent of concentration. For each ϵ, an average $\bar{K}$ was found and a plot of $\Delta\bar{K}/\bar{K}$ (where $\Delta\bar{K}$ is the average of the deviations of each individual K from the average $\bar{K}$) vs. ϵ was constructed. These plots always exhibited one minimum only (Figure 2, Supplementary Material). Sharp minima were obtained for a 1:1 THC·TRP model (THC in excess) with minimum at ϵ = 170–200, K = 4.8 M^{-1}; and a 2:1 THC·TRP model (THC in excess), the minimum at ϵ = 600 and K = 318 M^{-2}. According to this criterion, the plots assuming a 1:1 complex with TRP or 3IA in excess were always shallower, perhaps implying dimerization of TRP (or 3IA) prior to attachment of THC. If there is only one specie present, this criterion supports either a THC·TRP or THC_2·TRP complex.

Overall, the three criteria strongly suggest that the predominant complex under our experimental conditions (mostly THC in excess) is a single 1:1 complex.

Solvent Effects on K_{assoc}. There is excellent agreement in

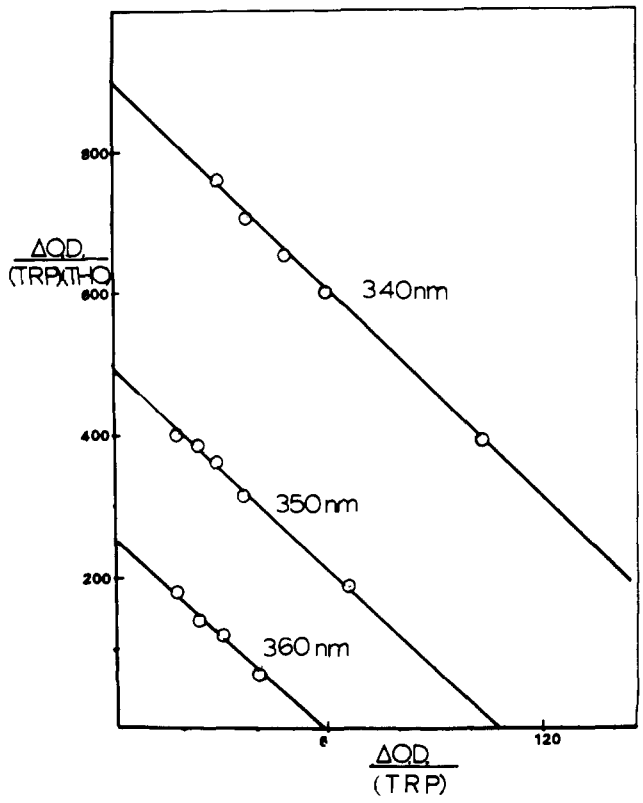


FIGURE 4: Scatchard plot of UV data at pH 5, THC in excess, 30 °C.

K's and ϵ's obtained by the B–H and Scatchard treatments at pH 5, with THC in excess over TRP (Table I, Supplementary Material), as shown in Figures 3 (Supplementary Material) and 4 as representative of the data treatment.

Table II presents the K_{assoc} values with THC in excess over TRP in H_2O–ethanol mixtures. In H_2O–dioxane mixtures, the trend of decreasing association with decreasing solvent dielectric constant is again found (Table III, Supplementary Material).

To prove that the observation also holds for other indole derivatives, the K_{assoc} of 3IA·THC was determined in H_2O–ethanol mixtures with 3IA in excess. Determinations were performed at pH 6.2 where both solutes are soluble enough. The K_{assoc} was slightly larger with 3IA in excess (5.3) than with THC in excess. Also, the large extinction coefficients calculated in this case could be due to 3IA dimerization (Dimicoli and Hélène, 1973), i.e., formation of some $(3IA)_2$·THC complexes (Table IV, Supplementary Material).

Further results include pH dependence of K_{assoc} between THC and TRP, indicating that K_{assoc} is nearly independent of pH but that the extinction coefficient changes with pH (Table V, Supplementary Material).

The temperature dependence of K_{assoc} at pH 5 was determined in a limited range (30, 35, 45 °C). The association is weaker at higher temperatures (Table VI, Supplementary Material). It is favored by enthalpy ($\Delta H = -4.7$ kcal according to B–H, -3.2 kcal according to Scatchard treatment) and disfavored entropically (~ -10 eu), as was found at a higher pH by Mieyal et al. (1969).

No significant break (nonlinearity) was observed in either the B–H or Scatchard plots in any of the above quoted results.

K_{assoc} between TRP and 2,3,4-trimethylthiazolium iodide was also measured at pH 6.0, 30 °C. B–H plot of the data gave a value of 5.5 ± 0.5, strongly implying that at this pH, at least,

TABLE II: Effect of Added Ethanol on K_{assoc} of THC·TRP (THC in Excess), pH 5.0, 30 °C.

Ethanol (%, v/v)	D^a	$K_{BH}{}^b$ (M^{-1})	$\epsilon_{BH}{}^b$	R^c	$K_{Sc}{}^d$ (M^{-1})	$\epsilon_{Sc}{}^d$	$R^{c,d}$
0	77.0	4.4	202	0.999	4.3	205	0.994
4.0	74.5	4.8	181	0.999	4.7	184	0.990
5.6	73.5	4.2	226	0.995	3.9	229	0.992
10.0	71.5	3.8	199	0.996	4.3	181	0.987
20.0	66.7	2.9	166	0.964	4.6	145	0.968
26.8	63.6	2.5	226	0.996	2.8	201	0.962
33.3	60.0	0.80	250	0.997	0.67	nd[e]	0.996
50.0	53.0	cannot be defined (K_{BH} and K_{Sc} both zero from slopes)					

[a] Dielectric constant. [b] Benesi–Hildebrand treatment. [c] Correlation coefficient. [d] Scatchard treatment. [e] nd, not defined.

the THZ ring is the predominant binding site of THC toward TRP.

The Quenching of Tryptophan Fluorescence by Thiamin. Upon excitation at 287 nm, the indole ring of tryptophan gives maximum fluorescence emission at 348 nm. We have found that this fluorescence could be quenched by THC. Depending on the concentration range of quencher, different quenching behavior was observed. At very low TRP ($<10^{-5}$ M) and THC ($<3 \times 10^{-4}$ M) concentrations, the Stern–Volmer plots were strictly linear (Table VII), i.e.

$$F_0/F = 1 + K_{SV}Q \quad (6)$$

where F_0 is the apparent fluorescence of TRP alone, F is the apparent fluorescence with quencher THC at concentration Q. However, at low concentrations of quencher

$$K_{SV} = V + k_q\tau_0 \quad (7)$$

where V is a static component, τ_0 is the lifetime of excited indole, and k_q is the collision rate constant, which determines the rate of quenching of excited indoles by quencher molecules (Shinitzky et al., 1966). At low quencher concentrations, presumably, a change from aqueous medium to one of higher viscosity (95% glycerol) should indicate the importance of the term $k_q\tau_0$ in eq 7. In our experiments, K_{SV} remained nearly identical in H_2O and 95% glycerol.

As the concentrations of quencher and of TRP were increased, the F_0/F vs. (Q) plots became distinctly nonlinear, indicating nonlinear (but higher order, as demonstrated by the upward curvature of the plot) dependence of quenching on THC concentration. The quenching data could be linearized (Eftink and Ghiron, 1976) by resolution into two contributions:

$$F_0/F = (1 + K_{SV}(Q))\exp(V(Q)) \quad (8)$$

a collisional (K_{SV}) and static (V) component. Upon rearranging;

$$F_0/F\exp(V(Q)) = 1 + K_{SV}(Q) = 1 + k_q\tau_0(Q) = \tau_0/\tau \quad (9)$$

where τ_0 and τ are the fluorescence lifetimes (of TRP) in the absence and presence of quencher, respectively. Hence, a plot of $F_0/F\exp(V(Q))$ vs. (Q) could be linearized by varying V until a "best" value (signified by the highest correlation coefficient of the assumed linear plot) was obtained.

Table VII lists such K_{SV} and V values at high TRP and THC concentrations. While V still depends on the concentration range of quencher, the data are clearly linearized.

TABLE VII: Fluorescence Quenching of Tryptophan by THC at 30 °C, 348 nm,[a] pH 6.0.

[TRP][b] (M)	[THC][c] (M)	K_{SV}[d] (M^{-1})	V[e] (M^{-1})	R[f]
1×10^{-5}	$2\text{-}12 \times 10^{-3}$	1099	70	0.997
1×10^{-5}	$6\text{-}33 \times 10^{-5}$	1640	0	0.996
1×10^{-4}	$6\text{-}33 \times 10^{-5}$	1360	0	0.999
8×10^{-5}	$6\text{-}33 \times 10^{-5}$	1090	0	0.988
7×10^{-5}	$6\text{-}33 \times 10^{-5}$	1050	0	0.993
5×10^{-5}	$6\text{-}33 \times 10^{-5}$	1053	0	0.993
3×10^{-5}	$6\text{-}33 \times 10^{-5}$	1123	0	0.997
1×10^{-3}	$6\text{-}33 \times 10^{-3}$	270 ± 30	36 ± 3	>0.999
1×10^{-2}	$6\text{-}33 \times 10^{-3}$	80 ± 20	35 ± 3	0.998
5×10^{-3}	$5\text{-}12.5 \times 10^{-2}$	50	6 ± 0.6	0.991
5×10^{-3}	$5\text{-}25 \times 10^{-2}$	35	4 ± 0.5	0.996
(50% v/v) EtOH				

[a] Excitation at 287 nm. [b] Always at constant concentration. [c] At variable concentration; range is given. [d] Stern–Volmer quenching constant. [e] Static component of quenching. [f] Correlation coefficient from linear least-squares plot, where V's are quoted obtained from exponential fit explained in text.

TABLE VIII: Quenching of Tryptophan Fluorescence by Thiamine Analogue at 30 °C and 348 nm.[a]

[TRP][b] (M)	Quencher[c] (M)	pH	K_{SV}[d] (M^{-1})	V[e] (M^{-1})	R[f]
	AMMPY[g]				
1×10^{-4}	$3\text{-}33 \times 10^{-4}$	8.45	1870	750	0.997
	AMMPY[g]				
1.5×10^{-4}	$3\text{-}33 \times 10^{-4}$	8.45	7690	400	0.995
	AMMPY[g]				
1.5×10^{-4}	$3\text{-}33 \times 10^{-4}$	3.95	1000	380	0.961
	AMMPY[g]				
1×10^{-3}	$3\text{-}13 \times 10^{-4}$	8.45	2000	150	0.948
10^{-3}	TTHC[h]	3.95	9350	1990	0.981
	$1.7\text{-}8.3 \times 10^{-4}$				
10^{-3}	THC	3.95	2370	150	0.970
	$1.7\text{-}8.3 \times 10^{-4}$				

[a] Excitation at 287 nm. [b] Always at constant concentration. [c] At variable concentration; range is given. [d] Collisional quenching (Stern–Volmer) constant. [e] Static quenching constant. [f] Correlation coefficient. [g] 4-Amino-2-methyl-5-methoxymethylpyrimidine. Thiothiamine (see structure in text).

Static quenching has been attributed to the presence of ground-state donor–acceptor complexes (in the present case THC·TRP, as shown by UV at high concentration of both THC and TRP), which compete with the uncomplexed tryptophan for the incident excitation, and which complexes "yield excited donor–acceptor complexes (and quenching) directly by absorption" (Birks, 1970, also Weller, 1961; Eftink and Ghiron, 1976).

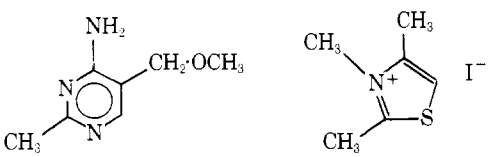
Perrin (1924) suggested the "active sphere" concept as an alternative model for static quenching. This model does not require formation of a bound complex between THC and TRP in the ground state. The "active sphere" is a volume of interaction around a quencher molecule, such that a fluorescent molecule excited within this volume is quenched instantaneously, while those outside this volume are unquenched (Birks, 1970).

The similarity of the magnitude of V and K_{assoc} obtained by the fluorescence and UV methods, respectively, at comparable, high concentration ranges, tempts one to adopt the former explanation. UV allows measurement of K_{assoc} only at high concentrations at which both THC and TRP have contributions to the absorption at 287 nm.

Since the ionic strength was not constant throughout these experiments, the decreased V at higher concentration may reflect a salt effect. V (as also indicated by K_{assoc} in the UV data) decreases with added ethanol.

It was thus clearly established that thiamin, just as pyridinium (Shifrin, 1968) and imidazolium (Shinitzky and Katchalski, 1968), quenches indole fluorescence.

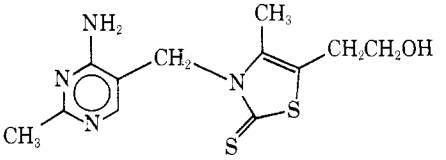
We also examined whether the pyrimidine or thiazolium ring was responsible for the observed quenching of TRP fluorescence. 4-Amino-2-methyl-5-methoxymethylpyrimidine (AMMPY) and 2,3,4-trimethylthiazolium iodide



were employed as models for the PY and THZ constituents, respectively, of THC. This pyrimidine analogue has a pK of 6.0 compared to 5.0 for the vitamin.

Table VIII presents fluorescence quenching data for AMMPY with TRP at pH 3.95 and 8.45. Surprisingly, the neutral pyrimidine ring is as efficient a quencher of the TRP fluorescence as the protonated one and both are more efficient than THC itself. While UV experiments in this laboratory failed to show significant interaction between the pyrimidine and TRP, the fluorescence data clearly imply that either protonated or neutral pyrimidine can quench TRP fluorescence. The THZ model employed appeared to be a very inefficient quencher.

Some experiments are also reported with thiothiamin



TTHC

a system possessing a neutral thiazolidene ring. At pH 3.95 (where this substrate is soluble enough), TTHC is a very much more efficient quencher of TRP fluorescence than is THC (both substrates have a protonated pyrimidine ring at this pH). Not only are both V and K_{SV} larger for TTHC, but there is also a shift of the maximum from 348 to 355 nm. This finding should be potentially useful, since thiothiamin pyrophosphate was recently claimed to be a transition-state analogue for the pyruvate dehydrogenase complex (Gutowsky and Lienhard, 1976).

Discussion

Solvent Effects on Association. All evidence from this laboratory points to the destabilization of the complex formed between THC and TRP in medium of low dielectric constant. The evidence at different pH's suggests that the state of ionization of the PY ring affects the complexation. Between pH 4 and 6 (bracketing the protonation pK_a of PY), the K_{assoc} is nearly pH independent, but the extinction coefficient changes. This could signal either a change in the site of interaction or a change in the geometry of the complex. Biaglow et al. (1969) presented qualitative data in favor of both the pyrimidinium- and thiazolium-ring binding to the indole nucleus, the latter being a donor, the positively charged rings acceptors in a stacked geometrical arrangement. The indole ring had been

previously shown to complex to imidazolium (Shinitzky and Katchalski, 1968), pyridinium (Shifrin, 1968), and nucleic bases (Dimicoli and Hélène, 1973) by UV, fluorescence, and nuclear magnetic resonance measurements. Both the magnitude and thermodynamic properties of the interactions in all cases are reminiscent of the stacking interactions found in the nucleic acid field.

Solvent effects on such stacking interactions have not been studied extensively. A study on nucleic acids (Hanlon, 1966) showed that nonaqueous solvents reduce the association tendencies. Douzou reported studies on flavin mononucleotide–indole interactions (1968) and concluded that both increased dielectric constant and viscosity enhance the association. Montenay-Garestier (1973) studied mixed aggregates of nucleic acid constituents with aromatic amino acids and found that aggregation was impeded by addition of organic solvents. The results from this laboratory are in accord with earlier ones on related stacking interactions with indoles; namely, the association invariably decreases with addition of organic cosolvent to water. Connors and Sun (1971) studied several complexes with different charge types in various solvent mixtures and found that the solvent contribution to complex stability could be correlated with surface tension of solvent, as also suggested by Sinanoglu (1968).

The very gradual decrease in K_{assoc} with smaller amounts of added ethanol perhaps indicates dependence on water-structuring effects. It has been shown that water possesses maximum structure at 6 mol % cosolvent (about 16% (v/v) ethanol in H_2O) and is less structured beyond that (Gordon, 1972).

On the Theoretical Origins of Complex Stability. We present theoretical models for the decreased associating tendency with decreased solvent dielectric constant. Theoretical studies on the origins of nucleic acid interactions in both stacking and hydrogen-bonding modes (De Voe and Tinoco, 1962; Pullman, 1968; among others) have implicated both electrostatic and dispersion forces in the stabilization of stacks. It was also shown that such interactions can be greatly enhanced by protonating one of the nucleic base partners (Jordan and Sostman, 1973). In such hemiprotonated stacks, electrostatics as well as dispersion forces play an important role. Hélène et al. (1973) studied the association of aromatic amino acids with poly(A), DNA, and tRNA and found dissociation upon increasing the ionic strength. Okuba et al. (1975) studied the interaction of 3-indoleacetate with NAD^+ and found decreased association with increased ionic strength. Both of these pieces of data were interpreted to mean that the complex was stabilized by electrostatic interactions. According to Mieyal et al. (1969), the strength of the interaction between THC and indole derivatives (in H_2O) was nearly independent of the charge on the indole derivative, perhaps implying that in THC–TRP dispersion forces are more important than electrostatic ones.

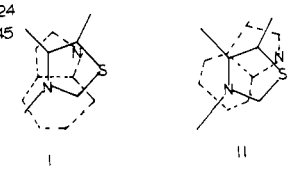
We have performed intermolecular potential calculations of the type previously outlined (Jordan and Sostman, 1973) on the thiazolium–indole stacking. Electrostatics appear to contribute less than dispersion for both thiazolium and the ylide (C2 deprotonated, the nucleophile in the enzymatic reactions) interacting with indole. Figures 5 and 6 (latter in the Supplementary Material) present some of the most favorable stacking geometries found, as well as the breakdown of the interaction energy into electrostatic (monopole–monopole, monopole-induced dipole) and dispersion (induced dipole-induced dipole) contributions. While the actual numbers are subject to uncertainty (due to basis set employed to obtain charges, assumption of a dielectric constant of 1, truncation of the multipole expansion, etc.), the relative trends are clear. The thiazolium ring interacts more favorably with indole (both electrostatic and dispersion terms) than does the ylide. According to this model, the decreased association in mixed solvents could be explained by enhanced solvent–solute interaction at the expense of solute–solute interactions due to the increased polarizability of solvent (i.e., ethanol or dioxane compared to water). This model would also account for the observed solvent effect were it pertinent to PY–TRP association.

PREFERRED THIAZOLIUM–TRYPTOPHAN ORIENTATIONS

INTERPLANAR DISTANCE 3.4 Å

INTERACTION ENERGY (kcal/mol)

	MONO	IND1	IND2	DISP	TOTAL
I	-1.08	-0.02	-1.0	-9.14	-11.24
II	-1.22	-0.02	-1.08	-9.13	-11.45



I II

FIGURE 5: Theoretical interaction geometries and energy contributions in the thiazolium–indole stacking interaction. IND 1 is monopole-induced dipole interaction in thiazolium; IND 2, monopole-induced dipole interaction in the indole ring.

Alternatively, a classical electrostatic model can be tested on the data. Kirkwood (1934) derived an equation for the free energy of transfer of an ion from a medium of unit dielectric constant (in vacuo) to one of dielectric constant D. We have derived an expression (see Supplementary Material) for ΔG transfer for the reaction: $THC^+ + TRP \rightarrow (THC\text{–}TRP)^+$. A $-\Delta G$ transfer (here found experimentally) is obtained going from aqueous ethanol to pure water if one assumes that the ionic radius of THC^+ changes more than that of $(THC\text{–}TRP)^+$ with the solvent change, i.e., if THC^+ is more highly solvated in water than the larger $(THC\text{–}TRP)^+$ complex ion.

Conclusions

Thiamin and its component aromatic rings have been demonstrated to be quenchers of tryptophan fluorescence. The quenching behavior could be analyzed in terms of static and dynamic contributions. While the dynamic term is clearly due to quenching of excited tryptophan by ground-state quencher, an acceptable interpretation of the static contribution implies interaction of ground-state tryptophan with the quencher. The interaction of TRP and THC as observed by UV has been shown to be disfavored by transfer of complex from aqueous medium to one of lower dielectric constant.

Finally, it is significant to note that a UV charge transfer band similar to the one here discussed has also been observed in this laboratory between apopyruvate decarboxylase and its cofactors. The origins of this spectral band are currently being characterized. The approaches here presented should be useful in studies of the binding sites of apoenzymes requiring the thiamin pyrophosphate cofactor.

Acknowledgment

We thank Ms. Christine Anderson for her able programming assistance; Merck, Sharpe and Dohme, Inc., Rahway, N.J., and Hoffmann-LaRoche Inc., Nutley, N.J. for their generosity in providing us with some vitamin B_1 intermediates; and, most of all, USPHS-NIH for financial support of our research.

Supplementary Materials Available

Tables I, III, IV, V, VI, and Figures 2, 3, 6 (13 pages). Ordering information is given on any current masthead page.

References

Ainsworth, S. (1961), *J. Phys. Chem. 65,* 1968.

Benesi, H. A., and Hildebrand, J. H. (1949), *J. Am. Chem. Soc. 71,* 2703.

Biaglow, J. E., Mieyal, J. J., Suchy, J., and Sable, H. Z. (1969), *J. Biol. Chem. 244,* 4054.

Birks, J. B. (1970), Photophysics of Aromatic Molecules, London, Wiley-Interscience, Chapter 9.

Coleman, J. S., Varga, L. P., and Martin, S. H. (1970), *Inorg. Chem. 9,* 1015.

Connors, K. A., and Sun, S. (1971), *J. Am. Chem. Soc. 93,* 7239.

Crosby, J., and Lienhard, G. E., (1970), *J. Am. Chem. Soc. 92,* 5707.

Crosby, J., Stone, R., and Lienhard, G. E. (1970), *J. Am. Chem. Soc. 92,* 2891.

Derenleau, D. A. (1969), *J. Am. Chem. Soc. 91,* 4050.

De Voe, H., and Tinoco, I., Jr. (1962), *J. Mol. Biol. 4,* 500.

Dimicoli, J-L., and Hélène, C. (1973), *J. Am. Chem. Soc. 95,* 1036, and references therein.

Douzou, P. (1968), in Molecular Associations in Biology, Pullman, B., Ed., New York, N.Y., Academic Press, p 447.

Eftink, M. R., and Ghiron, C. A. (1976), *Biochemistry 15,* 672, and references therein.

Gordon, J. E. (1972), *J. Am. Chem. Soc. 94,* 650.

Gutowski, J. A., and Lienhard, G. E. (1976), *J. Biol. Chem. 251,* 2863.

Hanlon, S. (1966), *Biochem. Biophys. Res. Commun. 23,* 861.

Hélène, C., Borazan, H., Dimicoli, J-L., Maurizot, J. C., Durand, M., and Toulme, J. J. (1973), in Dynamic Aspects of Conformational Changes in Biological Macromolecules, Sadron, C., Ed., Dordrecht, Holland, D. Reidel, p 349.

Janz, G. J., and Tompkins, R. P. T. (1972), Nonaqueous Electrolytes Handbook, Vol 1, New York, N.Y., Academic Press.

Job, P. (1928), *Justus Liebigs Ann. Chem. 9,* 113.

Jordan, F., and Sostman, H. D. (1973), *J. Am. Chem. Soc. 95,* 6544.

Katakis, D. (1965), *Anal. Chem. 37,* 876.

Kirkwood, J. G. (1934), *J. Chem. Phys. 2,* 351.

Krampitz, L. O. (1969), *Annu. Rev. Biochem. 38,* 213.

Mieyal, J. J., Suchy, J., Biaglow, J. E., and Sable, H. Z. (1969), *J. Biol. Chem. 244,* 4063.

Montenay-Garestier, T. (1973), *J. Chim. Phys. Phys. Chim. Biol. 70,* 1379.

Okuba, T., Ishiwatari, T., Mita, and Ise, N. (1975), *J. Phys. Chem. 79,* 2108.

Perrin, F. (1924), *C. R. Herbd. Seances Acad. Sci. 178,* 1978.

Pullman, B. (1968), in Molecular Associations in Biology, New York, N.Y., Academic Press, p 1.

Sable, H. Z., and Biaglow, J. E. (1965), *Proc. Natl. Acad. Sci. U.S.A. 54,* 808.

Scatchard, G. (1949), *Ann. N.Y. Acad. Sci. 51,* 660.

Shinitzky, M., and Katchalski, E. (1968), in Molecular Associations in Biology, Pullman, B., Ed., New York, N.Y., Academic Press, p 361.

Shinitzky, M., Katchalski, E., Grisaro, V., and Sharon, N. (1966), *Arch. Biochem. Biophys. 116,* 332.

Shifrin, S. (1968), in Molecular Associations in Biology, Pullman, B., Ed., New York, N.Y., Academic Press, p 323.

Sinanoglu, O. (1968), in Molecular Associations in Biology, Pullman, B., Ed., New York, N.Y., Academic Press, p 427.

Varga, L. P., and Weatch, F. C. (1967), *Anal. Chem. 39,* 1101.

Wallace, R. M. (1960), *J. Phys. Chem. 64,* 899.

Wallace, R. M., and Katz, S. M. (1964), *J. Phys. Chem. 68,* 3890.

Weller, A. (1961), in Progress in Reaction Kinetics, Vol. 1, Porter, G., Ed., Oxford, Pergamon Press, pp 189.