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Complex Formation Influence on Reaction Rate I

Effect of Caffeine on Riboflavin Base-Catalyzed Degradation Rate

By DAVID E. GUTTMAN

The rate of the base-catalyzed decomposition of riboflavin was investigated in aqueous solution in the absence and presence of caffeine at a number of different temperatures. It was found that at all temperatures the apparent velocity of the reaction was decreased by the presence of caffeine and that the magnitude of this effect was dependent on the concentration of caffeine in the system. A mathematical relationship, expressing the relative degree of stabilization as a function of the caffeine concentration and the dissociation constant for the complex, was derived by assuming that a 1:1 complex formed and that the complexed riboflavin did not undergo reaction with the base. Dissociation constants which were calculated for the complex from the kinetic data were found to be in close agreement with values previously obtained by nonkinetic techniques. Free energy, enthalpy, and entropy changes which characterize the association were calculated to be −2,000 cal., −5,750 cal., and −12 e.u., respectively. The energy of activation for the degradative reaction was found to be the same in the presence of caffeine as in its absence.

A NUMBER of investigations have demonstrated that molecular complex formation can influence the rate at which participating species undergo chemical reaction. For example, the rate of the coupling reaction between β-naphthol and *p*-diazobenzenesulfonic acid was found by Overbeek, Vink, and Deenstra (1) to be lowered by the addition of caffeine to the reaction medium. The lowering of the reaction velocity was explained on the basis of lowered reactivities of the reactants in the complexed forms since both phenol and the sulfonic acid were shown to form 1:1 complexes with caffeine. Similarly, Higuchi, Lachman, Ravin, and Guttman (2,3,4) showed that the rates of hydrolysis of local anesthetic esters were substantially reduced by the addition of complexing agents to their aqueous solutions. Their investigations indicated that the esters, in the complexed forms, did not undergo hydrolytic cleavage at the ester linkage.

Pharmaceutical interest in this particular manifestation of complex formation is quite naturally derived from the possibility of utilizing such behavior to stabilize labile medicinal agents. More comprehensive studies of this phenomenon would, therefore, be of value in amplifying and extending this interest and could, in addition, be of more fundamental importance in providing further insights into the nature of the bonding forces responsible for molecular associations. The extent to which the reactivity of a compound is affected by complexation might serve as an indication of the type of interactive mechanism that is primarily operant. Correlation and comparison of such studies with results obtained by nonkinetic methods might also serve to pinpoint functional groups as sites of contact between components in the complex. Alternatively, it is possible that a kinetic approach to the determination of the energetics of complex formation might be more direct and preferred in some cases to many of the classical approaches which are commonly used.

This communication summarizes the results of a preliminary investigation of the effect of mo-

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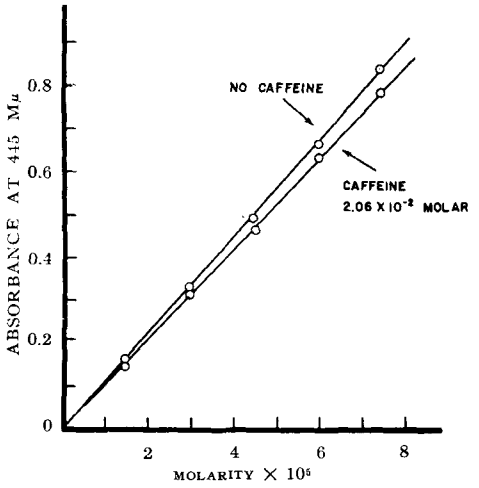

Fig. 1.—The relationship between absorbance and concentration for solutions of riboflavin.

lecular interactions on the velocities of reactions involving riboflavin. It is anticipated that riboflavin-containing systems will be useful models for the accumulation of pertinent information because of the well-known interactive tendencies of isoalloxazine derivatives and because the many modes of degradation and reaction of the vitamin will provide the opportunity to investigate the influences of complexation on the rates of reactions possessing quite different mechanisms. The specific objective of the present study was to investigate the influence of caffeine on the rate of the base-catalyzed conversion of riboflavin to urea and 1,2-dihydro-6,7-dimethyl-2-keto-1-D-ribityl-3-quinoxaline-carboxylic acid (5). No attempt was made to derive a mechanistic picture of the degradative reaction or a mechanistic explanation at the molecular level of the observed influence of complex formation on reactivity. The study will show, however, that the vitamin in its complexed form possessed negligible reactivity towards alkaline hydrolysis and that, consequently, pertinent information concerning energy changes which resulted from complexation could be obtained from kinetic studies.

EXPERIMENTAL[1]

Reaction systems were formulated in 0.05 *N* sodium hydroxide to contain approximately 1 × 10^{-4} moles $L.^{-1}$ of riboflavin and the desired concentration of caffeine. The reaction flasks were maintained at a constant temperature by immersion in a Sargent Thermonitor constant temperature bath. Five-milliliter samples were withdrawn from the flasks at various time intervals and immediately treated with exactly 5 ml. of 1 *N* acetic acid to quench the reaction. The absorbance of each sample was then determined at 445 mμ utilizing a Beckman model DU spectrophotometer. Studies were conducted in a similar manner on reaction systems containing 0.1 *N* sodium hydroxide. All riboflavin-containing solutions were protected from light at all times. The disappearance of riboflavin was thus followed by noting the decrease in absorbance at 445 mμ. The spectrophotometric assay appeared to be specific for the intact vitamin. Completely degraded solutions yielded negligible absorbance values at the selected wavelength. Although it has been shown by Harbury and Foley (6) that caffeine does affect the spectral characteristics of riboflavin in aqueous solution, preliminary studies which are illustrated in Fig. 1 showed that a direct proportionality existed between absorbance at 445 mμ and the concentration of the vitamin in both the absence and presence of caffeine. The absorbance values were, therefore, used directly in all of the kinetic plots as being representative of the vitamin concentrations.

RESULTS

Results typical of those obtained throughout the course of this study are graphically illustrated in Fig. 2. Here, semi-log plots are shown for two reaction systems formulated with and without caffeine. It can be seen that in both cases a pseudo first-order disappearance of the vitamin occurred. A number of studies showed that the linearity of the plots extended over time periods many times the half-life of the vitamin. Figure 2 makes apparent that the reaction occurred at a significantly reduced rate in the presence of caffeine. Similar studies were conducted at various temperatures and at various concentrations of caffeine. In all cases the data fit first-order plots and from such plots first-order rate constants

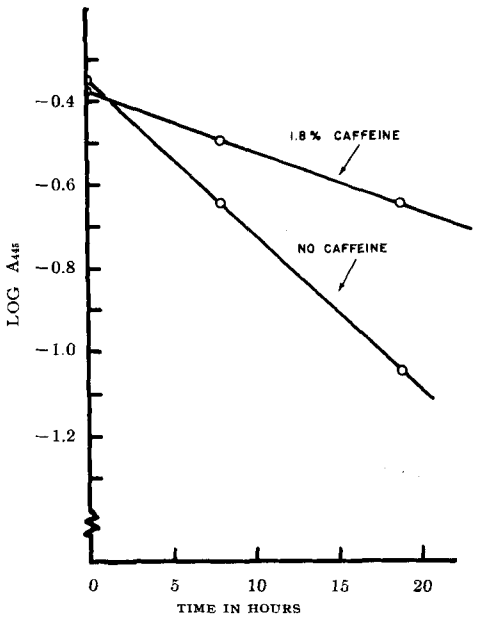

Fig. 2.—The first-order decomposition of riboflavin in 0.05 *N* sodium hydroxide at 35°.

[1] The author wishes to acknowledge the competent technical assistance of J. David McCallister.

were estimated. The results are summarized in Table I. Here, for each temperature investigated, are tabulated the molar concentration of caffeine in the system, the apparent rate constant observed for that system, and the ratio of the rate constant found in the absence of caffeine, k, to that found in the presence of caffeine, k_{app}. It can be seen that this ratio, which is indicative of the apparent reduction in vitamin reactivity in the presence of caffeine, is proportional to the caffeine concentration in the system.

DISCUSSION

A reasonable explanation for the decreased rate of vitamin disappearance effected by the presence of caffeine is that riboflavin in the complexed state possessed either a decreased or negligible reactivity toward the catalyst. If it is assumed that the latter was the case, then the overall mechanism for the process in the presence of caffeine is

$$\text{riboflavin:caffeine} = \text{caffeine} + \text{riboflavin}$$
$$\downarrow \text{OH}^-$$
$$\text{products}$$

Accordingly, the rate of disappearance will depend not on the stoichiometric concentration of riboflavin in the system but rather on the concentration of the noncomplexed specie.

If, as is indicated by the postulated mechanism, a 1:1 complex is formed, then it can be shown that the concentration of noncomplexed riboflavin is related to its stoichiometric concentration by the expression

$$R_f = \frac{K}{K + C_f} R_t \qquad \text{(Eq. 1)}$$

where R_f = molar concentration of noncomplexed riboflavin, K = dissociation constant for the complex, C_f = concentration of noncomplexed caffeine, and R_t = stoichiometric molar concentration of riboflavin. Since the rate equation for the decomposition reaction in the absence of caffeine is

$$-dR_t/dt = kR_t \qquad \text{(Eq. 2)}$$

then based on the preceding assumptions, in the presence of caffeine, it will be

$$-dR_t/dt = k \frac{K}{K + C_f} R_t \qquad \text{(Eq. 3)}$$

The apparent rate constants evaluated for reaction systems containing caffeine are thus related to the "true rate" constant by

$$k_{app} = k \frac{K}{K + C_f} \qquad \text{(Eq. 4)}$$

or more conveniently,

$$k/k_{app} = 1 + C_f/K \qquad \text{(Eq. 5)}$$

Because caffeine was maintained at a much higher concentration than riboflavin in all of the present studies, its stoichiometric concentration can be used as a close approximation of the C_f term in Eq. 5.

Equation 5 predicts a linear relationship between the ratio k/k_{app} and the molarity of caffeine and thus serves as a means to check the validity of the assumptions made. A plot of this relationship for three different temperatures is shown in Fig. 3. It is seen that, at least at lower concentrations of caffeine, a linear relationship existed. As the molarity of caffeine was increased, deviations from linearity became more pronounced. It is felt that this behavior does not negate the postulated mechanism but rather resulted from the self-complexing of caffeine which becomes increasingly significant at higher concentrations. This possibility is supported by the decreased curvature observed at higher temperatures and by the fact that a previous study on the solubilization of riboflavin by caffeine (7) yielded deviations of the same nature and of the same magnitude. It should be noted that the 35° curve of Fig. 3. includes experimental points obtained at two different concentrations of hydroxide ion. The close agreement obtained between the two different concentrations emphasizes that the stabilization did not result from neutralization or inactivation of catalyst by caffeine.

Dissociation constants for the complex were graphically estimated from the slopes of the curves obtained by the plot illustrated in Fig. 3. The initial straight-line portion of the curves were used for

TABLE I.—RATE CONSTANTS FOR THE BASE-CATALYZED DECOMPOSITION OF RIBOFLAVIN IN 0.05 N SODIUM HYDROXIDE IN THE ABSENCE AND PRESENCE OF CAFFEINE

°C.	Concn. Caffeine, mol./L. × 10^2	Rate Constant, $hr.^{-1}$ × 10^2	k/k_{app}
8	0	0.326	1.00
	0.778	0.244	1.34
	1.10	0.211	1.55
	2.36	0.171	1.91
	3.34	0.148	2.20
	3.94	0.138	2.36
	5.10	0.130	2.50
25	0	2.85	1.00
	0.472	2.42	1.18
	1.42	1.85	1.54
	2.34	1.69	1.69
	4.25	1.32	2.16
	6.60	1.15	2.48
30	0	5.25	1.00
	0.506	4.35	1.21
	1.44	3.50	1.50
	3.00	2.78	1.89
	4.24	2.39	2.20
	7.02	2.12	2.48
35	0	8.47	1.00
	0.943	6.60	1.28
	2.79	5.00	1.70
	4.76	4.11	2.06
	6.62	3.73	2.27
	8.50	3.26	2.52
35 (in 0.1 N NaOH)	0	13.8	1.00
	2.40	8.40	1.60
	7.20	5.68	2.43
50	0	33.8	1.00
	2.41	23.7	1.43
	4.70	20.2	1.84
	9.40	16.1	2.10
	14.2	15.5	2.18
70	0	198.	1.00
	2.40	151.	1.31
	4.70	138.	1.43
	9.40	110.	1.80
	11.8	104.	1.90
	14.2	109.	1.82
	16.6	96.0	2.06
	18.8	92.5	2.14

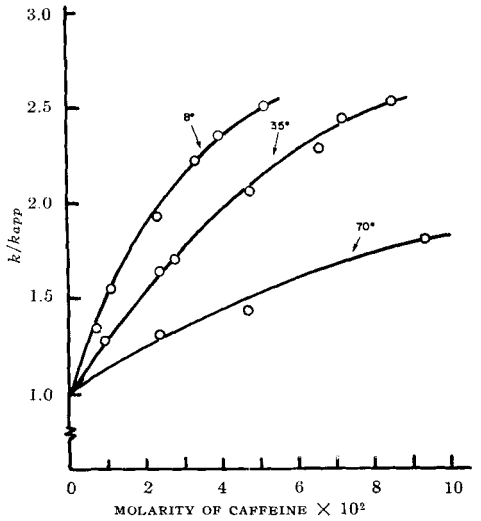

Fig. 3.—The effect of caffeine on the rate of decomposition of riboflavin in 0.05 *N* sodium hydroxide.

this purpose. The values obtained at various temperatures are tabulated in Table II.

The values obtained are in reasonable agreement with values derived by other workers who utilized different experimental methods. For example, the value for 20° was interpolated from this study to be 0.024 mol./L. as compared to a value of 0.019 mol./L. obtained by the spectrophotometric study of Yagi and Matsuoka (8) at the same temperature. Similarly, the solubility study at 30° of Guttman and Athalye (7) yielded a value of 0.0345 as compared to 0.0286 in the present study.

The temperature dependency of the dissociation equilibrium is graphically illustrated by the semi-log plot of Fig. 4. The enthalpy change $\Delta H°$ resulting from the association of caffeine with riboflavin was calculated to be −5.74 Kcal. The free energy change $\Delta F°$ and the entropy change $\Delta S°$ for the interaction at 35° were calculated to be −2.04 Kcal. and −12.0 e.u., respectively.

The temperature dependency for the degradative reaction, under the conditions used in this study, is shown by the Arrhenius plot of Fig. 5. Plotted here as a function of reciprocal absolute temperature are rate constants obtained at different temperatures for systems free of caffeine and also those obtained at corresponding temperatures for systems containing two different concentrations of caffeine. The apparent energy of activation was graphically estimated to be approximately 20.0 Kcal. in all cases. This observation gives further support to the assumption that the complexed form of riboflavin does not degrade at a perceptible rate.

This investigation has provided another example of a manifestation of complex formation that has possible practical and theoretical importance. In addition, the possibility of gaining information about the energetics of molecular interactions from kinetic studies has been demonstrated. Although quite equivocal, one might speculate that the reduction in the reactivity of riboflavin as well as the rather large

TABLE II.—DISSOCIATION CONSTANTS FOR THE RIBOFLAVIN-CAFFEINE COMPLEX

Temp., °C.	*K*, mol./L. × 10²
8	1.61
25	2.72
30	2.86
35	3.57
50	5.70
70	10.0

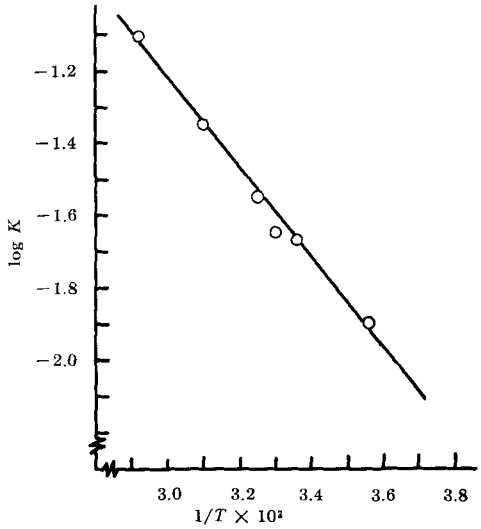

Fig. 4.—The temperature dependency of the interaction between riboflavin and caffeine.

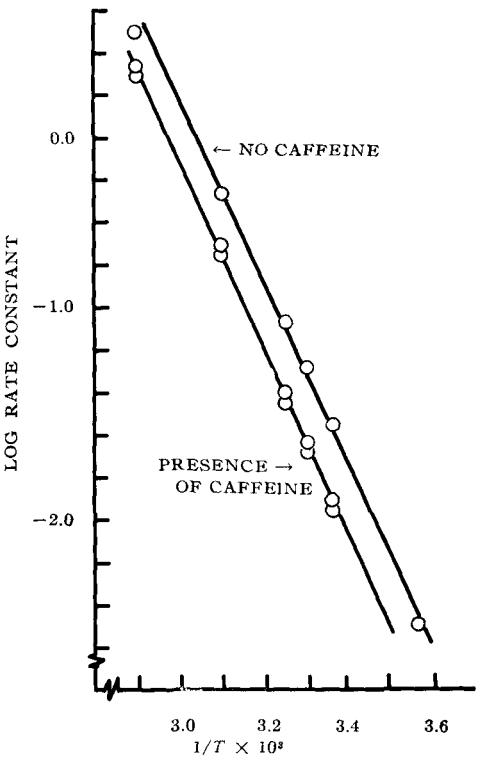

Fig. 5.—Arrhenius plot for the decomposition of riboflavin in 0.05 *N* sodium hydroxide

negative entropy change resulting from complexation supports the previously suggested involvement of a charge-transfer interactive mechanism with caffeine acting as a donor and riboflavin as the acceptor (6, 9). Such an interaction would possibly reduce the electrophilicity of riboflavin and might, therefore, reduce its reactivity by discouraging the nucleophilic attack of hydroxide ion, an attack undoubtedly involved in the hydrolytic cleavage of the isoalloxazine ring.

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Effect of Deuterium Oxide on Local Anesthetic Activity of Procaine

By S. V. SUSINA, F. D. HITER, F. P. SIEGEL, and M. I. BLAKE

Deuterium oxide was used as a solvent for procaine and the effect on the stability and local anesthetic activity was noted. The ED_{50} was determined by the method of Chance and Lobstein and comparison was made with aqueous procaine solutions. In water the ED_{50} was 1.8% while in deuterium oxide it was 1.0%. The LD_{50} was determined by intraperitoneal injection in mice. There appears to be no significant difference in the toxicity of procaine in either solvent. Stability studies indicate that procaine is more stable in deuterium oxide at pH and "apparent" pH values of 8.0, 8.5, and 9.0.

THE THERAPEUTIC efficacy of drugs instilled into the eye depends, among other factors, on the pH of the solution in which the drug is administered and on the stability of the drug under such conditions. Procaine is a typical example. These relationships may be adduced from kinetic data and pharmacological studies. Higuchi, *et al.* (1), have studied the influence of pH and temperature on the rate of procaine decomposition in aqueous solutions. It is apparent from their work that procaine deteriorates rapidly in the alkaline pH range. Inhibition of procaine decomposition by complex formation has also been studied (2).

Topical anesthetic activity of procaine depends primarily on the extent of availability of free base. At pH values below 7.0 procaine is relatively inactive topically. Hind and Goyan (3) have shown that at this pH the per cent of procaine free base is less than 1, and even at pH 7.4 it increases only to 3.4%, but at pH 9.0 it becomes 58.5%. The lipoid nature of the corneal epithelium controls the rate at which absorption of procaine will occur in the cornea. The greater the concentation of procaine free base in the lacrimal fluid, the greater should be the extent of procaine penetration and absorption from the cornea which should result in greater and more prolonged local anesthetic activity.

The present study was undertaken to determine the effect of deuterium oxide on the stability of procaine solutions at "apparent" pH values comparable to those in protium oxide (distilled water). In addition, comparison was made of the local anesthetic activity in both systems over the pH range 5.0 to 9.0.

The toxicity of deuterium oxide in biological systems was described by Morowitz and Brown (4). Katz (5) has reviewed the literature dealing with the chemistry and biology of deuterium oxide. Algae and other microorganisms have been cultured in 99.6% deuterium oxide (6). It has been demonstrated (5) that mice and rats can survive when up to about one-third of their body water is replaced by deuterium oxide. When the blood serum approaches about 20% deuterium oxide content, toxic manifestations become evident. Since it appears that deuterium oxide is toxic in animals only when the concentration

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