Indole–Catechol Charge Transfer Complexes I

Keyphrases □ Catechol—charge transfer complex with indoles □ Indoles—charge transfer complex with catechol □ Charge transfer complexes—interaction of catechol and indoles

To the Editor:

The involvement of charge transfer phenomena, based on UV absorption measurements, was hypothesized between catechol-containing substances and the nucleic acid bases in the mechanism of action of biogenic amines as well as in their storage in the adrenal medulla and other nerve endings (1, 2). In continuing efforts to elucidate this effect in nerve conduction, the interaction between (−)-tryptophan and catechol was studied by UV absorption and emission techniques in aqueous solutions.

Figure 1 shows that the mixture containing (−)-tryptophan and catechol is shifted toward longer wavelengths and is accompanied by an increase in absorbance from that representing the sum of the absorbances of the single pure substances, indicating complex formation[1]. The increase in absorbance is attributed to the (−)-tryptophan–catechol complex and is used to calculate the equilibrium constant by applying the Benesi–Hildebrand equation:

$$\frac{[A_0]}{A_\lambda^{AD}} = \frac{1}{\epsilon_\lambda^{AD}} + \frac{1}{K_{abs}\epsilon_\lambda^{AD}[D_0]} \qquad \text{(Eq. 1)}$$

where $[A_0]$ and $[D_0]$ represent the total concentrations of the reactants, A_λ^{AD} and ϵ_λ^{AD} are the absorbance and molar absorptivity at a given wavelength, and K_{abs} is the equilibrium constant. Equation 1 is based on the assumptions that a 1:1 complex is formed and that $[A_0] \ll [D_0]$. A plot of $[A_0]/A_\lambda^{AD}$ *versus* $1/[D_0]$, with $[A_0]$ kept constant, gave a straight line (Fig. 2). The equilibrium constant calculated from the slope and intercept of the plot, when treated by the least-squares method, was 0.178 ± 10% M^{-1} in 10^{-3} M cacodylate buffer at pH 5 and 15° while the molar absorptivity at the wavelength of the measurement (306 nm) was 99.2 ± 2%.

Fluorescence measurements frequently are used to study complex formation (3). To allow meaningful comparison, the system was studied by this method under similar conditions in neutral solutions. When (−)-tryptophan was excited at 275 nm, the maximum emission wavelength observed was 365 nm. The fluorescence of (−)-tryptophan was quenched upon addition of catechol[2] (Fig. 3). The fraction of the fluorescence intensity remaining is a function of the concentrations of both components.

The equilibrium constant obtained from the fluorescence measurements, K_{fluo}, is calculated from:

$$\frac{1-\beta}{\beta} = K_{fluo}\{[D_0] - [A_0](1-\beta)\} \qquad \text{(Eq. 2)}$$

where β represents the relative fluorescence intensity

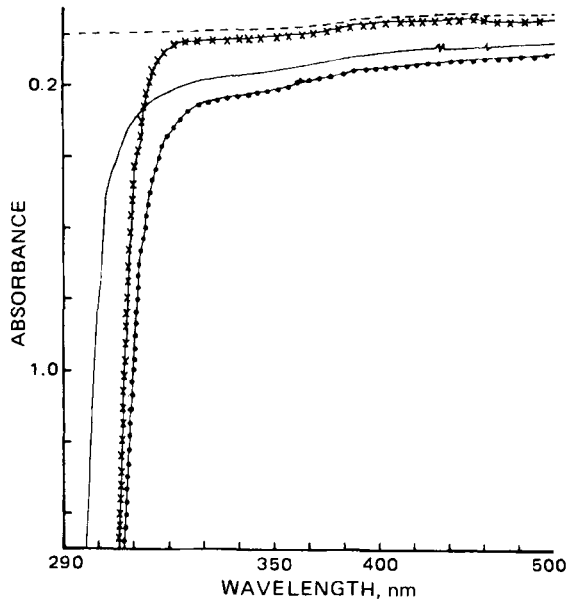

Figure 1—*Absorption spectra of 2 × 10^{-2} M (−)-tryptophan (-×-×), 1 M catechol (—), and 2 × 10^{-2} M (−)-tryptophan with 1 M catechol (-●-●); the broken line (- - -) represents the baseline. All solutions were in 10^{-3} M cacodylate buffer at pH 5. Measurements were made in 0.5-cm path length cells at 15°.*

observed. Equation 2 is based on the assumption that a 1:1 complex is formed; the complex does not fluoresce, and the fluorescence intensity is related linearly to concentration. Nearly equal K_{fluo} values (6.152 × 10^3 ± 17% M^{-1}) were found when fixed concentrations of (−)-tryptophan (10^{-5} and 10^{-4} M) and varying concentrations of catechol (up

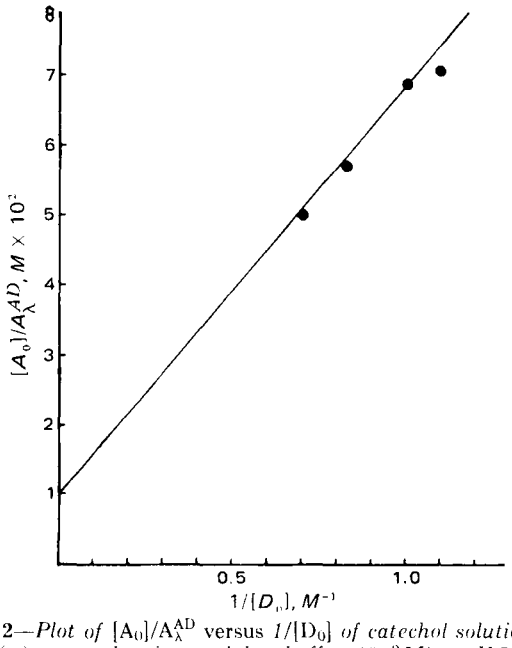

Figure 2—*Plot of $[A_0]/A_\lambda^{AD}$ versus $1/[D_0]$ of catechol solutions containing (−)-tryptophan in cacodylate buffer (10^{-3} M) at pH 5 and 15°. The total concentration of (−)-tryptophan, $[A_0]$, was 2 × 10^{-2} M; the total concentration of catechol, $[D_0]$, was 0.9–1.4 M; A_λ^{AD} represents the absorbance of the complex in a 1-cm path length cell at 306 nm.*

[1] Absorbances were measured with a Perkin-Elmer model 402 UV–visible spectrophotometer.

[2] Fluorescence measurements were made with an Aminco-Bowman model J4-8203G spectrophotofluorometer.

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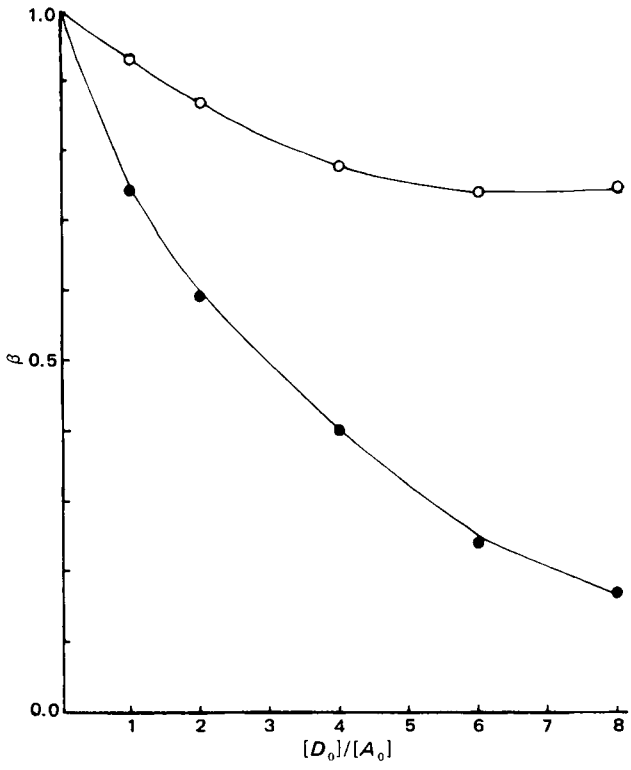

Figure 3—*Change in the relative fluorescence intensity of (−)-tryptophan, β, versus the molar ratio of catechol to (−)-tryptophan, $[D_0]/[A_0]$, at 10^{-5} M (-O-O) and 10^{-4} M (-●-●) (−)-tryptophan in cacodylate buffer (10^{-3} M) at pH 7 and 15°. The excitation wavelength was 275 nm. Fluorescence intensity was measured at 365 nm.*

to eightfold) were used. Furthermore, catechol did not have appreciable fluorescence at the highest concentration used (~5% from the total fluorescence intensity at the wavelength used).

In correlating the equilibrium constants obtained by the methods, a quenching coefficient, γ, is introduced in calculating the true (thermodynamic) equilibrium constant, K_t, from the simple mathematical model proposed:

$$K_{fluo} = \frac{[AD]\gamma_{AD}}{[A]\gamma_A[D]\gamma_D} = \frac{[AD]}{[A][D]}\left(\frac{\gamma_{AD}}{\gamma_A\gamma_D}\right) = K_t K_{quen} \qquad \text{(Eq. 3)}$$

$$K_{quen} = \frac{\gamma_{AD}}{\gamma_A\gamma_D} \qquad \text{(Eq. 4)}$$

where K_{quen} is the quenching constant. When K_{quen} was calculated by assuming $K_t \simeq K_{abs}$, it had a value of 3.4562×10^4.

Fluorescence quenching of tryptophan and some other fluorescing compounds was attributed to charge transfer interactions (4). In those studies, tryptophan was considered as an electron donor. It was suggested that the catechol-containing compounds act as electron donors when forming charge transfer complexes with nucleic acid bases in aqueous acidic solutions (1, 2). It may be reasonably concluded that the results presented in this work can be interpreted on the basis of charge transfer effects. It is difficult to assign the donor or acceptor species of the present molecules since precise theoretical data involving molecular orbital calculations of the highest occupied or lowest empty molecular orbitals are either inconsistent or unavailable and the values usually are expressed in terms of resonance units (uncertain quantities). However, it is assumed tentatively that catechol acts as an electron donor while (−)-tryptophan acts as an electron acceptor.

To explain the differences between the equilibrium constants obtained from the two methods as well as the appearance of the quenching constant, it is proposed that one pair of (−)-tryptophan–catechol out of several thousand pairs of molecules, within the concentration limits used, exists in close proximity in the form of a real complex while the rest remain isolated in solution.

On the other hand, to justify the charge transfer mechanism for the observed quenching, we propose a hypothesis of an electron pump through water. Experimental results indicated an observable quenching at concentrations as low as 10^{-5} *M* each (5.5%). Simple calculations revealed that the distance between molecules at 2×10^{-5} *M* is 436.26 Å; the distance between two molecules of water is 3.1 Å. Therefore, under this proposal, an electron can be pumped through ~140 molecules of water.

It is hoped that the results presented here and the theory advanced may help in understanding many important biological processes such as conduction through nerves and other excitable membranes.

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Mass Spectra of Dexamethasone and Betamethasone

Keyphrases □ Dexamethasone—mass spectral analysis, comparison with mass spectrum of betamethasone □ Betamethasone—mass spectral analysis, comparison with mass spectrum of dexamethasone □ Mass spectrometry—comparison of spectra of betamethasone and dexamethasone

To the Editor:

The application of mass spectrometry to the study of steroids provides a powerful tool for establishing the structure and spatial configuration of these complex molecules (1). In particular, the decomposition of steroidal alcohols under electron impact often is specific, with the position of the hydroxyl group affecting the fragmentation pattern of 17-hydroxysteroids, thus allowing discrimination between epimeric alcohols and their derivatives.

However, the occurrence of unwanted phenomena, *e.g.*,

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