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Evidence for a Solvent-Induced Change in Rate-Limiting Step in the Hydrolysis of Benzaldehyde Dimethyl Acetal

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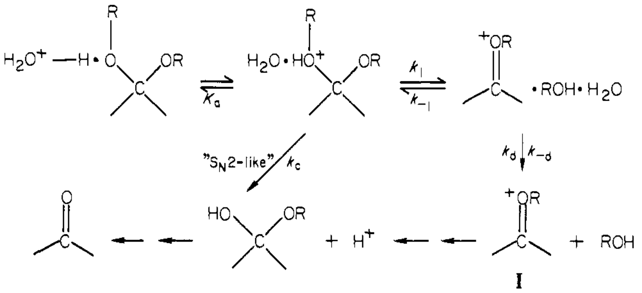
Abstract: Secondary deuterium isotope effects for the hydrolysis of the dimethyl acetal of benzaldehyde-*formyl-d* have been determined in mixed water–dioxane solvents varying between pure water and 70% dioxane, 30% water, by volume. Between 0 and 20% dioxane, the isotope effect (k^H/k^D) decreases from 1.12 to 1.06. Between 20 and 70% added dioxane, the isotope effects increase regularly from 1.06 to 1.15. The decrease in the apparent isotope effect at low concentrations of dioxane may be an artifact of the biphasic kinetics observed due to hemiacetal buildup in the more aqueous media. The increases in k^H/k^D that are observed between 20 and 70% dioxane are interpreted in terms of a change in rate-limiting step from C–O bond cleavage in water to rate-limiting diffusion apart of the alcohol–oxocarbonium ion encounter pair in high concentrations of dioxane. On the basis of this assumption, ratios of the rate constants for internal return and diffusional separation of the complex are estimated. The steady decrease in ρ and ρ^r within the series water and 50 and 70% dioxane also suggests an increase in cationic character in the rate-limiting transition state. Isolated resonance structure-reactivity parameters ($\rho^r/\rho_{eq}{}^r$) provide estimates of sp^2 character in rate-limiting transition states that are essentially identical with the estimates obtained from the secondary isotope effect studies.

Introduction

There is convincing evidence that oxocarbonium ions (I) occur as intermediates in the hydrolysis of certain simple acetals and ketals.[1-3] For acetophenone dimethyl ketals, these intermediates have been trapped by diffusion-controlled reaction with sulfite dianion[4] and directly observed in strong-acid solution.[5] The rate constants for reaction of these acetophenone oxocarbonium ions with water have been measured and vary with substituent between 7×10^6 and 4×10^8 s^{-1} (para OCH$_3$ to meta Br, respectively).[4,5] As the stability of oxocarbonium ions such as **I** decreases, the rate constant for attack of water and simple alcohols will increase. When the intermediate is sufficiently destabilized so that the reaction with simple alcohols is diffusion controlled, the rate-limiting step for the hydrolysis reaction will change from C–O bond cleavage to rate-limiting diffusion apart of the alcohol–oxocarbonium pair (k_{-d}, Scheme I).[6] If the alcohol–oxocarbonium ion pair becomes so unstable that its lifetime become less than the time required for a single bond vibration, then the reaction can no longer proceed through the stepwise pathway and the formation of hemiacetal *must* become concerted with alcohol departure.[4,6]

Secondary isotope effects are a method that is widely used to estimate central atom hybridization in rate-limiting transition states.[7-9] For complete conversion from sp^3 to sp^2, equilibrium isotope effects of 25–35% are observed for carbonyl addition reactions.[9,10] In the case where diffusional separation of an alcohol–oxocarbonium ion encounter pair has become the rate-limiting step in acetal hydrolysis, a full equilibrium isotope effect would be anticipated based on the sp^2 hybridization of the oxocarbonium ion. Under conditions where C–O bond cleavage is rate limiting, smaller hybridization changes would occur and a smaller secondary isotope effect would be observed. Secondary deuterium isotope effects therefore provide a method for observing and quantitating lifetime-dependent changes in mechanism during the hydrolysis of simple acetals.

Scheme I



In order to ascertain the importance of diffusional separation, we have examined the secondary deuterium isotope effects and structure-reactivity parameters for the hydrolysis of benzaldehyde dimethyl acetals in mixed water–dioxane solvents varying between pure water and 70% dioxane, 30% water, by volume.

Experimental Section

Materials. Substituted benzaldehyde dimethyl acetals were prepared by reaction of the carbonyl compound with trimethyl orthoformate in methanol[4,9] by using *p*-toluenesulfonic acid as catalyst and were purified by recrystallization or distillation. Glass distilled water was used throughout. Benzaldehyde-*formyl-d* was prepared by the cyanide cleavage of benzil in the presence of D$_2$O.[11] Isotopic purity was estimated to be greater than 98% by NMR. Dioxane was purified by refluxing over sodium followed by distillation. Identical rate constants were obtained by using reagent grade dioxane without further purification as with the purified materials. Stock solutions of the substituted acetals were prepared in methanol at about 0.01 M.

Kinetic runs were performed in 3.0-mL spectrophotometer cells containing 10–20 μL of the desired acetal stock solution and 3.0 mL of the dioxane–water solvent mixture containing 10^{-3} M HCl. Dioxane–water solvent mixtures were prepared by volume and are expressed as the percentage of dioxane in the total volume. Reactions were initiated by the addition of acetal stock solution to the temperature-equilibrated reaction cell.

Kinetic Measurements. The rates of appearance of substituted benzaldehydes were followed at suitable wavelengths by using a Hitachi 100-60 spectrophotometer equipped with an automatic cell changer and a digital printout. Constant temperature was maintained at 25 °C by means of a thermostated cell compartment. First-order rate constants were determined from semilogarithmic plots of ($A_t - A_\infty$) against time and, with the exception of the data in water and 10% dioxane, were linear for over 3 half-times. In water and in 10% dioxane a minor induction period was evident in semilogarithmic plots; first-order rate constants

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Table I. Solvent Effects on the Hydrolysis of Benzaldehyde Dimethyl Acetal[a]

solvent, % dioxane[b]	k^H/k^D [c]	k_{H^+}/M^{-1} s^{-1} [d]	k_{-1}/k_{-d}
0	1.118 ± 0.02 1.061 ± 0.01[e]	33.0	0.20
10	1.097 ± 0.01	24.6	0.30
20	1.056 ± 0.01	14.8	0.44
30	1.085 ± 0.015	8.27	0.80
40	1.117 ± 0.015	4.00	1.7
50	1.131 ± 0.008	1.69	3.9
60	1.144 ± 0.015	0.73	9.0
70	1.151 ± 0.01	0.16	41

[a] At 25 °C in the presence of 10^{-3} M HCl. [b] Solvent composition by volume. [c] Observed isotope effect for the hydrolysis of benzaldehyde-*formyl-d* dimethyl acetal. Errors are given as 90% probability limits. [d] Second-order rate constant for acid-catalyzed hydrolysis, based on proton activity as measured by the glass electrode. [e] At pH 5.3.

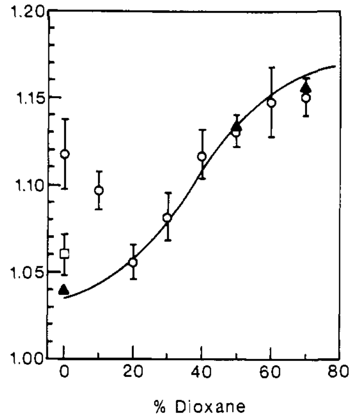

Figure 1. Dependence of the observed isotope effect for the hydrolysis of benzaldehyde-*formyl-d* dimethyl acetal on the percentage of dioxane in the dioxane–water mixed solvents. The solid line is calculated by using eq 2; see text. Triangles (▲) are values of k^H/k^D estimated from ρ^r/ρ^r_{eq} ratios. The square (□) is the isotope effect observed in aqueous solution at pH 5.3 by using 20 mM citrate buffer.

were obtained from the best linear portions in the latter half-times. The pH of each cell was measured at the end of each kinetic run with a Corning Model 130 pH meter equipped with a combined glass electrode. Isotope effect studies were performed as sets of parallel runs in duplicate. Generally each isotope effect reported is the average of 4–8 determinations. Standard deviations given are expressed based on t curves to 90% probability. Experiments using solvent compositions that yield high and low isotope effects were performed in random order. On replicate determinations, the isotope effect maxima and minima were reproducible to within the error limit stated. Values of ρ and ρ^r were determined by systematically varying the ratio ρ^r/ρ in equation 3 to obtain the best fit as judged by the highest correlation coefficient of the line determined by linear least-squares analysis of the data.

Results

The effect of added dioxane on the α-deuterium isotope effect for the hydrolysis of benzaldehyde dimethyl acetal is complex (Table I and Figure 1). Although the differences between the maxima and minima in the figure are not large, they are significantly larger than the experimental errors estimated from replicate determinations. The general shape of the curve can be rationalized on the basis of the mechanism given in Scheme I.

In water at pH 3.05, ionic strength 10^{-3}, and 25 °C, we have found the kinetic isotope effect to be $k^H/k^D = 1.12 \pm 0.02$. This is slightly larger than the value of 1.09 reported by Cordes and co-workers at ionic strength 0.5, 25 °C, using phosphate or acetate buffers (pH not specified).[1,12] The accurate determination of isotope effects for this reaction in water is complicated by hemiacetal buildup during hydrolysis[13] which gives rise to biphasic kinetics for the appearance of benzaldehyde. The rate of hemiacetal hydrolysis is pH dependent and becomes fast relative to the hydrolysis of the parent acetal in the pH range 4–5.[13] The isotope effect at pH 5.3 was found to be 1.06 ± 0.01. The greatest error in determining the isotope effects at low pH no doubt arises from attempting to describe the appearance of the second product in a set of two consecutive reactions by using the simple first-order treatment.

Table II. Substituent Effects for the Hydrolysis of Benzaldehyde Dimethyl Acetals in 70% Dioxane[a]

substituent	k_{H^+}/M^{-1} s^{-1} [b]
para OCH_3	10.9
para CH_3	1.08
-H	0.16
para Cl	2.94×10^{-2}
meta Br	3.76×10^{-3}

[a] In 70% dioxane, 30% water, by volume at 25 °C, in the presence of 10^{-3} M HCl. [b] Apparent second-order rate constant for the acid-catalyzed hydrolysis reaction.

As the solvent is changed from water to 20% dioxane, biphasic kinetics is no longer observed for the appearance of benzaldehyde. This is consistent with Jensen's observation[13] that the solvent effect on hemiacetal hydrolysis is smaller than the effect observed for hydrolysis of the parent acetal. Thus, as the concentration of dioxane is increased, the rate constant for acetal hydrolysis decreases faster than the rate constant for the hydrolysis of the hemiacetal. The decrease in the isotope effect from 1.12 in water to 1.06 in 20% dioxane may simply be an artifact of the biphasic kinetics in the aqueous media. As the solvent is changed from 20 to 70% dioxane, by volume, the observed isotope effects increase regularly from 1.06 to about 1.15.

Rate constants for the specific-acid-catalyzed hydrolysis of substituted benzaldehyde dimethyl acetals in 70% dioxane, 30% water, are given in Table II. These rate constants are poorly correlated by using "standard" Hammett σ scales but are well correlated by eq 3 in which polar and resonance substitutent effects are separated.[10] Varying the ρ^r/ρ ratio to obtain the best correlation coefficient around the linear least-squares line gives $\rho = -4.0$ and $\rho^r = -2.0$.

Discussion

Isotope Effects. The equilibrium isotope effect for the formation of an oxocarbonium ion from an O-protonated acetal can be estimated to be about 1.24 on the basis of the value that is observed for the addition of cyanide anion to benzaldehyde.[10] The isotope effect on the protonation equilibrium can be estimated to be about $K_a^H/K_a^D = 0.95$, on the basis of the values that are observed for the ionization of mono-, di-, and trimethylamines in water at 25 °C.[10,14] The maximum *observed* isotope effect for the formation of an sp^2 oxocarbonium ion from an acetal is then about (0.95)(1.24) = 1.17. The increase in the isotope effect from 1.06 in 20% dioxane to 1.15 in 70% dioxane is consistent with a mechanism in which the composite sp^2 character of the rate-limiting transition state has increased to essentially the full equilibrium value in the highly nonpolar solvent mixtures. In the past,[1,12] results such as these have been interpreted in terms of a variable-transition-state mechanism, a more highly destabilized oxocarbonium giving rise to a "later transition" state. However, a mechanism such as this is only tenable when the C–O bond-breaking process is entirely rate limiting. Rate constants for the addition of water to oxocarbonium ions derived from acetophenone have been estimated from trapping experiments[4] and directly measured in strong-acid solution.[5] On the basis of these values, the rate constant for the addition of water and simple alcohols to benzaldehyde oxocarboniums has been estimated to be about 10^9 M^{-1} s^{-1}.[4] This can be converted into the rate constant for the

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internal return reaction (k_{-1}, Scheme I) by multiplying by the equilibrium constant for the formation of an encounter complex. Taking $k_d = 5 \times 10^9$ M^{-1} s^{-1} and $k_{-d} = 5 \times 10^{11}$ s^{-1},[4,6] we can estimate k_{-1} can to be about 10^{11} s^{-1}. This is only fivefold smaller than the rate constant taken for diffusional separation of the encounter complex, and separation is therefore rate limiting to the extent given by eq 1. For oxocarbonium ions that are less

$$k_{\mathrm{obsd}} = \frac{[\mathrm{H}]}{K_a} \frac{k_1 k_{-d}}{k_{-1} + k_{-d}} \tag{1}$$

stable than those derived from benzaldehyde, k_{-1} will be larger and k_{-d} will become rate limiting. Under these conditions, the full equilibrium isotope effect on the hydrolysis reaction (1.17) will be observed. The large isotope effects (1.15) that are observed in the high concentrations of dioxane are consistent with a mechanism involving rate-limiting separation of the alcohol–oxocarbonium ion encounter pair under these conditions. It should be pointed out that the above treatment merely established the *ratio* of k_1 to k_{-d}. If the rate constant for diffusion apart of the oriented oxocarbonium ion–alcohol complex is less than 5×10^{11} s^{-1}, the value of k_{-1} will also be proportionately less. In all probability this will be the case; the ratio $k_{-1}/k_{-d} = 0.2$ is probably a reasonable estimate, but k_{-1} is most likely *less* than 10^{11} s^{-1}.

If the decrease in k_{obsd} that is observed as the concentration of dioxane is increased (Table I) is largely due to the increase in k_{-1} relative to k_{-d}, the ratios k_{-1}/k_{-d} in the various solvent mixtures can be estimated. On the basis of the ratio $k_{-1}/k_{-d} = 0.2$ in water, the estimated ratios increase regularly to about 41 in 70% dioxane (Table I). The dependence of the observed isotope effect on this ratio is given by eq 2. The isotope effect on K_a can

$$(k^{\mathrm{H}}/k^{\mathrm{D}})_{\mathrm{obsd}} = (k_1^{\mathrm{H}}/k_1^{\mathrm{D}})(K_a^{\mathrm{D}}/K_a^{\mathrm{H}})(k_{-1}^{\mathrm{H}}/k_{-d}^{\mathrm{D}}) \frac{k_{-1}^{\mathrm{D}} + k_{-d}^{\mathrm{D}}}{k_{-1}^{\mathrm{H}} + k_{-d}^{\mathrm{H}}} \tag{2}$$

be estimated to be about 0.95, as before, and the effect on k_{-d} can be taken as unity. The true isotope effects for k_1 and k_{-1} for this reaction are not known but they must vary between the equilibrium limits of 0.8 and 1.24. Varying the effects on k_1 and k_{-1} between these limits and using the ratios k_{-1}/k_{-d} in Table I, we obtained the best fit to the data by using 1.07 and 0.87 for the effects on k_1 and k_{-1}, respectively (both as $k^{\mathrm{H}}/k^{\mathrm{D}}$). The line calculated by using these values appears as the smooth curve in Figure 1. With the exception of the data in water and 10% dioxane in which biphasic kinetics is observed, the calculated line provides a very good fit to the experimental data. The assumptions that k_{-1} varies as the simple inverse of k_{obsd} and that the extent of bond breaking in the k_1 transition state remains constant as the concentration of dioxane is varied are no doubt oversimplifications. However, the fit of the data to this simple model is sufficiently good so that a more complex interpretation is not warranted.

Structure-Reactivity Effects. Rate constants for the specific-acid catalyzed hydrolysis of benzaldehyde dimethyl acetals in 70% dioxane are poorly correlated by "standard" Hammett σ scales but are well correlated by eq 3 in which polar and resonance

$$\log k/k_0 = \rho(\sigma^{\mathrm{n}} + (\rho^{\mathrm{r}}/\rho)(\sigma^+ - \sigma^{\mathrm{n}})) \tag{3}$$

substituent effects are separated.[4,10,15] The best fit of the data in Table II is obtained by using $\rho = -4.0$ and $\rho^{\mathrm{r}} = -2.0$ (Figure 2). The rate constants for the hydrolysis of the diethyl acetals of benzaldehydes in water[16] and 50% dioxane[17] are also best described by this equation giving $\rho = -2.6$ and $\rho^{\mathrm{r}} = -0.9$ in water and $\rho = -3.4$ and $\rho^{\mathrm{r}} = -1.8$ in 50% dioxane (these data are replotted in Figure 2). The point for the para NO_2 compound in water deviates from this line; however, the large isotope effect of 1.15 that is observed[12] suggests that diffusional separation may already be largely rate limiting for this compound, giving rise to the negative deviation.

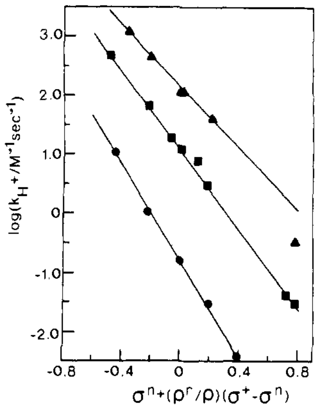

Figure 2. Hammett plot for the hydrolysis of benzaldehyde diethyl acetals in water (▲) and 50% dioxane (■) and for the hydrolysis of benzaldehyde dimethyl acetals in 70% dioxane (●). The data from water and 50% dioxane are replotted from ref 16 and 17; the σ scale is calculated by using eq 3.

For the addition of cyanide anion to substituted benzaldehydes, we have previously reported[10] that the ratio of the kinetic and equilibrium values of ρ^{r} gives an estimate of transition-state rehybridization that is comparable to the estimate that is obtained from α-deuterium isotope studies. This is presumably because the resonance parameter ρ^{r} measures the gain or loss of resonance interacting ability of substituents in a transition state or an equilibrium. The changes in resonance interacting ability appear to be a linear function of central atom rehybridization, as measured by α-deuterium isotope effects. For the hydrolysis of benzaldehyde acetals, the value of $\rho_{\mathrm{eq}}^{\mathrm{r}}$ can be estimated to be about −2.2 on the basis of the value that is observed for the formation of acetophenone oxocarbonium ions in aqueous solution.[4] The ratios $\rho^{\mathrm{r}}/\rho_{\mathrm{eq}}^{\mathrm{r}}$ are then 0.40, 0.82, and 0.91 for the reaction in water and 50 and 70% dioxane, respectively. Taking the equilibrium isotope effect to be 1.24,[10] the predicted isotope effects (after correction for the isotope effect on ionization of 0.95)[14] are 1.041, 1.135, and 1.157 for water and 50 and 70% dioxane, respectively. These predicted values are shown as the solid triangles in Figure 1. The agreement between the observed and predicted values is extremely good at the high concentrations of dioxane. The small value that is predicted in water reinforces the suggestion that the true isotope effect in water is being masked by problems associated with hemiacetal buildup. The agreement between the observed values of the kinetic isotope effects and the values predicted by ρ^{r} ratios again suggests that the use of ρ^{r} ratios will provide a useful supplement or alternate to isotope effects in the study of transition state structures.

The values of k_{-1} for the hydrolysis of simple aliphatic aldehydes and glycosides, which form less stable oxocarbonium ions than the benzaldehydes, would be expected to be very large and diffusional separation would be expected to be largely rate limiting for these compounds. Consistent with this suggestion are the large isotope effects of 1.15 and 1.17[1,12] that are observed for the hydrolysis of para NO_2 benzaldhyde and propionaldehyde acetals in water. Large isotope effects of about 1.14 are also observed for the hydrolysis of glycosides both in free solution and by the enzyme lysozyme.[18,19] These large effects have been considered as evidence that the enzyme-catalyzed reaction is proceeding by a stepwise oxocarbonium pathway, the rationale being that an S_N2-like pathway would exhibit much smaller isotope effects.

A changeover to an S_N2-like mechanism for acetal hydrolysis *must* occur when the oxocarbonium ion intermediate is so unstable that the rate constant for internal return approaches or exceeds $h/kT = 6 \times 10^{12}$ s^{-1} or the limit of 10^{13} s^{-1} which is the rate constant for a single-bond vibration. In the present work, the rate

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constant for internal return in 70% dioxane is estimated to be 40 times larger than k_{-d}. The fact that k_{-d} is an *apparent* dissociation rate constant for an oriented complex held together by secondary valence forces makes it impossible to accurately compare k_{-d} to the limiting values for internal return (k_{-1}). The observation of a large isotope effect of 15% does not require that k_{-d} be much less than k_{-1} since Kirby[20] has observed similar effects of 5–16% for S_N2 displacement reactions involving formaldehyde methyl phenyl acetals. The large negative ρ of −4 that is observed in 70% dioxane suggests that if the reaction *is* S_N2-like, the incoming and leaving groups are widely separated in space. This large amount of effective charge on the central carbon would be expected to have a significant effect on the zero-point energies for the two states under consideration, giving rise to the large isotope effect. If displacement reactions involving acetals commonly exhibit late, loose transition states such as this, large isotope effects such as those that are observed with glycosides would be anticipated.

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Photophysics of Aqueous Tryptophan: pH and Temperature Effects

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Abstract: The fluorescence decay kinetics of aqueous tryptophan and 3-methylindole have been determined as a function of pH and temperature by using a picosecond dye laser–single photon counting system with a time resolution of 50 ps. At pH 11, tryptophan exhibits a single exponential decay, with a lifetime of 9.1 ns at 18 °C. However, at pH 7 the decay is faster and definitely nonexponential; the values obtained from a biexponential fit to the data at pH 7 are τ_1 = 0.43 ns, τ = 3.32 ns, and f = 0.19 at 18 °C. The behavior of a 3-methylindole closely resembles that of tryptophan at pH 11. A model for the photophysics of aqueous tryptophan is presented in which the excited-state decay constant at pH 11 (where the amino acid side chain is not protonated) is given by the superposition of three independent processes: fluorescence, intersystem crossing, and photoionization; of these processes only photoionization is temperature sensitive (E^* = 51 kJ mol^{-1}). In the region pH 4–8, where tryptophan exists in the zwitterion form, a new nonradiative process is introduced, which involves intramolecular proton transfer from the $-NH_3^+$ group to the excited indole ring. The apparent activation energy for intramolecular quenching (E^* = 16 kJ mol^{-1}) suggests that it is a predominantly diffusion-controlled process. It is proposed that the nonexponential decay observed for aqueous tryptophan at pH 7 arises from transient terms in the rate constant for intramolecular quenching. Quantum yields calculated from this model compare well with experimental values.

Introduction

The fluorescence of proteins is usually dominated by that of the tryptophan residues.[1] Both the fluorescence lifetime and quantum yield of a tryptophan residue are strongly influenced by the nature of its local environment, and this sensitivity is widely exploited through the use of tryptophan as an intrinsic fluorescence probe for the structure and conformation of proteins and polypeptides in solution.[2] Interpretation of the results of such experiments requires an understanding of the excited-state decay processes in tryptophan, and their response to environmental perturbations. However, despite extensive investigations[3] two fundamental questions remain unanswered. (1) Can an isolated tryptophan residue be characterized by an exponential decay law? (2) What are the *principal* excited-state decay routes for tryptophan in aqueous solution?

Excitation of proteins that contain a few tryptophan residues per molecule yields nonexponential fluorescence decay kinetics;[4,5] the decays can be described as a superposition of a number of exponential components, each of which is assumed to correspond to a tryptophan residue (or group of residues) in a different environment. However, nonexponential decays have also been observed for a number of proteins and peptides containing a *single* tryptophan residue;[6,7] here, the nonexponentiality is attributed to the presence of multiple conformations of the molecule. Each of these explanations for nonexponentiality is based on the implicit assumption that the fluorescence decay of an isolated tryptophan residue is strictly exponential. Yet this assumption has not been verified. Recently, Rayner and Szabo[8] investigated the fluorescence decay of aqueous tryptophan at pH 7 using the conventional single photon counting technique (excitation by a weak spark lamp of 2–3 ns duration). The fluorescence emission was shown to follow a biexponential decay law with components having lifetimes of 3.14 and 0.51 ns. In a previous paper[9] we also reported the observation of a nonexponential fluorescence decay for aqueous

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