J. Am. Chem. Soc. **1981**, *103*, 3709-3713

in order to generate a greater appreciation of the relative importance of the possible effects.

One final point that emerges from this work is that the present model suggests that within a family of reactions (say the benzyl system) there are two parameters which govern reactivity. These are $(I_D - A_A)$, the ionization potential of the donor less the electron affinity of the acceptor, and $E_{\sigma\sigma^*}$, the excitation energy of the C-X bond to be cleaved. Thus, substituents may be classified in terms of their effects on these two parameters and rates may be correlated with them. This approach may lead to a direct check on the structure of the transition state and may provide necessary information about the degree of charge transfer from the nucleophile to the substrate, $[N\cdot^+(R\dot{-}X)^-]$ and/or on the extent of its carbenium ion character ($N{:}\ R^+\ {:}X^-$).

Acknowledgment. We thank Professors R. A. More O'Ferrall and D. J. McLennan and a reviewer for helpful suggestions. S.S.S. thanks the Ministry of Absorption for financial support.

The pH Jump: A Rapid Modulation of pH of Aqueous Solutions by a Laser Pulse

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Abstract: The first electronically excited state of compounds like 8-hydroxypyrene-1,3,6-trisulfonate or 2-naphthol-3,6-disulfonate is characterized by an acid dissociation constant ($pK^* = 0.5$) much lower than that of the ground state ($pK^0 = 7$). Thus excitation of such compounds is equivalent to introduction of a strong acid to the solution. In this study we utilized high-intensity (~50 mJ) short laser pulse (50 ns full width at half-maximum) to acidify aqueous solutions by exciting the above proton emitters. The discharged protons were detected both by their reaction with various pH indicators or by following the reprotonation of the proton emitter after its decay to the ground state. The maximal proton concentrations determined by either method are comparable and in typical experiments amount to 10^{-5}–5×10^{-5} M. The maximal proton concentration is built up during the laser pulse and decays to the initial level within a few microseconds. The protonation of pH indicators is a second-order diffusion-controlled reaction; the measured rate constants are compatible with the values calculated according to Debye's equation for diffusion-controlled reactions. Though the proton pulse is short, groups which have been protonated during the pulse remain in their protonated state for much longer periods, which are proportional to their intrinsic acid dissociation constant. The applicability of this method as a perturbing system for fast kinetics is discussed.

Introduction

The rapid diffusion of a proton in aqueous solution hampers kinetic studies of protonation. Thus in most cases only equilibrium studies can reveal the mechanism by which the proton participates in the chemical reaction. Though the electric field jump[2] is suitable for measuring rates of protonation, it is limited to pure water and cannot be applied to complex biochemical reactions.

In a recent publication[3] we described a new method which can generate within 50 ns some 10^{-4} M of protons. This proton pulse, called the pH jump, is achieved by irradiation of aqueous solutions of certain naphthol derivatives with an intense laser pulse. As a consequence, these compounds are pumped to their first excited singlet state which is characterized by a pK^* value much lower than that of the ground state (pK^0). Thus the laser pulse converts the proton emitter from a weak acid to a strong acid and within 100 ps[4] it dissociates releasing the protons to the water. Of the many compounds which on excitation exhibit a pK shift,[5,6] we selected two for our studies: 8-hydroxypyrene-1,3,6-trisulfonate and the 2-naphthol-3,6-disulfonate which, because of their negative charges, cannot permeate phospholipid bilayers.[3]

While the previous reports[3,7] were more descriptive in nature, the present one analyzes the postpulse events and quantitates the concentration of the ejected protons, the kinetics of protonation of the various protonatable groups present in the solution, and the final relaxation of the system to its equilibrium, prepulse state.

As will be demonstrated, the proton pulse can lower the pH by 3–5 pH units, it can be carried out in the presence of electrolytes, and the monitoring of the reaction is rather simple. These properties render the laser-induced pH jump as a convenient method for perturbing chemical and biochemical reactions by a brief intense proton pulse.

Materials and Methods

The optical arrangements for excitation and monitoring of the sample were described before.[3] A passively *Q*-switched ruby laser generated a giant pulse at 694.3 nm of about 1 J, with 30 ns full width at half-maximum. The pulse was passed through a KDP crystal generating the second harmonic frequency at 347.2 nm. The red light was filtered so that only the UV frequency irradiated the sample cell.

Absorbance changes of the sample were monitored either at 632.8 nm (using the line of a continuous He-Ne laser (Spectra-physics 2 mW)) or at 442 nm (using the He-Cd laser). The interrogating beam was passed either perpendicular or nearly colinear with the excitation pulse, collimated, and directed into the entrance slit of a Jarral-Ash 250-mm monochromator. The changes in the sample absorbance were detected by a Philips TVP 56 or an RCA 1P-28 photomultiplier, with rise time of 1–2 ns, traced on an oscilloscope (Tektronix 454 or a 7623A) and photographed. The changes in transmittance were converted to absorbance units.

Energy-dependence studies were carried out using a Molectron UV14 nitrogen laser (8 mJ/pulse, 10-ns pulse width), and the transient absorbancy changes of the monitoring beam (632.8 nm) were recorded by a Biomation 8100 transient recorder coupled to a Nicolet 1170 signal averager. The energy of the excitation pulse was measured by a Molectron energy meter placed at the place of the observation cell.

8-Hydroxypyrene-1,3,6-trisulfonate was Eastman Kodak laser grade. 2-Naphthol-3,6 disulfonate was Merck, Sharp and Dohme preparation, recrystallized before use.

Results

Energy Dependence of the Light-Driven Proton Pulse. In order to evaluate the nature of the photochemical reaction involved in

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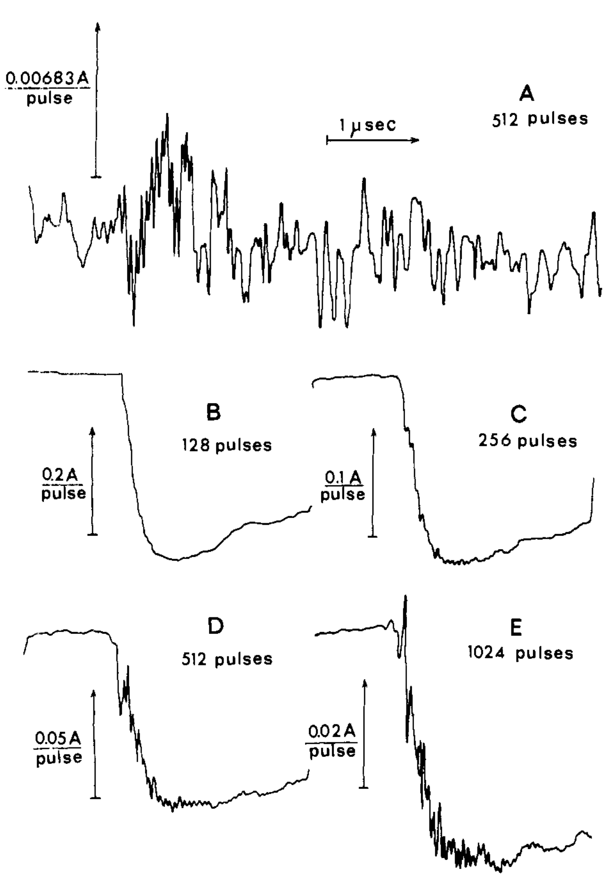

Figure 1. Transient absorbance changes of 40 µM solution of bromocresol green in H_2O, pH 6.0, following an excitation pulse at 337 nm and monitored at 632.8 nm in the absence (A) or the presence (B–E) of 2-naphthol-3,6-disulfonate. The excitation energies (in MW/cm^2 pulse) and the number of pulses were: (A) 1.9 MW/cm^2, 512 pulses; (B) 1.9, 128; (C) 0.95, 256; (D) 0.47, 512; (E) 0.24, 1024. The excitation beam (1 × 10 mm) hit the front surface of a 10 × 10 four-face quartz cuvette, and the monitoring light (1 mW He–Ne laser beam 1 mm in diameter) was passed perpendicularly to the excitation pulse about 0.5 mm behind the front surface. The excitation pulse was attenuated by UV filters of known absorbance, and the energy was measured by placing the energy meter instead of the observation cell. In order to avoid accumulation of stable photoproduct in the irradiated volume (~0.03 mL) the content of the cell (3 mL) was stirred by a magnetic stirrer. The output of the detecting photomultiplier was recorded by a Biomation 8100 transient recorder and accumulated in a Nicolet 1170 signal averager.

the pH jump, the light-driven reaction was carried out at varying light intensities. In order to avoid accumulation of possible stable unidentified photoproducts, the content of the cell was vigorously stirred with the aid of a magnetic stirrer. The amount of total energy used to the experiment did not exceed 400 mJ. The results are given in Figure 1. In the absence of a proton emitter molecules, no transient absorptions were recorded (Figure 1A).

Addition of 2-naphthol-3,6-disulfonate (1 mM) to the indicator solution caused a large transient absorbance of ~0.3 A/pulse. Attenuation of the excitation pulse by UV filters lowered the magnitude of the recorded signals (Figure 1B,C–E). The dependence of the recorded signal on the light intensity fits a linear log–log plot. Below 1 MW/cm^2, the slope of the line is 1, indicating a single photon excitation of the 2-naphthol derivative (see Figure 2). Above this power level a slight decrease of the slope was measured, probably reflecting saturation of the responsive system or possibly a parasitic triplet–triplet absorption. Because of this deviation the rest of the experiments were carried out at power flux of 1 MW/cm^2 or less. In the rest of the experiments described below, a low repetition rate ruby laser was used. Consequently the total excitation energy never exceeded 400 mJ.

Monitoring of the pH Jump by pH Indicators. Figure 3 depicts a typical oscillogram of a pH-jump experiment. The reaction mixture contained 8-hydroxypyrene-1,3,6-trisulfonate as a proton emitter and bromocresol green (pK = 4.7) as the pH indicator. The changes in the concentrations of the alkaline form of the indicator (λ_{max} 610 nm) were measured at 632.8 nm (ϵ_{632} 33.10^3 M^{-1} cm^{-1}) using a He–Ne laser. Figure 3A depicts the rapid protonation of the indicator which is followed by the dissociation of the proton (Figure 3B,C) to the prepulse state.

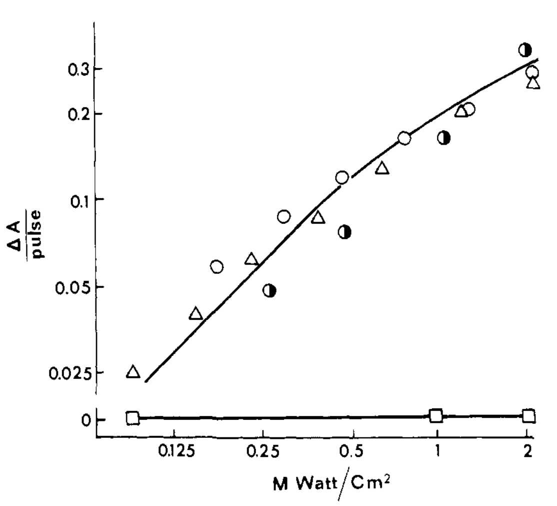

Figure 2. Correlation between the maximal size of the signal and the excitation pulse intensity. The experimental points (△, ○ ◑) are the results of experiments carried out, as exemplified in Figure 1: (□) measurements carried out in absence of 2-naphthol-3,6-disulfonate.

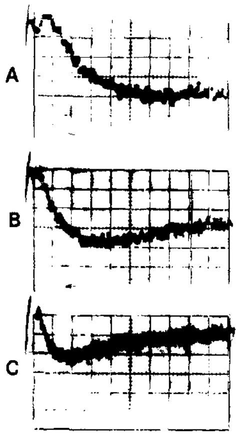

Figure 3. Typical oscillogram of a laser-induced pH jump; 135 µM of the proton emitter 8-hydroxypyrene-1,3,6-trisulfonate and 50 µM of bromocresol green (0.1 M $NaClO_4$, pH 6.1), were placed in a 5-mm quartz cuvette and pulsed by the second harmonic frequency (347.2 nm) of a ruby laser. Absorbance changes due to protonation of the indicator were monitored at 632.8 nm using continuous-wave He–Ne laser. The maximal deflection in the upper trace ($\Delta A = -0.255$) corresponds to protonation of 14% of the present indicator. The sweeping rates were 100, 200, and 500 ns/cm for upper, middle, and bottom tracing, respectively.

The dissociation of the protonated indicator is a pseudo-first-order reaction[8] ($k_{off} = 2.75 \times 10^5$ s^{-1}) independent of the indicator concentration (see Figure 4). A similar rate constant was measured with 2-naphthol-3,6-disulfonate as the proton emitter ($k_{off} = 3.6 \times 10^5$ s^{-1}).

The protonation of the pH indicators is a diffusion-controlled reaction. Using the expression $k_{on} = k_{off}/K_{diss}$, the k_{on} values of various indicators were measured and compared with values

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[8] k_{on} is the second-order rate constant of protonation. k'_{off} and k_{off} are the apparent and the true first-order rate constants of acid dissociation; ϕOH, ϕOH^*, ϕO^-, ϕO^{-*} are the ground state and first electronically excited state of the proton emitter in its acid and alkaline forms, respectively. In$^-$ and HIn are the alkaline and acidic forms of the indicator respectively. $(H^+)_{in}$, $(H^+)_t$, and $(H^+)_{eq}$ are the initial proton concentration, proton concentration at time t, and the equilibrium prepulse concentrations, respectively.

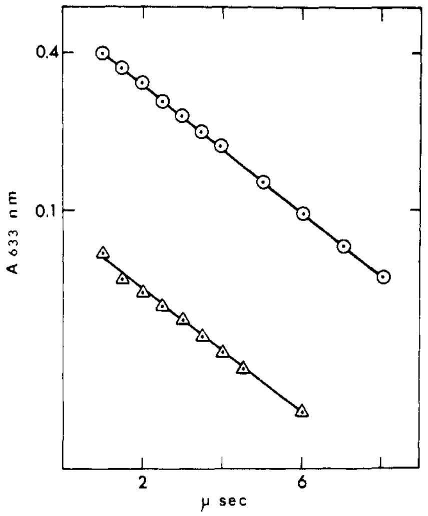

Figure 4. Kinetic analysis of disappearance of the protonated form of bromocresol green after laser pulsing of 8-hydroxypyrene-1,3,6-trisulfonate solution. The pulsed solution contained bromocresol green (16 μM (Δ) or 72 μM (○), 97 μM of 8-hydroxypyrene-1,3,6-trisulfonate (10 mM $NaClO_4$, pH 6.2). The reaction was monitored at 632.8 nm using the He–Ne laser.

Table I. Kinetic Parameters of Protonation and Acid Dissociation of Indicators in Solution Measured by the pH-Jump Method[a]

indicator	pK	log k_{off} (measd)	log k_{on} (calcd)	log k_{on} (est by other methods)
bromocresol green	4.7	5.5–5.8	10.2–10.5	10.85,[b] 10.7[c]
bromocresol purple	6.3	4.5	10.8	10.85,[b] 10.9[c]
neutral red	6.8	4.5	11.3	10.6[b]
bromothymol blue	7.0	4.15	11.15	10.85[b]
phenol red	7.1	4.6	11.7	10.85,[b] 10.4[d]
8-hydroxypyrene-1,3,6-trisulfonate	7.7	3.70	11.4	11.27[e] 11.3[b]

[a] The rate of dissociation was calculated from the decay kinetics of HIn measured as in Figures 3 and 4. Log k_{off} was calculated from log k_{on} = pK log k_{off}. [b] Calculated according to Debye[9] for ionic strength of I = 10 mM. [c] Taken from ref 10. [d] Taken from ref 11, 0.1 M KNO_3. [e] Taken from ref 12.

calculated according to Debye[9] or as reported in the literature (Table I).

Determination of the Ejected Proton Concentrations by Its Reaction with an Indicator. The duration of the excitation pulse is too short to allow equilibration between the proton and the indicator. Thus the initial concentration of the protonated indicator cannot be used to calculating the initial concentration of H^+. Similarly, the maximal concentration of HIn does not coincide with maximal proton concentration. In such nonequilibrium systems the proton concentration can be calculated from the velocity of HIn formation.

The variation in the concentration of HIn (Figure 5) is given by the differential equation:

$$-\frac{d(In^-)}{dt} = \frac{d(HIn)}{dt} = k_{on}(In^-)(H^+) - k'_{off}(HIn) \qquad (1)$$

The velocity of the reaction (V_t), k'_{off}, k_{on}, and the transient concentrations $(HIn)_t$ and $(In^-)_t$ are all measurable values. Thus, as long as $(H^+)_t >> (H^+)_{eq}$, the concentration of $(H^+)_t$ at any time is given by:

$$(H^+)_t = \frac{(d(HIn)/dt)_t + k'_{off}(HIn)_t}{(In^-)_t k_{on}} \qquad (2)$$

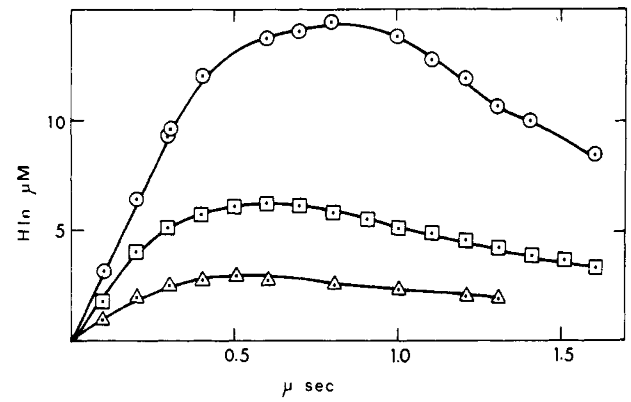

Figure 5. Build-up and decay of the protonated form of bromocresol green following a laser pulse of 8-hydroxypyrene-1,3,6-trisulfonate. The pulsed solution contained 8-hydroxypyrene-1,3,6-trisulfonate (97 μM) and bromocresol green; 16 (Δ), 43.5 (□), or 72 μM (○) (10 mM $NaClO_4$, pH 6.2).

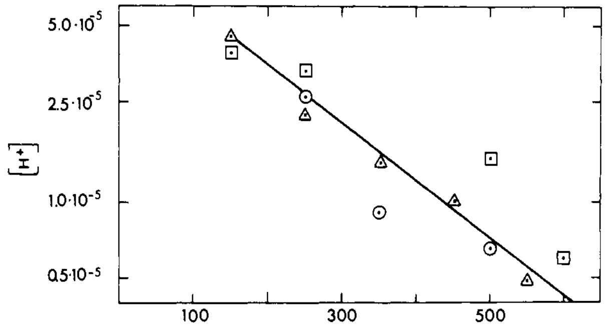

Figure 6. Kinetic analysis of the decay of free protons concentration following a laser pulse of 8-hydroxypyrene-1,3,6-trisulfonate. The proton concentration was calculated according to eq 2 using the data presented in Figure 5.

The maximal proton concentrations were calculated from the initial rates of protonation, where the second term of the numerator is negligible. Typical results, using various proton emitters and pH indicators, are given in Table II.

In the last experiment presented in Table II, 8-hydroxypyrene-1,3,6-trisulfonate was used as the pH indicator. This experiment was performed deliberately at lower excitation energy in order to minimize the formation of stable photoproducts[13,14] from 2-naphthol-3,6-disulfonate, which interfere with the monitoring of the protonation of this indicator at 442 nm (the He–Cd laser line). As seen in the fifth column, the velocity of the reaction increases with the indicator concentration, as expected for a second-order bimolecular reaction, while the resulting proton concentration is constant (last column). Doubling the proton emitter concentration increases the maximal proton concentration by the same factor. These two findings indicate that the formation of HIn is indeed a second-order reaction.

Equation 2 is applicable for calculaing the proton concentration at any time after the pulse. The results of such an analysis, using the data presented in Figure 5, are given on Figure 6. The decay of the free proton concentrations follows pseudo-first-order kinetics with a rate constant of $k = 3.5 \times 10^6\ s^{-1}$.

The reaction controlling the decay of the free proton concentration is the diffusion-controlled recombination of H^+ with the ground-state ionized proton emitter, ϕO^-. As in the experiments described in Figure 6 the initial pH was well below pK^0 of the proton emitter (see Table II), we can approximate that after the pulse $[\phi O^-] \approx [H^+]$. The relaxation time for the recombination between these species is $\tau^{-1} = k_{on}([\phi O^-] + [H^+])$. From the data

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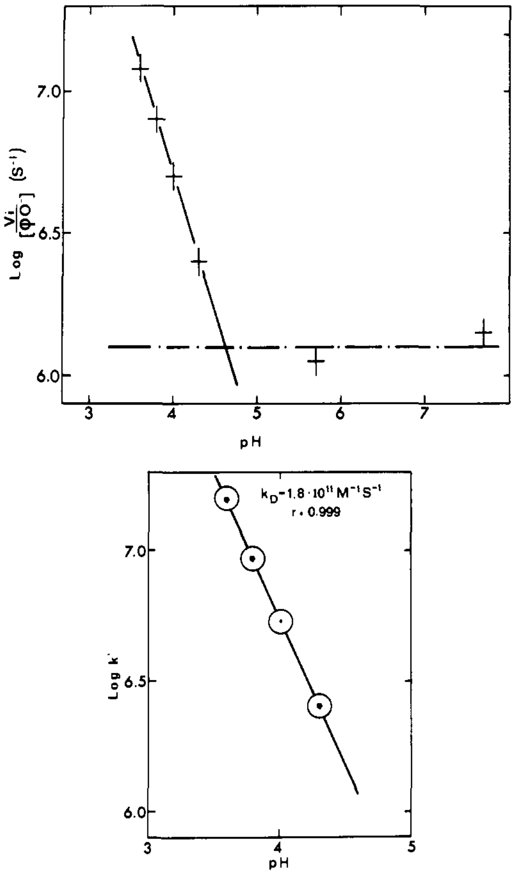

Figure 7. (A, top) Estimation of the concentration of ejected protons by kinetic analysis of the reprotonation of the proton emitter. 8-Hydroxypyrene-1,3,6-trisulfonate solution (115 μM) at the indicated pH values was pulsed by the second harmonic frequency of the ruby laser and the reprotonation of the ionized form was followed at 442 nm using a He–Cd laser. The initial velocity of the reaction was measured and divided by the pyrenate concentration in the pulsed solution. The intersection of the two lines is at pH 4.6. (B, bottom) Estimation of the second-order rate constant of protonation of the anionic form of 8-hydroxypyrene-1,3,6-trisulfonate. The results are taken from the experiment described in Figure 7A, where $(H^+)_{eq} >> (H^+)_{ej}$. The pseudo-first-order rate constants corrected for the ionic strength (Forster and Volkers[12]) are drawn vs. pH. The intercept corresponds to $k_{on} = 1.8 \times 10^{11}$ M^{-1} s^{-1}.

given in Table II and Figure 6 we can estimate the rate constant of recombination of H$^+$ with the anionic form of 8-hydroxypyrene-1,3,6-trisulfonate to be 1.6×10^{11} M^{-1} s^{-1}.

Determination of the Ejected Proton Concentration by Monitoring the Reprotonation of the Proton Emitter. In the absence of a pH indicator the only group with which the ejected proton can react at the end of the pulse is the proton emitter itself. As the perturbed system is far from equilibrium, the reaction of ϕO^- with H$^+$ will follow a simple undirectional rate equation:

$$\frac{d(\phi OH)}{dt} = k_{on}(\phi O^-)((H^+)_{eq} + (H^+)_{ej}) \qquad (3)$$

where $(H^+)_{eq}$ is the prepulse proton concentration and $(H^+)_{ej}$ is the concentration of ejected protons. It is clear that as long as $(H^+)_{ej} >> (H^+)_{eq}$ the velocity of recombination will be independent of the prepulse pH. On the other hand, once $(H^+)_{eq} >> (H^+)_{ej}$ the velocity of the reaction will be linear with $(H^+)_{eq}$ (Figure 7). The intersection of these two lines is a good estimate of $(H^+)_{ej}$. This simple determination of the ejected proton concentration is applicable as long as the initial pH falls in the range $pK^* < pH_{eq} < pK^0$.

The applicability of this technique is demonstrated in Figure 7A where the velocity of recombination of ϕO^- with H$^+$ is related to the prepulse proton concentration. The estimated concentration of the ejected protons, given by the intersection of the two lines at pH 4.6, is in good agreement with the data given in Table II.

Under conditions where $(H^+)_{eq} >> (H^+)_{ej}$ the rate of protonation of ϕO^- will follow pseudo-first-order kinetics with apparent rate constant k':

$$\log k' = \log k_{on} + \log (H^+)_{eq}$$

Drawing this function, with proper correction for the ionic strength, allows estimation of the rate constant of protonation of the alkaline form of 8-hydroxypyrene-1,3,6-trisulfonate. As demonstrated in Figure 7B the extrapolated value corresponds to 1.8×10^{11} M^{-1} s^{-1}, in high agreement with the reported value[12] (see also Table I).

Discussion

Kinetic Analysis of the pH Jump in Aqueous Solution. The pH-jump technique[3,7] is capable of generating 10^{-5}–10^{-4} mol of protons within a few nanoseconds. To our knowledge no other method can cause, under controlled conditions, such a large pH change in such a short time. Consequently, before the method can be used for perturbation of other systems, the dynamics of the perturbing species, the protons, must be clearly understood. The ejection of the proton from the excited proton emitters is completed within 100 ps.[4] In the present study we looked at the reaction of the proton after the acid dissociation, using a pH indicator as a model for a protonatable group.

The equilibria involved in the generation and detection of the pH jump are as follows:[15]

$$\text{I. } \phi OH \xrightarrow{h\nu} \phi OH^* \rightarrow \phi O^{*-} + H^+$$

$$\text{II. } \phi O^{-*} \rightarrow \phi O^- + (h\nu)$$

$$\text{III. } \phi O^- + H^+ \rightarrow \phi OH$$

$$\text{IV. } In^- + H^+ \leftrightarrow HIn$$

The decay times of the first electronically excited singlet state of 8-hydroxypyrene-[1,3,6]-trisulfonate and 2-naphthol-3,6-disulfonate are 6 and 20 ns, respectively.[4] Thus, during the excitation pulse, the proton-emitter molecules cannot undergo many cycles consisting of excitation, dissociation, relaxation to the ground state, and reprotonation. The excited singlet state lifetime is about 10–30% of the pulse length, and as long as the proton emitter is in its excited state its protonation is thermodynamically unfavored. Besides this thermodynamic restriction, there are also kinetic limitations on the reprotonation of ϕO^{-*} during the duration of the pulse. At $(H^+) = 10^{-4}$–10^{-5} M, the time constant of recombination (0.1–1 μs) is much longer than the laser pulse duration. The combination of these conditions provides an effective mechanism for accumulation of the proton emitter during the pulse in its excited dissociated form. This accounts for the high yield of protons measured per pulse (Table II, expts 1–5).

In a typical experiment 10^{-4} M of indicator reacts with 10^{-5} M of ejected protons; thus the average distance between the H$^+$ and indicator molecules is ~270 Å. This distance is too long to be covered, even by the highly mobile proton, during the 30-ns of the laser pulse. Indeed most of the HIn is formed after the pulse (Figure 3A). The short lifetime of ϕO^{-*} and its relative slow mobility (respect to H$^+$) ensure that no interaction of ϕO^{-*} with the indicator takes place.

The proton concentration generated during the pH jump is 2–4 orders of magnitude higher than the equilibrium, prepulse level. Thus, if we limit our observation period to the time where the reactants concentrations are far from their final equilibrium concentrations, we can consider the reaction to be practically unidirectional, which simplifies the kinetic analysis. As documented in Figure 6, the decay time of the free proton is about 200 ns; thus 1 μs after the pulse the pH is nearly back to its equilibrium level. Still, as seen in Figure 5 and Table I, we can measure about 20% of the total indicator in its protonated state many microseconds after the pulse (50 μs for the high pK indicator

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[15] The nonradiative decay of ϕOH^* is not considered in this discussion as it does not alter the conclusion.

Table II. Estimation of Maximal Proton Concentration in pH-Jump Experiments

	proton emitter	μM	indicator	μM	dHIn[a]/dT, M/s	H_{max}^+,[b] μM mol	pH equilibrium $t = \infty$	pH initial $t \rightarrow 0$
1.	8-hydroxypyrene-1,3,6-trisulfonate	97	bromocresol green	16	9.25	11.6	6.2	4.93
2.		97	bromocresol green	43	24.4	11.3	6.2	4.94
3.		97	bromocresol green	72	35	9.7	6.2	5.01
4.		194	bromocresol green	230	500	43.5	6.2	4.36
5.		194	bromocresol green	700	1800	51.4	6.2	4.29
6.	2-naphthol-3,6-disulfonate	2300	bromocresol green	38	61	32.1	7.5	4.49
7.		2700	bromothymol blue	24	41	34.1	8.6	4.46
8.		2300	8-hydroxypyrene-1,3,6-trisulfonate	100	12.3	0.6	9.0	6.22

[a] The maximal velocity recorded in the table was measured during the first 100 ns. [b] The proton concentration was calculated according to eq 2 using $k_{on} = 5 \times 10^{10}$ M^{-1} s^{-1} for bromocresol green and bromothymol blue and 2×10^{11} M^{-1} s^{-1} for hydroxypyrenetrisulfonate.

bromothymol blue). At that time the concentration of the free protons already reached the prepulse, equilibrium level. Consequently, HIn concentration is very far from its equilibrium concentration, and its dissociation is also unidirectional.

Dissociation of the Protonated Indicator. Examination of Table I reveals that k_{on} calculated as k'_{off}/K_{diss} deviates in some cases from the value calculated according to Debye.[9] The deviations are attributed to a direct proton transfer from the protonated indicator to the anionic proton emitter

$$\mathrm{HIn} + \phi O^- \xrightarrow{k_{PT}} \mathrm{I\bar{n}} + \phi OH$$

Consequently k'_{off} in eq 1 is a sum of two terms $k'_{off} = k_{off} + k_{PT}[\phi O^-]$. As the value of k_{on} in Table I was calculated by the measured value (k'_{off}) and not according to the true value (k_{off}), deviations are expected especially when $k_{PT}[\phi O^-]$ is not negligible with respect to k_{off}. Limiting our present discussion to the sulfonphthalein indicators (bromocresol green, bromocresol purple, bromothymol blue, and phenol red; see Table I), we observe that the higher the pK of the indicator (i.e., the slower its rate of acid dissociation), the bigger the deviation between the measured and the predicted values of k_{on}. The error is within experimental accuracy for bromocresol green and increases to sevenfold for phenol red. The direct proton transfer calls for encounter between the protonated indicator and the anionic proton emitter. Such encounter is affected by the electrostatic repulsion between $(\mathrm{HIn})^-$ (bearing a charge of -1 for the sulfonphthaleins) and the -3 or -4 charges of the two proton emitters used in this study. Because of the large radius of encounter of ~12 Å, direct proton transfer is a rather fast reaction with a rate of encounter (not corrected for steric factor) of $\sim 2 \times 10^9$ M^{-1} s^{-1} (calculated according to Debye[9] at $I = 0$). This constant contribution of the direct proton transfer to the decay of HIn explains why we observe the deviation of k_{on} mostly with slow dissociating indicators where k_{off} is already comparable to $k_{PT}[\phi O^-]$. In contrast to the sulfonphthaleins a highly negatively charged molecule like 8-hydroxypyrene-1,3,6-trisulfonate (used as indicator, see bottom line in Table I) is not expected to participate in any direct proton transfer with the triply charged anionic form of 2-naphthol-3,6-disulfonate. The electrostatic repulsion ($Z_1Z_2 = +9$) is strong enough to prevent the encounter between them. Indeed, in this case in spite of the slow rate of acid dissociation, $k'_{off} = 5 \times 10^3$ s^{-1} we observe no contribution of $k_{PT}[\phi O^-]$ to k'_{off}, and k_{on} calculated as $k'_{off}/K_{diss} = 2.5 \times 10^{11}$ M^{-1} s^{-1} is very close to the values calculated from the data presented in Figure 6 (1.6×10^{11} M^{-1} s^{-1}), Figure 7 (1.8×10^{11} M^{-1} s^{-1}), and the value measured by Forster and Volker.[12] (1.85×10^{11} M^{-1} s^{-1}), or as calculated according to Debye[9] (2×10^{11} M^{-1} s^{-1}).[12]

Properties of a Proton Emitter to be Used in the pH Jump Experiment. In the present study we used two proton emitters: 8-hydroxypyrene-1,3,6-trisulfonate and 2-naphthol-3,6-disulfonate. Yet any molecule with p$K^* <$ pK^0 can be used as a possible proton emitter for a pH-jump experiment. In this section we shall consider the properties required from an efficient proton emitter. The first request is an intense absorption band of the acidic form, at the excitation wavelength, and minimal absorbance of the anion at the same wavelength both in its ground and excited states. Otherwise the formation of the anion will absorb the excitation energy with consequent drop in yield. There are many compounds with p$K^* <$ pK^0, but as discussed by Schulman[5] only those with a fast dissociation constant are suitable for our purpose. If the dissociation time is comparable to the lifetime of the first electronically excited singlet state, there will be a marked drop in the efficiency of the pulse-induced proton ejection.[5] The radiative lifetimes of the first electronically excited singlet state of aromatic compounds are in the nanosecond range; hence an efficient proton ejecting molecule should dissociate in the subnanosecond or even picosecond range. As shown in a previous publication,[4] such compounds have a p$K^* < 2$.

The amount of ejected protons is a function of the proton emitter absorption cross section, its concentration, and the laser pulse energy. Thus, under constant experimental conditions the number of protons ejected by each pulse is constant. In order to maximize the change in the pH, the initial pH should be a little alkaline. Still the initial pH cannot exceed the pK^0 of the proton emitter; if this pH is exceeded, the concentration of ϕOH will sharply decline; what is more, the proton emitter will act as a buffer. Such limitations may lead to a selection of compounds with high pK^0 values. This tendency should be carefully considered regarding the ability of the anionic form (ϕO^-) to participate in hydrolytic reactions. A compound like hydroxypyrenate trisulfonate (pK = 7.7) can regain a proton only by recombination with a free proton or by a direct proton transfer from another protonated group. The interaction with H_2O to form the acidic pyrene and OH^- is very slow ($k_{hyd} = 1$ s^{-1}, calculated from the data given by Forster and Volker[12]). On the other hand, the anionic form of proton emitters with higher pK^0 are strong bases and can undergo a rapid hydrolysis. In such a case the proton pulse will be followed by nearly an equivalent amount of OH^-, formed by rapid hydrolysis, a reaction which lowers the efficiency of the desired event.

In the present study we analyzed the main characteristics of the laser-induced proton pulse: the concentration of the ejected protons, their relaxation time, the kinetic response of the pH indicator, and the effect of spurious reactions such as direct proton transfer or hydrolytic attack by the ionized proton emitter. The recognition and understanding of these reactions are the first step for the future use of the pH jump as a general method of perturbing chemical reactions.

Acknowledgment. This work was supported in part by the Israeli Commission for Basic Research.