ELSEVIER

Biophysical Chemistry 73 (1998) 13–22

Biophysical Chemistry

An experimental methodology for measuring volume changes in proton transfer reactions in aqueous solutions

Cristiano Viappiani[a,b,*], Stefania Abbruzzetti[a,b], Jeanne R. Small[c], Louis J. Libertini[d], Enoch W. Small[d]

[a] *Istituto Nazionale per la Fisica della Materia, Parma, Italy*
[b] *Dipartimento di Fisica, Università di Parma, Parma, Italy*
[c] *Department of Chemistry and Biochemistry, Eastern Washington University, MS 74, Cheney, WA, USA*
[d] *Quantum Northwest Inc., Spokane, WA, USA*

Received 13 September 1997; received in revised form 22 January 1998; accepted 22 January 1998

Abstract

A fast perturbation in proton concentration can be induced in aqueous solution using a pulsed ultraviolet laser and suitable photolabile compounds which, upon photoexcitation, irreversibly release protons. The volume change and the rate constant for the reaction of the photodetached protons with proton-accepting groups in solution can be monitored using time resolved photoacoustics. A typical proton concentration jump of 1 μM can be obtained with a 200-μJ laser pulse at 308 nm. Reaction dynamics from 20 ns to 5 μs can be easily followed. The methodology we establish represents a direct, time-resolved measurement of the reaction volume in proton transfer processes and an extension to the nanosecond–microsecond range of traditional relaxation techniques, such as stopped-flow. We report example applications to reactions involving simple molecules and polypeptides. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Photoacoustics; Volume changes; Proton transfer; Laser pH jump

---

1. Introduction

Proton transfer (PT) between adjacent reactants in solutions is a commonly found reaction in a variety of chemical and biochemical processes, including enzymatic reactions, in which protons can be reagents, products or intermediates. PT reactions are difficult to study since they are generally very fast, but recent development of suitable techniques has allowed their initial experimental characterisation [1].

PT reactions often involve a change in the number of charges on going from reactants to

---

* Corresponding author, Dipartimento di Fisica, Macedonio Melloni, vile delle Scienze, 43100 Parma, Italy. E-mail: viappiani@vaxpr.fis.unipr.it

0301-4622/98/$19.00 © 1998 Elsevier Science B.V. All rights reserved.
PII S0301-4622(98)00108-2

products and, in most cases, this is known to generate volumetric effects in the solution [2–4]. The observed volume changes for the formation or disappearance of point charges can be partly explained by means of the classical electrostrictive theory [5].

From a thermodynamic point of view, the response of chemical reactions to pressure can be described by means of a transition state complex theory in which pressure plays the same role on the volume profile as temperature does on the energetics of the reaction:

$$\text{reactants (R)} \rightarrow \text{transition state} (\ddagger) \rightarrow \text{products (P)} \tag{1}$$

$$\Delta V^{\ddagger} = V^{\ddagger} - V_{\mathrm{R}} \tag{2}$$

$$\Delta V = V_{\mathrm{P}} - V_{\mathrm{R}} \tag{3}$$

Reaction volumes, ΔV, are generally studied by means of partial molar volume measurements via density data, or measured directly with the aid of a dilatometer. The reaction volume can also be determined considering the effect of pressure on the equilibrium constant K of the reaction [2–4]:

$$\Delta V = -RT\frac{\partial \ln K}{\partial P} \tag{4}$$

The activation volume, $\Delta V^{\ddagger}$, can be determined from the measured rate constant, k, of the reaction in a very similar way [2–4]:

$$\Delta V^{\ddagger} = -RT\frac{\partial \ln k}{\partial P} \tag{5}$$

However, several solvent parameters affecting the proton transfer reactions are influenced by the increase of pressure, e.g. the dielectric constant and the viscosity therefore rendering difficult the characterisation of these reactions.

Time resolved photoacoustics (PA) is an alternative method for studying structural volume changes (also termed reaction volumes) as well as enthalpic changes accompanying light-induced reactions [6–8]. The time profile of the pressure waves, generated by the photo-induced processes, can be used to determine the rate constants and the enthalpic and volumetric changes of the different reactions, provided that the time constants are sufficiently distinct [6,8,9]. Several charge transfer reactions have been studied by means of time resolved photoacoustics, e.g. intra- and inter-molecular electron transfer reactions [10–14] and proton transfer reactions [15,16]. Studies of photoinitiated reactions of biological relevance using photothermal methods have been recently reviewed by Schulenberg and Braslavsky [17].

The overall photoacoustic signal is generally composed of a thermal component, due to heat released in the de-excitation processes, and a structural part, due to the reaction volumes of the photoinitiated reactions. In aqueous solutions, these two contributions can be separated by means of temperature-dependent experiments [7,8,18–20].

We have recently described an experimental methodology that allows the direct time resolved monitoring of ΔV for a proton transfer reaction in solution [16]. We applied the concept of laser-induced pH jump, elaborated by the group of Gutman in a series of works in which several different systems were investigated (see Gutman and Nachiel [1] and references therein). Essentially, an intense, ultraviolet nanosecond-laser pulse is used to photolyze a solution containing an organic compound (e.g. an aromatic alcohol) which, upon excitation, becomes a very strong acid and rapidly releases a proton to the solution. The reaction of the photodetached proton with a reactant in solution was monitored by Gutman et al. by means of transient absorption spectroscopy and time-resolved fluorescence.

In our experiments we have monitored directly the structural volume change for the protonation reactions by means of time-resolved photoacoustics (vide supra). In this work we demonstrate the usefulness of this approach for studying the reaction volumes of a number of PT reactions that so far have been difficult to investigate. We report the determination of the reaction volume for proton detachment upon photoexcitation of aqueous solutions of two *o*-nitrobenzyl compounds, namely *o*-nitrobenzaldehyde and 2-hydroxyphenyl-1-(2-nitro)phenylethylphosphate (NPE caged proton), and the following protonation reactions with hydroxide and the ε-amino groups of poly-L-lysine. We have previously investigated the case of *o*-nitrobenzaldehyde in pure water [16]. In this paper, we report on the results obtained with the NPE caged proton and compare the reaction volume for the photodetachment of protons with the previously reported value for the analogous reaction in *o*-nitrobenzaldehyde. The effect of the ionic strength on the reaction volumes for the photodetachment of protons and the reaction volumes and the rate constants for the reaction with hydroxide to form water has been considered. A remarkable advantage of photoacoustics lies in the fact that there is no need to monitor any optical property of a chromophore and the technique can be applied to any reaction, provided the rate constant and the extent of the volume change are within the experimentally accessible ranges.

2. Materials and methods

2.1. Chemicals

Evans blue and *o*-nitrobenzaldehyde were from Aldrich. Poly-L-lysine (PLL) hydrochloride (mol wt. 24 000) was from Sigma. 2-hydroxyphenyl-1-(2-nitro)phenylethylphosphate was a kind gift of Dr Ernest McGoran. Evans blue was used as reference compound in all the PA experiments.

The compounds we used to photoinduce the fast acid pulse belong to the general class of *o*-nitrobenzyl compounds. Upon photoexcitation these compounds undergo a subnanosecond intramolecular hydrogen abstraction that leads to the formation of the nitronic acid [21–23]. The latter species is a very strong acid and rapidly deprotonates on the nanosecond time scale. These compounds act as 'caged proton' [23]. The caged proton forms we used are *o*-nitrobenzaldehyde and 2-hydroxyphenyl-1-(2-nitro)phenylethylphosphate (NPE).

2.2. Experimental setup and data acquisition

The experimental setup is essentially the same as the ones we have previously described [24,25]. Excitation is achieved via a XeCl excimer laser (Lambda Physik, EMG 50, $\lambda = 308$ nm). The beam is not focused and is attenuated by means of a set of neutral density filters and shaped by a rectangular slit (height 1-cm, width 280-μm). The laser pulse energy is monitored by an energy meter (Laser Precision RJ-7620) equipped with a pyroelectric probe (Laser Precision RJ-765). The pressure changes are detected by a piezoelectric PZT transducer (Panametrics, V 103). The signal is then amplified (60 dB) and fed into a digital oscilloscope (LeCroy 9450A, 300 MHz, 400 Ms s^{-1}). The data is transferred to a personal computer for the numerical analysis. The sample holder was temperature controlled using a circulating water bath that ensured ±0.1°C stability inside the cuvette. The temperature inside the cuvette is monitored by a Pt100 digital thermometer.

Prior to deconvolution, the photoacoustic waveforms are normalised for the absorbance of the solution at the excitation wavelength and the laser pulse energy.

Depending on the laser pulse energy, signals were acquired either as single shots (high-laser pulse energy) or as the average of several (typically 100) traces. The dynamic range accessible to the instrumentation is remarkably broad, allowing the laser pulse energy to be varied between 2 and 200 μJ in a linear response regime [16].

2.3. Data analysis

The protocol we use for fast and precise data acquisition takes advantage of the strong temperature dependence of the thermoelastic parameters of aqueous solutions [7,8,17–20]. The photoacoustic signal is proportional to the overall volume change induced in solution by absorption of light and can be written as the sum of a thermal and a structural contribution. The signal of thermal origin is mediated by the thermal expansion coefficient of the solution, β. In aqueous solutions, this term can be suppressed by acquiring the photoacoustic signal at the temperature $T_{\beta=0}$ where β is zero [6]. In pure water $T_{\beta=0}$ is 3.9°C [26]. We estimate the extent of the structural volume changes using a two-temperature method [27].

The volumetric and kinetic information is retrieved from the experimental waveforms by comparing the signal, *S(t)*, obtained for the sample at $T_{\beta=0}$, with the signal for a calorimetric reference, *R(t)*, acquired at a slightly higher temperature where $\beta \neq 0$, reproducing the instrumental transfer function:

$$S(t) = R(t - t_0) \otimes H(t) \tag{6}$$

where *H(t)* represents the time derivative of the volume change function. *R(t)* is time-shifted with respect to *S(t)* due to the change in the speed of sound for the temperature difference between reference and sample. $T_{\beta \neq 0}$ must be close enough to $T_{\beta=0}$ so that the isothermal compressibility, κ, may be considered unchanged [27]. *H(t)* at $T_{\beta=0}$ is assumed to be a sum of exponentials:

$$H(t) = \sum_i \frac{\varphi_i}{\tau_i} \mathrm{e}^{-t/\tau_i} \tag{7}$$

where φ_i represents the amplitude of transient *i* (proportional to the volume change ΔV_i) and τ_i its lifetime. The extent of the structural volume change, ΔV_i, is calculated from φ_i as:

$$\Delta V_i = E_\lambda \left(\frac{\beta}{C_p \rho} \right)_{T_{\beta \neq 0}} \varphi_i \tag{8}$$

where E_λ is the molar energy content of the laser pulse energy and $(\beta/C_p \rho)_{T_{\beta \neq 0}}$ is the thermoelastic parameter of the solution at $T_{\beta \neq 0}$. The parameter $(\beta/C_p \rho)_{T_{\beta \neq 0}}$ for water was calculated from literature data [26] or determined experimentally using a comparative method [8]. It is important to note that the volume changes, ΔV_i, are calculated per mole of absorbed photons. In order to get the volume change for a mole of reacted species it is necessary to divide ΔV_i by the quantum yield of the process [17].

Deconvolution of experimental data was performed using the program Sound Analysis 3000 (Quantum Northwest Inc., Spokane, WA), a least-squares iterative reconvolution based on the Marquardt algorithm. The principles of deconvolution in photoacoustics have been already explained in detail in the literature [9,28,29]. For any given number of components, the program searches for the φ_i and τ_i which minimize the sum of the squares of the residuals. The goodness of fitting is judged by examination of the residuals and the autocorrelation function [28,29]. Accessible lifetimes with the above described instrumentation range from approx. 20 ns to 5 μs.

The minimum detectable volume change for a fast, sub-resolution process is approx. 0.2 ml mol^{-1}, but the sensitivity decreases on decreasing the rate constant of the reaction [29]. The errors affecting the volume changes and the lifetimes are determined by performing a statistical analysis on the recovered parameters in multiple acquisitions under identical experimental conditions.

3. Applications and testings

3.1. Proton solvation

The photoacoustic instrumentation has been found to give signals which are linear with laser pulse energy over a wide range of pulse energies. This is seen for data, such as that shown in Fig. 1.

Fig. 1 reports the signal observed for *o*-nitrobenzaldehyde at 3.9°C in water at pH 6.7.

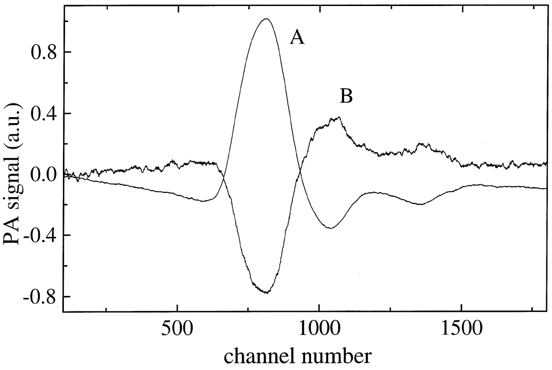

Fig. 1. Observed photoacoustic signal upon photolysis of *o*-nitrobenzaldehyde in water at pH = 6.7. Signals are normalised for the absorbance at the excitation wavelength and the laser pulse energy. Curve A (average of 100 shots) is the reference compound Evans blue at $T = 6.0$°C, curve B (single shot) is *o*-nitrobenzaldehyde at $T = 3.9$°C. Excitation was at 308 nm with laser pulse energy of 20 μJ. Each channel corresponds to 2.5 ns. Best fit was obtained with a single exponential decay with $\varphi_1 = -0.69 \pm 0.13$ and lifetime below the experimental resolution.

We have previously shown that proton release from *o*-nitrobenzaldehyde is accompanied by a prompt (lifetime below a few nanoseconds) contraction, $\varphi_1 = -0.69 \pm 0.13$ [16], related to the solvation of the newly formed charges [2–4]. Similarly, a contraction of $\varphi_1 = -0.42 \pm 0.04$ follows photoexcitation of the NPE caged proton at pH 6.75. The extent of the observed contractions can be determined using Eq. (8) as $\Delta V_1 = -2.10 \pm 0.16$ ml mol^{-1} for *o*-nitrobenzaldehyde [16] and $\Delta V_1 = -1.61 \pm 0.13$ ml mol^{-1} for the NPE caged proton. These values are independent of the laser pulse energy between 2 and 200 μJ and indicate that the proton concentration jump is linear with energy over at least two orders of magnitude.

In order to calculate the volume change for a mole of reacted species (i.e. for a mole of photodetached protons), a correction for the proton detachment quantum yield, Φ_{H^+}, must be performed. Assuming for *o*-nitrobenzaldehyde a value of $\Phi_{H^+} = 0.4$ [30], the extent of the volume change for the solvation of the newly formed ions is calculated as $\Delta V_{solv} = \Delta V_1 / \Phi_{H^+} = -5.2 \pm 0.2$ ml mol^{-1}. Values of Φ_{H^+} for the NPE caged proton reported in the literature are 0.4 ± 0.1 [31], 0.095 (308 nm, nanosecond excitation) [32] and 0.29 ± 0.02 (300–350 nm, flashlamp excitation) [32]. Using $\Phi_{H^+} = 0.4$ we get $\Delta V_{solv} = -4.0 \pm 1.3$ ml mol^{-1}, whereas assuming $\Phi_{H^+} = 0.095$ we obtain $\Delta V_{solv} = -16.9 \pm 1.3$ ml mol^{-1}. Experiments conducted with aqueous solution of *o*-nitrobenzaldehyde in the presence of 100 mM NaCl show that $\Delta V_{solv} = -4.9 \pm 0.5$ ml mol^{-1}.

The concentration of the photodetached protons immediately after the laser pulse can be estimated from the laser pulse energy, the absorbance of the solution at the excitation wavelength (typically 0.2), the illuminated volume (50 μl) and Φ_{H^+}, as ranging from 0.01 μM for a 2-μJ pulse, to approx. 1 μM for a 200-μJ pulse.

3.2. Reaction with hydroxide

When the pH of an aqueous solution of *o*-nitrobenzaldehyde is raised to approx. nine, the prompt contraction for ion solvation is followed by a large expansion [16] of $\Delta V_2 = 9.80 \pm 0.16$ ml mol^{-1}, with an apparent rate constant that increases upon increasing the pH of the solution. We have attributed this process to the reaction of photodetached protons with hydroxide according to the scheme:

$$H^+ + OH^- \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} H_2O \qquad (9)$$

The extent of the volume change for water formation, after correction for $\Phi_{H^+} = 0.4$ is $\Delta V_W = 24.5 \pm 0.4$ ml mol^{-1} [16], in excellent agreement with the previously reported value of 24.6 ml mol^{-1} at 5°C [33]. The observed volume change becomes $\Delta V_2 = 9.13 \pm 0.19$ ml mol^{-1} in the presence of 100 mM NaCl. This gives a reaction volume for water formation $\Delta V_W = 22.83 \pm 0.47$ ml mol^{-1}, which is in very good accordance with the literature value $\Delta V_W = 22.84$ ml mol^{-1} at 5°C in 100 mM NaCl [2].

The rate for Eq. (9) can be estimated by plotting the apparent rate constant vs. the hydroxide concentration (Fig. 2) [16]. The linear plot evidences a pseudo-first order kinetics, with hydroxide in large excess. The extrapolated bimolecular rate constant is $k = (4.9 \pm 0.4) \times 10^{10}$ M^{-1} s^{-1} in plain water and $k = (1.5 \pm 0.5) \times 10^{10}$ M^{-1} s^{-1} in the presence of 100 mM NaCl. These findings are

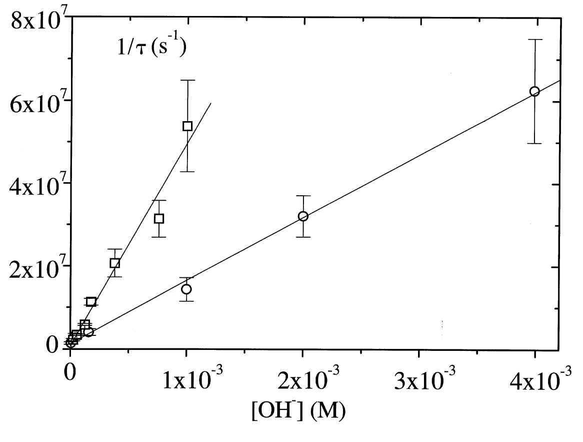

Fig. 2. Apparent rate constant, $1/\tau$ vs. hydroxide concentration for the reaction of photodetached protons with hydroxide. The caged proton was *o*-nitrobenzaldehyde and the excitation wavelength 308-nm. Open squares refer to plain water data [16] and circles to a solution containing 100 mM NaCl. Each channel corresponds to 2.5 ns.

in very good accordance with literature data [1] and correctly reproduce the expected dependence on ionic strength.

Fig. 2 also shows that this method will access reaction lifetimes down to 20 ns, corresponding to an apparent rate constant of 5×10^7 s^{-1}.

Similar results have been obtained with the NPE caged proton in water, as shown in Fig. 3. The prompt contraction is followed by a large expansion presumed to be due to the bimolecular reaction with hydroxide.

The apparent rate constant for the expansion also shows a linear trend, with a slope $k = (1.0 \pm 0.9) \times 10^{10}$ M^{-1} s^{-1}, a bit smaller than the one already evidenced in Fig. 2. The measured $\Delta V_2 = 2.4 \pm 0.2$ ml mol^{-1} can be used to estimate the Φ_{H^+} for the NPE caged proton from the known reaction volume $\Delta V_W = 24.5 \pm 0.4$ ml mol^{-1}. We obtain $\Phi_{H^+} = 0.10 \pm 0.01$, a value close to the previously reported quantum yield of 0.095 [32]. Using this value we can calculate the prompt contraction accompanying ion solvation as $\Delta V_{solv} = -16.1 \pm 2.9$ ml mol^{-1}.

3.3. Reaction with poly-L-lysine

An experiment that demonstrates the potentiality of the methodology in biophysical applications is shown in Fig. 4, where we report the apparent lifetimes for the binding of the photodetached protons to poly-L-lysine (PLL) as a function of the concentration of the polypeptide.

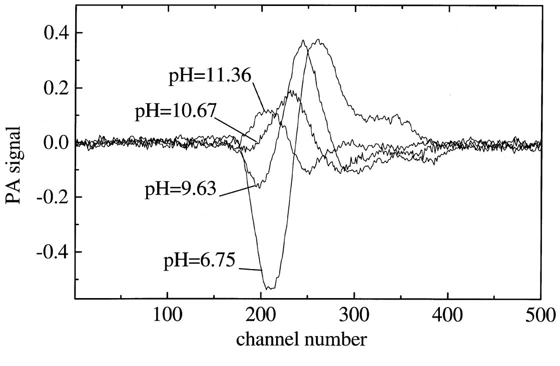

Fig. 3. Photoacoustic signal of NPE caged proton in water at $T = 3.9$°C, at various pH values. Signals are normalised for the absorbance at the excitation wavelength and the laser pulse energy. Each channel corresponds to 10 ns. Excitation wavelength was 366.6 nm. The experimental apparatus used for these data is described by Kurian et al. [42].

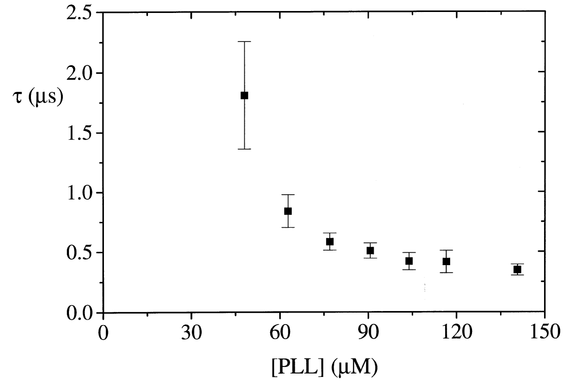

Fig. 4. Apparent lifetimes for the protonation reaction of poly-L-lysine in aqueous solution at pH 9.5. The laser pulse energy used in the experiment was 20 μJ. The caged proton was NPE. Photoexcitation was at 308 nm. The proton jump is accompanied by a prompt contraction due to proton detachment (not shown) and a further, slower negative volume change $\Delta V_2 = -1.6$ ml mol^{-1} for the protonation of the ε-amino groups of the lysine residues. The apparent lifetime becomes shorter upon increasing the concentration of the polypeptide.

The reactions occurring after photoexcitation and detachment of a proton are summarized below:

$$H^+ + OH^- \underset{k_2}{\overset{k_1}{\rightleftarrows}} H_2O \tag{10}$$

$$H^+ + PLL \underset{k_4}{\overset{k_3}{\rightleftarrows}} PLLH^+$$

The NPE caged proton was used in this experiment. The extent of the negative volume change for proton detachment reported above is scarcely affected by addition of PLL to the solution, indicating that the photolysis process is not altered by the addition of the polypeptide. A slower contraction of approx. -1.6 ml mol^{-1} and rate τ is detected in addition, which becomes faster (Fig. 4) as the concentration of PLL is raised, showing that a bimolecular reaction between the photodetached protons and the polypeptide is occurring.

Dividing the observed contraction (-1.6 ml mol^{-1}) by the proton detachment quantum yield for the NPE caged proton (0.095), an upper limit of $\Delta V_{\mathrm{PLL}} = -16.8$ ml mol^{-1} can be obtained for the extent of the reaction volume for protonation of PLL.

4. Discussion

4.1. Proton solvation

The values of ΔV_{solv} for *o*-nitrobenzaldehyde and the NPE caged proton reported above are substantially different. In both cases, the extent of ΔV_{solv} we have calculated assumes that the processes competing with the deprotonation of the acid–nitro intermediate give rise to negligible volume changes, or to volume changes that rapidly revert when the excited state relaxes.

We have already observed [16] that, in the case of slightly acidic solutions of *o*-nitrobenzaldehyde, ΔV_{solv} can be partly explained by means of the Drude–Nernst model [5]. We assumed for the solvated proton a partial molar volume $V_{\mathrm{H}^+} = -1.5$ ml mol^{-1} [34] and, for the negative charge left on the anion, which is localised on an oxygen atom, the electrostrictive contribution was estimated as -2.3 ml mol^{-1}. An alternative determination of $V_{\mathrm{H}^+} = -3.4$ ml mol^{-1} can be done applying directly the Drude–Nernst equation (Eq. (11) to the proton using a radius $r = 1.2$ Å:

$$V_{\mathrm{anion}} = -\frac{N_A}{4\pi\varepsilon_0}\frac{e^2}{2r\varepsilon_r}\frac{\partial \ln \varepsilon_r}{\partial P} \qquad (11)$$

In this equation ε_r represents the relative dielectric constant of the medium, P is the pressure and e is the solvated charge. The value of -3.4 ml mol^{-1} is close to the average value of -3.9 ml mol^{-1} that can be obtained from the data tabulated by Millero for the molar volume of H^+ [35]. Although the values reported by Millero refer to infinite dilution, these partial volumes can be used for our data, taken at neutral pH and in the absence of dissolved salts, without any further correction, since the effect of the ionic strength is negligible [36]. Furthermore, we assume we can neglect the changes in the intrinsic volume when *o*-nitrobenzaldehyde is converted into nitrosobenzoic acid, since both molecules should have similar size. Under these assumptions, the molar volume of the photoproducts is mainly determined by the electrostrictive forces and a value of $\Delta V_{\mathrm{solv}} = V_{\mathrm{H}^+} + V_{\mathrm{anion}} = -5.7$ ml mol^{-1} can then be calculated, which is more consistent with the experimentally determined one. The value of $\Delta V_{\mathrm{solv}} = -16.1 \pm 2.9$ ml mol^{-1}, observed for the NPE caged proton, is obviously not accountable on the basis of a simple electrostrictive model. We cannot, at this stage, assign the source of such a large contraction upon solvation of the newly formed charges. However, some general comments can be made. The solvation of the photodetached proton is likely to be independent of the specific *o*-nitro compound used in the experiment and therefore its contribution is likely to be the same in both photo-induced reactions. The major differences between the two processes are likely to be found in the different specific interactions with the solvent the two *o*-nitro compounds have. In particular, 2-hydroxyphenyl-1-(2-nitro)phenylethylphosphate may show peculiar interactions with the solvent through the hydrogen bonds at the phosphate and the phenyl groups. Moreover, the presence of a second benzene ring on the NPE caged proton may decrease locally the dielectric constant of the medium, thus contributing to increase the extent of the volume change.

4.2. Reaction with hydroxide

The large value of the reaction volume for water formation is determined by the fact that water has a positive molar volume of $V_{\mathrm{H_2O}} = 18$ ml mol^{-1} [3] whereas the reactants are characterised by negative partial molar volumes, since the bulk solvent around the ions shrinks and gives rise to remarkable contractions. Using for V_{H^+} the value -3.4 ml mol^{-1}, the partial molar volume of hydroxide can be estimated as $V_{\mathrm{OH}^-} = \Delta V_W - V_{\mathrm{H_2O}} + V_{\mathrm{H}^+} = -3.1 \pm 0.5$ ml mol^{-1}, comparable to the partial molar volume of H^+. This value is approximately one-half the value reported by Millero [35].

The addition of NaCl to the aqueous solution of *o*-nitrobenzaldehyde reduces the extent of ΔV_W, the volume change upon water formation. This effect is consistent with an increased dielectric constant of the medium in the Drude–Nernst Eq. (11). Estimates of single partial molar volumes in this case are not possible because the dielectric properties of the solvent are not available. Unfortunately the value of ΔV_{solv} for the NPE caged proton could not be used to establish a similar correlation, since the observed changes were of the same order of the uncertainty on ΔV_{solv}.

The rate constant of Eq. (9) decreases at higher ionic strength as predicted by the Debye–Hückel theory for reactants bearing charges of opposite sign [37].

4.3. Reaction with poly-L-lysine

The data presented on PLL represent a preliminary result showing how the technique can be used to monitor events that affect the structure of polypeptides in solution. PLL is characterised by a pH sensitive secondary structure. In particular, the polypeptide folds into an α helix at pH 12 in neat water and completely unfolds into a random coil at neutral pH [38]. The unfolding process is due to the strong electrostatic repulsive forces between the protonated ε-amino groups. The pK_a of the ε-amino groups is approx. 10.7 [39], whereas the pK of the helix-coil transition lies below this value and depends on the ionic strength of the solution [39,40].

Some considerations can be done on the observed lifetimes for the contraction measured in the presence of PLL, to show that they are compatible with the reaction of photodetached protons with the ε-amino groups of PLL. The rate constant k_3 in Eq. (10) should be of the order of a diffusion controlled reaction with rate constant 5×10^9 M^{-1} s^{-1} [1] and is not expected to be influenced by the ionic strength, since only one of the reactants (the proton) is charged. The actual concentration of protonable species, i.e. of protonable ε-amino groups, can be estimated by considering the fact that the number of residues is approx. 165 per PLL molecule and at pH 9.5 only approx. 10% of them are protonated. Therefore the concentration of protonable groups in our experiments was approx. 1 mM, resulting in apparent lifetimes of hundreds of nanoseconds, which are compatible with the values determined experimentally. We cannot perform, at the moment, any quantitative interpretation of the rate constants shown in Fig. 4 because of the presence of the slower competitive reaction with hydroxide. The lack of a suitable kinetic model prevents the estimate of the reaction volume. Assuming, however, that the competition of hydroxide in binding protons is not overwhelming under our experimental conditions, the slower contraction should not differ substantially from the estimate of $\Delta V_{\text{PLL}} = -16.8$ ml mol^{-1}, given above. The sign of this volume change is consistent with the contractions accompanying the protonation of amines in aqueous solutions [2,4]. For instance, the protonation of NH_3 to form NH_4^+ is accompanied by a contraction of -6.6 ml mol^{-1} [4] and the reaction $MeNH_2 + H^+ \rightarrow MeNH_3^+$ is characterised by a volume change of -2.4 ml mol^{-1} [4]. However, the extent of the observed ΔV_{PLL} is larger than any of the tabulated reaction volumes for amines [2,4]. The possible involvement of a local disruption of the secondary structure of PLL upon binding of protons is an event which likely occurs but is difficult to quantitate at this stage. Recent laser T-jump data have evidenced that the structural events accompanying the helix-coil transition in a short (21 residues) polypeptide are characterised by an apparent lifetime of 160 ± 60 ns [41]. The data presented in Fig. 5, where the apparent rate constant $1/\tau$ is plotted vs. 1/[PLL], suggest that, at high PLL concentrations, a limiting lifetime is reached of approx. 250 ± 50 ns. A 'simple' bimolecular reaction would show a normal hyperbolic behaviour, at least within the experimental resolution. Since our experimental resolution is approx. 20 ns, the saturation in the rate constant must be due to some event which cannot be separated from the protonation event, possibly a structural change in PLL conformation. As the laser pulse energy produced approx. 0.1 μM of photodetached protons, at most one proton is bound per PLL molecule. Therefore it is unlikely that the structural events involve large portions of the polypeptide. It is more realistic to assume that the local structure around the ε-amino which gets protonated is changed, thus explaining the larger reaction volume observed. Further experiments are under way in order to

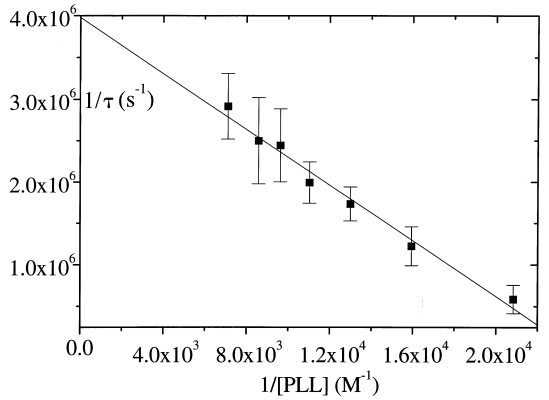

Fig. 5. Rate constant $1/\tau$ vs. 1/[PLL] for data shown in Fig. 4. The data show a linear trend with a limiting rate at infinite [PLL] of 250 ± 50 ns.

clarify the nature of the observed events and possibly discriminate the protonation process from the secondary structure change.

Finally, we wish to note that in the above reported reactions of photodetached protons with hydroxide and poly-L-lysine no laser pulse energy dependence was observed for any of the kinetic parameters. This observation is in accordance with the pseudo-first order kinetics expected in the presence of large excess of the proton accepting molecules.

Acknowledgements

The authors acknowledge Dr Ernest McGoran for the synthesis of 2-hydroxyphenyl-1-(2-nitro)phenylethylphosphate.

References

[1] M. Gutman, E. Nachliel, Biochim. Biophys. Acta. 1015 (1990) 391–414.
[2] T. Asano, W.J.L. Noble, Chem. Rev. 78 (1978) 407–489.
[3] W.J. leNoble, H. Kelm, Angew. Chem. 19 (1980) 841–856.
[4] R. VanEldick, T. Asano, J.L. Noble, Chem. Rev. 89 (1989) 549–688.
[5] P. Drude, W. Nernst, Z. Phys. Chem. 15 (1894) 79–85.
[6] S.E. Braslavsky, G.E. Heibel, Chem. Rev. 92 (1992) 1381–1410.
[7] J.B. Callis, W.W. Parson, M. Gouterman, Biochem. Biophys. Acta 267 (1972) 348–362.
[8] K.S. Peters, T. Watson, K. Marr, Ann. Rev. Biophys. Chem. 20 (1991) 343–362.
[9] J.E. Rudzki, J.L. Goodman, K.S. Peters, J. Am. Chem. Soc. 107 (1985) 7849–7854.
[10] J.L. Habib Jiwan, M.T. Indelli, F. Scandola, S.E. Braslavsky, Recl. Trav. Chim. Pays-Bas 114 (1995) 542–548.
[11] J.L. Habib Jiwan, A. Chibisov, S.E. Braslavsky, J. Phys. Chem. 99 (1995) 10246–10250.
[12] J. Feitelson, D. Mauzerall, J. Phys. Chem. 100 (1996) 7698–7703.
[13] D.C. Mauzerall, J. Feitelson, R. Prince, J. Phys. Chem. 99 (1995) 1090–1093.
[14] B. Wegewijs, J.W. Verhoeven, S.E. Braslavsky, J. Phys. Chem. 100 (1996) 8890–8894.
[15] J.R. Small, E. Kurian, Spectroscopy 10 (1995) 27–33.
[16] G. Bonetti, A. Vecli, C. Viappiani, Chem. Phys. Lett. 269 (1997) 268–273.
[17] P.J. Schulenberg, S.E. Braslavsky, Progress in photothermal and photoacoustic science and technology, in: A. Mandelis, P. Hess (Eds.), Life and Earth Sciences, vol. 3. SPIE press, 1997.
[18] H. Arata, W.W. Parson, Biochim. Biophys. Acta 636 (1981) 70–81.
[19] D.R. Ort, W.W. Parson, J. Biol. Chem. 253 (1978) 6158–6164.
[20] W.W. Parson, Methods Enzymol. 88 (1982) 272–276.
[21] R.W. Yip, Y.X. Wen, D. Gravel, R. Giasson, D.K. Sharma, J. Phys. Chem. 95 (1991) 6078–6081.
[22] H. Schupp, W.K. Wong, W. Schnabel, J. Photochem. 36 (1987) 85–97.
[23] J.A. McCray, D.R. Trentham, Ann. Rev. Biophys. Chem. 18 (1989) 239–270.
[24] A. Losi, C. Viappiani, P.R. Crippa, Photochem. Photobiol. 59 (1994) 596–602.
[25] A. Losi, R. Bedotti, C. Viappiani, J. Phys. Chem. 99 (1995) 16162–16167.
[26] R.C. Weast, CRC Handbook of Chemistry and Physics, CRC Press, Boca Raton, 1971.
[27] T. Gensch, S.E. Braslavsky, J. Phys. Chem. 101 (1997) 101–108.
[28] J.R. Small, L.J. Libertini, E.W. Small, Biophys. Chem. 42 (1992) 24–48.
[29] Small J.R., Numerical computer methods, in: L. Brand, M.L. Johnson (Eds.), vol. 210, Academic Press, San Diego, 1992, pp. 505–521.
[30] M.V. George, J.C. Scaiano, J. Phys. Chem. 84 (1980) 492–495.
[31] J.A. McCray, D.R. Trentham, Biophys. J. 47 (1985) 406a.
[32] S. Khan, F. Castellano, J.L. Spudich, et al., Biophys. J. 65 (1993) 2368–2382.
[33] F.J. Millero, E.V. Hoff, L. Cahn, J. Sol. Chem. 1 (1972) 309–327.
[34] R.M. Noyes, J. Am. Chem. Soc. 86 (1964) 971–979.
[35] Millero F.J., Water and aqueous solutions, in: R.A. Horne (Ed.), Wiley-Interscience, New York, London, Sidney, Toronto, 1972, pp. 519–595.

[36] K.S. Pitzer, J. Phys. Chem. 77 (1973) 268–277.

[37] K.J. Laidler, J.H. Meiser, Physical Chemistry, The Benjamin/Cummings Publishing Company, 1982.

[38] C.R. Cantor, P.R. Schimmel, Biophysical Chemistry part II: Techniques for the Study of Biological Structure and Function, W.H. Freeman and company, New York, 1980.

[39] C. Tanford, Physical Chemistry of Macromolecules, Wiley, New York, 1961.

[40] G. Weber, Protein Interactions, Chapman and Hall, New York, London, 1991.

[41] S. Williams, T.P. Causgrove, R. Gilmanshin, et al., Biochemistry 35 (1996) 691–697.

[42] E. Kurian, F.G. Prendergast, J.R. Small, Biophys. J. 73 (1997) 466–476.