Volume Changes Related to Triplet Formation of Water-Soluble Porphyrins. A Laser-Induced Optoacoustic Spectroscopy (LIOAS) Study†

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The production of triplet states in basic solution of tetrakis(tetrasulfonated-phenyl)porphyrin ($TPPS_4$) is accompanied by a contraction of (10 ± 3) mL/mol as determined by laser-induced optoacoustic spectroscopy (LIOAS) at different temperatures. The contraction is reverted as the triplet decays. The volume changes should mainly originate in changes in the solvation sphere due to the entire process of ground state to triplet transition (for the contraction) and back to the ground state (for the expansion). The presence of oxygen has no major effect on the contraction and the expansion observed. The present study shows that $TPPS_4$ is not suitable as a calorimetric reference, contrary to our own hypothesis in Heihoff et al. *Photochem. Photobiol.* **1990**, *51*, 635−641. It was also observed that triplet state formation of methylene blue and rose bengal is accompanied by a structural volume change of −1.5 and 1 mL/mol, respectively.

Introduction

We have recently reported that the production of triplet states by charge recombination of the triplet radical pair in a stable and fully active D1-D2-cyt *b*-559 reaction center complex without quinones was accompanied by an expansion of 4.4 mL/mol.[1] We attributed this expansion to reorganization of the protein around the triplet radical pair within its lifetime of ca. 100 ns and persisting for times longer than 10 μs after the 8 ns laser pulse.[1] The method used was laser-induced optoacoustic spectroscopy (LIOAS), by which it is possible to determine enthalpies, quantum yields, absorption coefficients, as well as structural volume changes of transient species in the nasosecond to microsecond time range.[2,3]

After absorption of a laser pulse, two processes lead to the volume changes in the sample, which in turn create the total pressure pulse detected by the piezoelectric transducer in LIOAS. The two processes are radiationless deactivation (giving rise to heat dissipation, ΔV_{th}) and structural changes (ΔV_r).[2−4] The thermal term ΔV_{th} depends on the thermoelastic parameters of the solution.[4]

Owing to the strong temperature dependence of the thermal expansion coefficient, β, in aqueous solutions, it is possible to separate the two contributions to the pressure wave by performing LIOAS measurements at different temperatures.[1−6] In non-aqueous media structural volume changes have been determined by using a series of homologous solvents with variable thermoelastic properties.[2,7] Thus, the contribution of this effect to the total pressure should not be neglected a priori in any case.

The relationship between the volume changes observed and the molecular properties is starting to emerge for some of the systems studied. Recently, we have been able to correlate the structural volume changes observed upon light-induced intramolecular electron transfer in a series of ruthenium−cyano complexes with specific solute−water interactions.[8] In a previous report,[9] we identified the structural volume changes observed upon isomerization of cyanines (as models for isomerizable chromophores) with reorganization of the solvent (a water/ethanol mixture in this case) around the isomerized dye possessing a dipole moment different from that of the starting molecule. Reports from the group of Mauzerall, on the other hand, explained the contractions observed using LIOAS in photosynthetic reaction centers[10] as well as in intermolecular electron transfer reactions[11] by the Drude−Nernst equation for electrostriction. The expansions and contractions determined in our laboratory for intermolecular electron transfer reactions could not be simply explained by this latter effect[12] nor could electrostriction explain our results on intramolecular electron transfer in systems that can be understood as models for the electron-transfer reaction in photosynthetic units.[8] A general theoretically based concept is still missing that correlates the values for the structural volume changes with molecular properties. As a further step toward the understanding of the volume changes observed in supramolecular biological systems, a variety of chromophores similar to those in the supramolecular structures and in various media should be studied using LIOAS.

We decided to study the volume change upon triplet state production and decay for a number of water-soluble molecules, porphyrins among them. In the present report, the origin of the surprisingly large structural volume changes in tetrakis(tetrasulfonated-phenyl)porphyrin, $TPPS_4$, is analyzed in detail. $TPPS_4$ is a well-known water-soluble porphyrin used as a standard compound for $O_2(^1\Delta_g)$ generation and as a fluorescence imaging agent for biological tissues.[13] Careful literature searching showed that the data on $TPPS_4$ aggregation and photophysics needed re-evaluation, since contradictions were evident, e.g., fluorescence quantum yields ranging from 0.08[14] to 0.16[15] were reported. Since the photophysical parameters and the knowledge about the aggregation were required for the correct interpretation of the optoacoustic experiments, a study of these properties was necessary.

Already in 1969 Callis et al.[16] suggested the use of photoacoustic spectroscopy to determine triplet state production yields and/or triplet energy levels. However, neither these authors nor those using pulsed-laser techniques for the study of triplet states by optoacoustics[17] considered that structural volume changes could be produced concomitant with the production and decay of a triplet state. The question of the errors introduced in the LIOAS data by ignoring the value of the structural volume change is addressed in this paper.

† Dedicated to Professor Kurt Schaffner on the occasion of his 65th birthday.

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Materials and Methods

Tetrakis(tetrasulfonated-phenyl)porphyrin ($TPPS_4$) was purchased from Porphyrin Products (Logan, Utah). Methylene blue (3,7-bis[dimethylamino]phenazothionium, MB), rose bengal (4,5,6,7-tetrachloro-2′,4′,5′,7′-tetraiodofluorescein, RB), bromocresol purple (5,5′-dibromo-*o*-cresolsulfonaphthalein, BCP), and deuterated water were from Fluka (Buchs, Switzerland). Evans blue (EB) was from Sigma-Aldrich (Deisenhofer). Tetrapropylporphycene (TPrPn)[18] was synthesized by the group of Professor E. Vogel (Universität Köln). All measurements were carried out in 10 mM Tris-buffer (pH 7.8). Absorption spectra were measured using a UV-2102 spectrophotometer (Shimadzu).

For the determination of the aggregation constant K_D of $TPPS_4$, the absorption spectra were measured of a 100 μM stock solution in 10 mM Tris-buffer (pH 7.8), as well as of solutions obtained by dilution with aliquots of 33% buffer. This procedure was repeated ca. 20 times. The final solution measured was 5×10^{-8} M. A dimerization model was used to describe the aggregation of $TPPS_4$. The absorbance $A(\lambda)$ at various wavelengths as a function of the total concentration was analyzed using the regular equation containing the absorption coefficients of monomer (measured for the most dilute solution) and dimer at each wavelength and K_D.[19] The absorbances at 413, 516, 570, and 635 nm were simultaneously fitted to afford the values of the absorption coefficients for the dimer and the K_D value.

For the LIOAS experiments we used the setup already described.[2,6,20] The second harmonic of a Q-switched Nd:YAG laser (Spectron Lasers, Rugby, UK, 8 ns pulses) was used as an excitation source ($\lambda_{exc} = 532$ nm) or to pump a dye laser (rhodamin 6G, $\lambda_{exc} = 570$ nm).

As beam-shaping elements, either pinholes (0.7−1 mm diameter) or slits (0.5−1 mm) were used. The resulting acoustic transit times, τ_a', were in the range 330−700 ns. The use of slits permits the enlargement of the irradiated area up to the full detector limit.[10] The measured temperature deviated at most 0.2 °C from the actual value in the excited volume.

The piezoceramic (Pb−Zr−Ti, Vernitron) detector was spring-loaded on the cuvette wall parallel to the laser beam direction.[2,20] Time-resolved analysis of the data was performed with an iterative deconvolution program based on the Marquardt algorithm,[21,22] either with an IBM-compatible PC (program SOUND Analysis 3000, Quantum Northwest) or with the VAX system with an adapted version of the same program.

Steady state fluorescence spectra were measured with a Spex Fluorolog equipment as described previously.[23] Tetrapropylporphycene (TPrPn) in toluene was used as a fluorescence standard ($\Phi_f = 0.38$[24]). The fluorescence quantum yield of $TPPS_4$ was calculated by comparing its spectrally integrated emission to that of the reference solution after correcting for the difference in refractive index of the solvents.

The emission of $O_2(^1\Delta_g)$ in the near-IR spectral region ($\lambda_{max} = 1270$ nm) was observed with a liquid nitrogen-cooled germanium detector (EO817FP, NorthCoast, rise time of 200 ns) as described earlier.[1,25] The data of the time-resolved phosphorescence detection (TRPD) were fitted by a biexponential function, eq 1, including the generation and decay of $O_2(^1\Delta_g)$.

$$L(t) = \frac{a\tau_\Delta}{\tau_\Delta - \tau_T}[\exp(-t/\tau_\Delta) - \exp(-t/\tau_T)] \qquad (1)$$

In eq 1, τ_T and τ_Δ are the lifetimes of the O_2-quenched triplet state and the $O_2(^1\Delta_g)$, respectively. The amplitude a was used for the determination of the quantum yield of $O_2(^1\Delta_g)$ formation, Φ_Δ.

The methods used for LIOAS signal handling have been described in several publications.[1−3,8,9,26−28]

A deconvolution technique was used for a detailed description of the time evolution of the slower heat release. In this case the function modeling the system (eq 2) describes the pressure time evolution (i.e., the time derivative of the heat release and volume change function) in the sample, which is then convoluted with a prompt instrumental response obtained by measuring the signal from a calorimetric reference.[2]

$$H(t) = \sum_i \frac{\varphi_i}{\tau_i} \exp(-t/\tau_i) \qquad (2)$$

In eq 2, φ_i is the reference-normalized pre-exponential factor for the ith component and τ_i is the corresponding lifetime.

When the deconvolution method is used, the two contributions (ΔV_{th} and ΔV_r; see Introduction) to each of the pre-exponential factors of the multiexponential decay function of the volume changes can be considered additive if it is assumed that the kinetic behavior of the structural volume changes is the same as that of the thermal deactivation processes. Furthermore, the additional assumption should be validated that the several processes in the slow decay steps either are parallel or, if sequential, are separated enough in time to permit the assignment of each time constant to an elementary reaction step. When this latter assumption does not hold, more detailed kinetic schemes should be used.[22]

Thus, for each of the components of the multiexponential fit, eq 3 may be written, provided the above assumptions are fullfilled.[21,22,26−28]

$$\varphi_i E_\lambda = q_i + \Phi_i \Delta V_{R,i}[c_p\rho/\beta] \qquad (3)$$

The total volume change for each species is equal to its production yield, Φ_i, times its molar volume change $\Delta V_{R,i}$ multiplied by the number of Einsteins absorbed (n^s), i.e., $\Delta V_{r,i} = n^s\Phi_i\Delta V_{R,i}$. The thermoelastic parameters, c_p, the specific heat, ρ, the mass density, and β of the solution are identical with those of the solvent, since the experiments are performed in dilute solutions. The term q_i is the enthalpic term containing the energy difference between the species ($i - 1$) and i times the efficiency of the ith step.

Since in aqueous solutions β strongly depends on the temperature, the separation of ΔV_{th} and ΔV_r is possible by measurements in a relatively narrow temperature range (typically 5−30 °C). In pure water, $\beta = 0$ at 3.9 and in pure D_2O at 11.2 °C.[29] In buffer solutions this temperature is slightly lower owing to the distortion of the ordered water structure by the salts. The separation of ΔV_r and ΔV_{th} by this method assumes that the former term is not temperature dependent in the narrow range analyzed.

A calorimetric reference shows no signal at the temperature at which $\beta = 0$, since all the absorbed energy is released as heat and the molecules return to the ground state in times much shorter than the integration time, i.e., in less than 1 ns in our case. Therefore, for the calorimetric reference $\Delta V_r = 0$. Bromocresol purple (BCP) and Evans blue (EB) in Tris buffer were used as calorimetric references. The ratio of thermoelastic parameters ($c_p\rho/\beta$) for the solutions employed was determined by comparing the LIOAS signal amplitudes for the calorimetric reference in buffer and in water.[9]

The absorption spectrum of BCP exhibited a remarkable temperature dependence that is attributed to the temperature-dependent pH of the Tris buffer. The pH changed from 7.8 at room temperature to 8 and 7.1 at 40 and 5 °C, respectively. Since BCP shows its pH transition between 5.2 and 6.8,[30] it should change its absorption spectra with temperature in Tris buffer. In fact, when the temperature is lowered, the 590 nm maximum decreases and a new band appears at 430 nm. An isosbestic point is observed at 490 nm. The absorbance change at λ_{exc} 532 nm between room and the lowest temperature was ca. 13%. The absorbance of BCP was corrected for this effect. The $TPPS_4$ solutions showed a negligible temperature-dependent absorbance in the temperature range studied.

With the sensitizers studied in this work, the deconvolution procedure was used, since it is known that the triplet states in the presence of air have lifetimes of several microseconds,[31] while the $O_2(^1\Delta_g)$ lifetime is 3.2 μs in water,[32] i.e., the decaying species were expected to contribute to the pressure evolution within the integration time of the experiment.

The analysis of data at variable temperatures may be achieved in two manners. (i) Sample and reference solutions are measured at various temperatures (several-temperature method, ST), i.e., at various values of the ratio $c_p\rho/\beta$. Plotting the amplitudes of the fitting function (φ_i) vs $c_p\rho/\beta$ affords the structural volume change from the slope and the enthalpy change $\alpha_i = q_i/E_\lambda$ (eq 3) from the intercept. (ii) The temperature at which $\beta = 0$ is found by measuring reference solutions; then sample solutions are measured at $T_{\beta=0}$ and at another $T_{\beta\neq0}$ (two-temperatures method, TT). The deconvolution procedure is performed using the reference signal at $T_{\beta\neq0}$ for both sample signals (at $T_{\beta\neq0}$ and $T_{\beta=0}$). For $T_{\beta=0}$ an option to time shift the reference signal vs the sample signal was used. Simple equations permit the evaluation of the enthalpy change and of the structural volume change for the transient in the time analyzed, using again the amplitudes of the fitting function (see Appendix). The ST-method experiments imply one measurement at each temperature, while the TT-method experiments imply three to five measurements at each of the two temperatures. In the tables (vide infra) all data are presented and the average is used. In all cases it is required to ensure that the signals show a linear dependence with the laser fluence with a zero intercept.[2]

Simple energy balance considerations lead to eq 4 in which the molar energy of the exciting laser absorbed by the system, E_λ, is dissipated throughout the four possible processes undergone by the excited molecules, *i.e.*, in fluorescence, in prompt heat release by vibrational relaxation (to S_1 and T_1) and internal conversion ($S_1 \rightarrow S_0$), in loss during energy transfer, and in stored heat. Phosphorescence from the $TPPS_4$ triplet, as well as from $O_2(^1\Delta_g)$, are known to have yields $<10^{-3}$ [14,33] and are therefore neglected.

$$E_\lambda + \Phi_f E_S + q_1 + q_2 + q_3$$

$$E_\lambda = \Phi_f E_f + [E_\lambda - E_S + \Phi_f(E_S - E_f) + (1 - \Phi_f - \Phi_T)E_S + \Phi_T(E_S - E_T)] + [(\Phi_T - \Phi_\Delta)E_T + \Phi_\Delta(E_T - E_\Delta)] + \Phi_\Delta E_\Delta \quad (4)$$

The first term on the right side of eq 4 is the energy lost through radiation, *i.e.*, through fluorescence from the singlet excited state of $TPPS_4$. The second term is the prompt heat release, q_1, including vibrational relaxation $S_n \rightarrow S_1$, nonradiative deactivation $S_1 \rightarrow S_0$, and $S_1 \rightarrow T_1$ transition. The third term, q_2, corresponds to the heat release due to the short-microsecond component (*vide infra*), *i.e.*, the triplet deactivation including the triplet-to-O_2 energy transfer step. The last term, q_3, is the energy stored by $O_2(^1\Delta_g)$ with the molar energy content E_Δ.

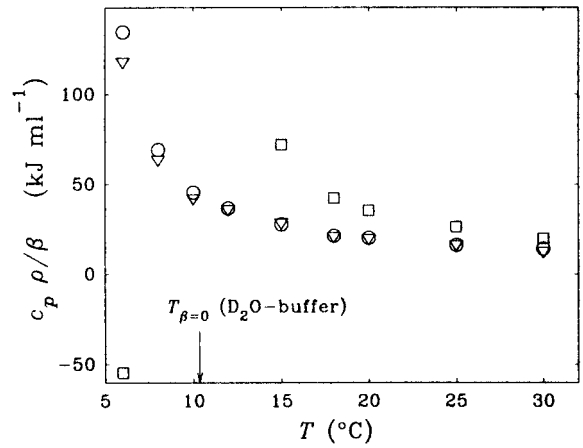

Figure 1. Thermoelastic parameters calculated from LIOAS measurements[9] for (□) 10 mM D_2O-based Tris-buffer (pD 8.0), (▽) 10 mM H_2O-based Tris-buffer (pH 7.8), and (○) neat water at several temperatures. BCP was used as a calorimetric reference in both buffer solutions and EB in neat H_2O. $A_{BCP} = A_{EB} = 0.13$; $\lambda_{exc} = 532$ nm.

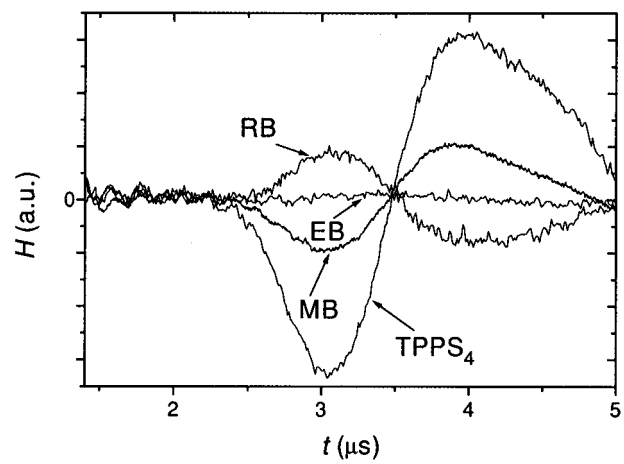

Figure 2. Energy-normalized LIOAS signals from EB, $TPPS_4$, RB ($\lambda_{exc} = 532$ nm), and MB ($\lambda_{exc} = 570$ nm) at $T_{\beta=0}$ (2.7 °C) in air-saturated 10 mM Tris-buffer (pH 7.8). Absorbances were matched to 0.15 at the respective excitation wavelength.

Results

For the analysis of the data it was necessary to determine the thermoelastic parameters of the solutions in the range 6−40 °C. For 10 mM Tris buffer, $T_{\beta=0}$ was 2.7 °C in H_2O and 10.2 °C in D_2O using EB and BCP as calorimetric references (see Figure 2). The data obtained for these buffers using BCP as a calorimetric reference are depicted, together with those for neat H_2O from the literature,[34] in Figure 1. The comparative measurements in H_2O were made with EB as the reference compound.

The calorimetric references (EB and BCP) gave no LIOAS signal at $T_{\beta=0}$ (Figure 2), while $TPPS_4$ and MB showed a negative signal at this temperature and RB a positive signal. The TT procedure (2.7 and 21 °C) without deconvolution (using amplitudes only) yielded total structural volume changes of −4.5, −1, and 1 mL/mol, respectively. Since at 21 °C a decay of ca. 1−2 μs was observed for all sensitizers, these total structural volume changes may be taken as approximate numbers only for the prompt structural volume change, i.e., for the production of the triplet state per mole of absorbed photons (vide infra).

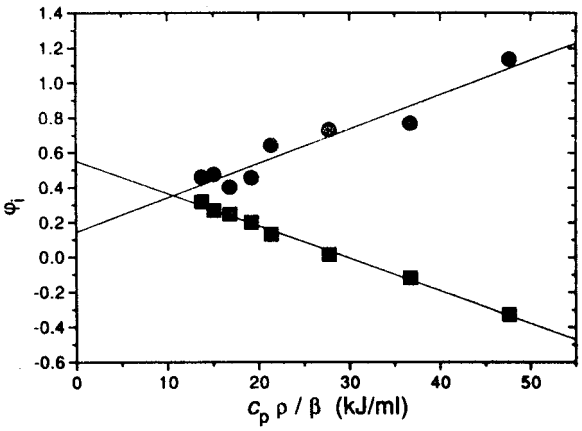

Figure 3. ST experiment with $TPPS_4$ in air-saturated 10 mM Tris-buffer (pH 7.8) with BCP as reference. Plot of pre-exponential factors from deconvolution (■) φ_1 = prompt and (●) φ_2, fixed at 2.5 μs, vs $c_p\rho/\beta$; λ_{exc} = 532 nm.

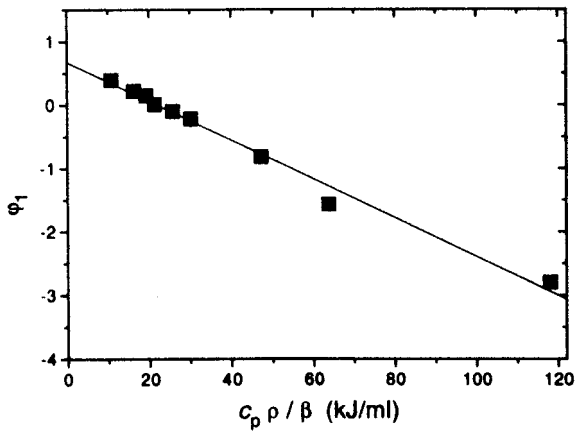

Figure 4. ST experiment with $TPPS_4$ in argon-bubbled 10 mM Tris-buffer (pH 7.8) with BCP as reference. Plot of pre-exponential factor φ_1 from deconvolution vs $c_p\rho/\beta$; λ_{exc} = 532 nm.

To understand this phenomenon, we focused on $TPPS_4$, the sample with the most pronounced ΔV_r. For aereated solutions of $TPPS_4$, the deconvolution procedure afforded three components: a prompt (<1 ns), a 1−2 μs, and a 3−6 μs component. ST measurements with solutions of $TPPS_4$ in 10 mM Tris-buffer (pH 7.8) between 6 and 40 °C were carried out. Owing to the variation in sign and relative contributions of the components, it was difficult to analyze the data at all temperatures with three components. Since the structural volume change was the main interest of this study, the two longer lifetimes were fixed to be one with a 2.5 μs decay. In Figure 3, the dependence of the amplitudes of the two lifetime components (prompt and 2.5 μs) on the ratio $c_p\rho/\beta$ is depicted. A prompt contraction of (4.5 ± 0.5) mL/mol was determined followed by an expansion of (4.5 ± 1.5) mL/mol concomitant with the 2.5 μs decay. This simplified treatment of the data may introduce systematic errors of variable importance at different temperatures, affecting mostly the ST data.

When the same experiment under argon atmosphere is carried out, only a prompt signal could be found, indicating a contraction of (7 ± 1) mL/mol (Figure 4).

Deconvolution analysis of ST measurements with $TPPS_4$ in deuterated Tris-buffer (10 mM, pD 8.0) under air afforded two components. One was prompt and the second had a decay lifetime between 1.5 and 1.9 μs. The prompt signal gave a contraction of (6.5 ± 1) mL/mol, while the second component yielded an expansion of (6 ± 2) mL/mol (Figure 5). The

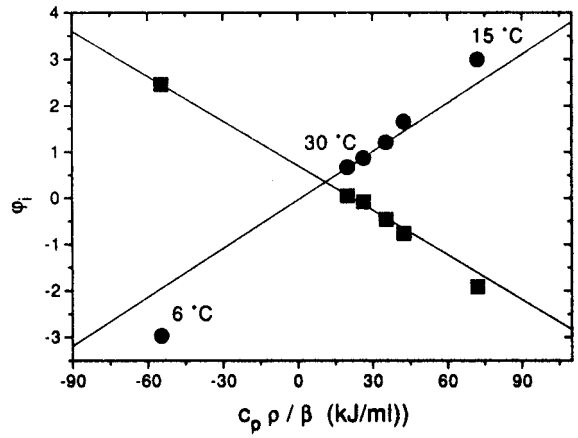

Figure 5. ST experiment with $TPPS_4$ in air-saturated D_2O-based 10 mM Tris buffer (pD 8.0) with BCP as reference. Plot of pre-exponential factors from deconvolution (■) φ_1 prompt and (●) φ_2 vs $c_p\rho/\beta$; λ_{exc} = 532 nm. The value at 15 °C for φ_1 and the values at 6 and 15 °C for φ_2 were not used for the fitting.

lifetimes and volume changes derived from all three plots (experiments under air and under argon in H_2O-based solutions and under air in D_2O-based solutions) of the amplitudes vs the ratio of thermoelastic parameters are listed in Table 1. The respective intercepts α_i^m are collected in Table 2. Furthermore, Tables 1 and 2 contain the results from TT experiments ($T_{\beta=0}$ = 2.7 and $T_{\beta\neq0}$ = 8 °C for H_2O-based solutions and $T_{\beta=0}$ = 10.2 and $T_{\beta\neq0}$ = 6 °C for D_2O-based solutions) with the three solutions (see eqs 5 and 6 in Appendix). In the discussion we use the average values for $\Phi_i\Delta V_{R,i}$ = −5.6 and 5.7 (air/H_2O), −6.1 (Ar/H_2O), and −5.9 and 5.8 mL/mol (air/D_2O).

The lifetime of the fast microsecond component was influenced by the amount of molecular oxygen in deuterated and in non-deuterated solution (Figure 6). In the former medium in which the slow microsecond component was not detectable owing to its long lifetime, the fast microsecond component decreased from 1.9 μs under air to 400 ns under O_2. A similar effect was observed in the H_2O-based solutions. However, in this case the fitting procedure was difficult owing to the similarity between the lifetimes of the quenched triplet state and $O_2(^1\Delta_g)$ produced by energy transfer. When various O_2:N_2 ratios are used and when the lifetime of the long-microsecond component at 3.2 μs (which equals the value of τ_Δ in water; see Figure 7A) is fixed, a linear relationship between the rate constant of the fast-microsecond component and [O_2] was obtained (inset in Figure 6). When a Stern−Volmer analysis of the data was performed and when the value of 0.218 × 10^{-4} [35] for the molar fraction of O_2 in water at 30 °C (a similar value is assumed in D_2O) was used, a value of $k_q = 2.3 \times 10^9$ M^{-1} s^{-1} was obtained for the O_2-quenching constant of the $TPPS_4$ triplet in H_2O-buffer and a value of $k_q = 1.9 \times 10^9$ M^{-1} s^{-1} was obtained in D_2O-buffer, in good agreement with the literature value for this constant.[36] This agreement indicates that the fast-microsecond component corresponds to the triplet decay and supports the assignment of the contraction concomitant to the very fast component to the production of the $TPPS_4$ triplet state.

The time constant for $O_2(^1\Delta_g)$ formation and its lifetime, τ_Δ, were obtained by TRPD as well (eq 1, Figure 7A). For $TPPS_4$ in 10 mM aqueous Tris buffer, a rise time of 1.8 μs and a decay of 3.2 μs were obtained. The rise time corresponds to the triplet-to-O_2 energy-transfer rate and the decay to the $O_2(^1\Delta_g)$ decay. $TPPS_4$ in 10 mM D_2O Tris buffer yielded a rise time again of 1.8 μs, and a decay of 60 μs (Figure 7B). These measurements were performed at ca. 15 °C. The values of τ_Δ determined by

TABLE 1: Lifetimes (τ_i) and Structural Volume Changes Per Absorbed Einstein ($\Phi_i\Delta V_{R,i}$) and Per Mole of Photoproduced Species ($\Delta V_{R,i}$) from LIOAS Measurements[a]

method	buffer, gas	τ_1 (ns)	$\Phi_1\Delta V_{R,1}$ (mL/mol)	$\Delta V_{R,1}$ (mL/mol)	τ_2 (μs)	$\Phi_2\Delta V_{R,2}$ (mL/mol)	$\Delta V_{R,2}$ (mL/mol)	τ_3 (μs)	$\Phi_3\Delta V_{R,3}$ (mL/mol)
ST	H_2O, air	10	−4.5	−7.5	0.8−2.5	4.5	7.5	3−6	0
TT	H_2O, air	10	−6.7	−11	1.8−2.0	7.1	11.5	3−5	0
ST	H_2O, Ar	10	−6.9	−11.5	n.o.	n.o.	n.o.	n.o.	n.o.
TT	H_2O, Ar	10	−5.4	−9	n.o.	n.o.	n.o.	n.o.	n.o.
ST	D_2O, air	10	−6.5	−10.5	1.5−1.9	6	10	n.o.	n.o.
TT	D_2O, air	10	−5.4	−9	1.8−2.0	5.7	9.5	n.o.	n.o.

[a] $TPPS_4$ in 10 mM Tris-buffer (pH 7.8 or pD 8.0, respectively); n.o. = not observable; $\Phi_1 = \Phi_2 = \Phi_T = 0.61$ (see Calorimetric Calculations section) was used to determine $\Delta V_{R,1}$ and $\Delta V_{R,2}$. The errors of $\Phi_i\Delta V_{R,i}$ are ±2 mL/mol.

TABLE 2: Fractional Heat Release, α_i^m, for $TPPS_4$ (eqs 3 and 5) in 10 mM Tris-Buffer (pH 7.8 or pD 8.0, Respectively) and Calculated Fractional Heat Release, α_i^c (See Text)[a]

	process	solvent	method	i	α_i^c	α_i^m
1	triplet formation	H_2O, air	ST	1	0.52	0.55
2	triplet decay	H_2O, air	ST	2	−0.17	n.p.
3	$O_2(^1\Delta_g)$ decay	H_2O, air	ST	3	0.59	n.p.
4	triplet + $O_2(^1\Delta_g)$ decay	H_2O, air	ST	2	0.43	0.18
5	triplet formation	H_2O, air	TT	1	0.52	0.59
6	triplet decay	H_2O, air	TT	2	−0.17	n.p.
7	$O_2(^1\Delta_g)$ decay	H_2O, air	TT	3	0.59	n.p.
8	triplet + $O_2(^1\Delta_g)$ decay	H_2O, air	TT	2	0.43	0.26
9	triplet formation	H_2O, Ar	ST	1	0.52	0.69
10	triplet formation	H_2O, Ar	TT	1	0.52	0.59
11	triplet formation	D_2O, air	ST	1	0.52	0.58
12	triplet decay	D_2O, air	ST	2	0.17	−0.07
13	triplet formation	D_2O, air	TT	1	0.52	0.55
14	triplet decay	D_2O, air	TT	2	0.17	0.06

[a] n.p. = analysis not possible. The errors of α_i^m are ±0.05.

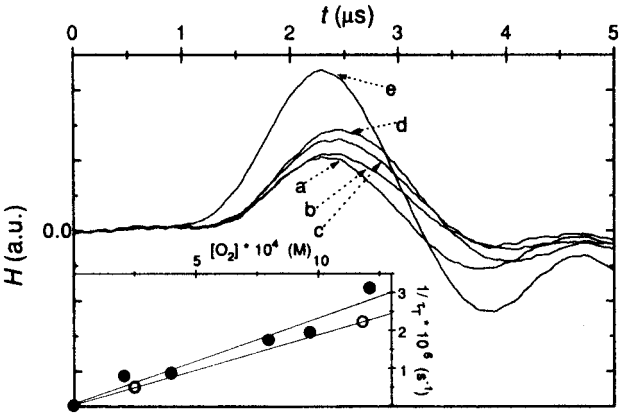

Figure 6. LIOAS signals of (e) BCP under air and of $TPPS_4$ under (a) 0 atm O_2, (b) air, (c) 0.5 atm O_2, (d) 1 atm O_2 in H_2O-based Tris-buffer (pH 7.8); $\lambda_{exc} = 532$ nm. Inset shows a plot of $1/\tau_T$ vs $[O_2]$ in (○) D_2O- and (●) H_2O-based 10 mM Tris-buffer (pD 8.0 and pH 7.8, respectively) obtained at 30 °C from deconvolution with the respective model functions, i.e., (○) prompt and triplet decay and (●) prompt, fixed $\tau_\Delta = 3.2$ μs, and triplet decay.

TRPD in H_2O- (3.2 μs) and in D_2O-based (60 μs) buffers agree well with the literature data (3.1−3.5 and 60−68 μs, respectively).[37]

Comparing the amplitudes obtained using eq 1 for the fitting of the TRPD data for rose bengal as reference sensitizer ($\Phi_\Delta = 0.76 \pm 0.03$)[38] and for $TPPS_4$ in D_2O buffer at pD 8.0 afforded a value of $\Phi_\Delta = 0.62 \pm 0.05$, in good agreement with the average literature value of $\Phi_\Delta = 0.57 \pm 0.14$.[38]

At pH 7.8 a fluorescence quantum yield of $\Phi_f = 0.060 \pm 0.005$ (average of six measurements) was determined for $TPPS_4$ (2 μM) by using as a fluorescence standard TPrPn. This value agrees reasonably well with the literature value of $\Phi_f = 0.08$,[14] while $\Phi_f = 0.16$ [15] was reported without mention of the

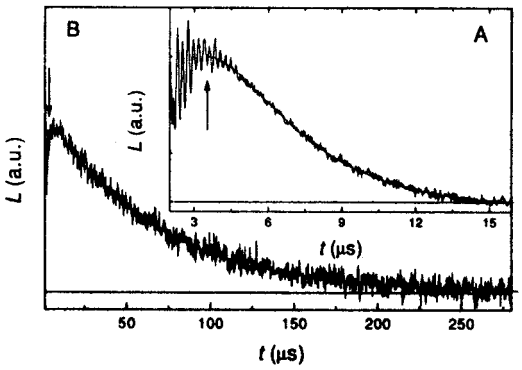

Figure 7. Time-resolved near-IR emission at 1270 nm of $O_2(^1\Delta_g)$ sensitized by 8 μM $TPPS_4$ in air-saturated (A) H_2O-based 10 mM Tris-buffer (pH 7.8) and (B) D_2O-based 10 mM Tris-buffer (pD 8.0); $\lambda_{exc} = 532$ nm. Fit functions obtained by using eq 1; the lifetimes were (A) $\tau_\Delta = 3.2$ and $\tau_T = 1.8$ μs, (B) $\tau_\Delta = 60$ and $\tau_T = 1.5$ μs. The arrows indicate the starting point for the fitting procedure.

reference standard used. The average fluorescence energy, $E_f = 174$ kJ/mol was calculated from the fluorescence emission spectrum.

The ST method required the measurements in the range 6−40 °C. To evaluate the effect of aggregation in this temperature range, the value of K_D was determined. At concentrations <10 μM only small deviations from the Lambert−Beer behavior were observed. At higher concentrations K_D values of 4000 M^{-1} at 22 °C and of 9500 M^{-1} at 7 °C were obtained, in line with the observation that for porphyrins aggregation increases at lower temperatures.[19,38] The values of the molar absorption coefficient for the dimer, ϵ_D, are 80−150% (depending on the wavelength) of those for the monomer ϵ_M, similar to the case of other water-soluble porphyrins.[39]

The value of K_D was verified by measuring Φ_f at various $TPPS_4$ concentrations (2−60 μM). To keep $A < 0.06$, the excitation wavelength was varied from 355 nm to visible wavelengths. The reasonable underlying assumption was that Φ_f is independent of excitation wavelength. For concentrations <30 μM, the spectra did not change ($\lambda_{em} = 645$ and 702 nm) and the value $\Phi_f = 0.060 \pm 0.005$ (average of data at three concentrations) was obtained. At 61 μM a value $\Phi_f = 0.052$ was determined, concomitant with the appearance of a shoulder at 660 nm in the emission spectrum attributed to the dimer fluorescence.

Discussion

Dimerization. Although it is known that the dimerization of $TPPS_4$ is salt and temperature dependent (similar to other water-soluble porphyrins),[39,40] the literature values for K_D are not fully consistent. While Fleischer et al.[41] found no evidence of aggregation at room temperature in a range of ionic strength, $I = 0-0.1$, Krishnamurthy et al.[42] reported a value of $K_D =$

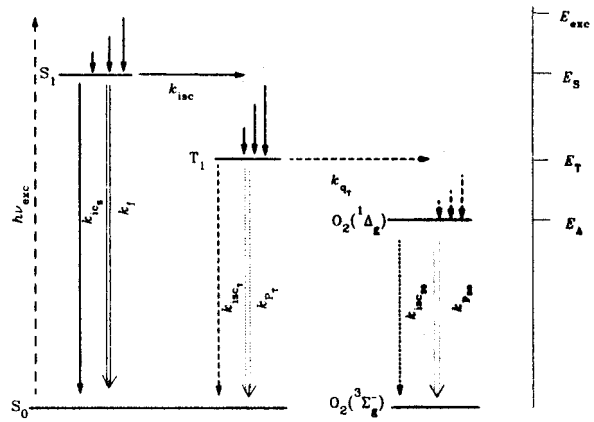

Figure 8. Energy diagram of photosensitized $O_2(^1\Delta_g)$ generation. Solid arrows represent the process included in φ_1, arrows with long dashes the processes included in φ_2, and dotted arrows the processes included in φ_3.

96 000 M^{-1} at room temperature and $I = 0.06$ and Sutter et al.[43] reported $K_D = 21\,000$ M^{-1} at $I = 0.11$. Our value $K_D = 4000$ M^{-1} at 22 °C and $I = 0.01$ is more in line with those by Fleischer et al.[41] and Sutter et al.[43] The fact that no decrease in the value of ϕ_f was found below 30 μM agrees with our calculation of K_D using absorption data.

By use of $K_D = 9500$ M^{-1} at 7 °C and 4000 M^{-1} at 22 °C, the calculated dimer concentrations under our conditions result in 15% at 7 °C and 5% at 22 °C. It is possible that the deviations from linearity observed, e.g., in Figures 3 and 5 at the lower temperatures (higher absolute $c_p\rho/\beta$ values), are due to some influence on the results of the small amount of dimers. By use of the linear parts in Figures 3−5, the intercepts are in reasonable agreement with the otherwise known parameters of $TPPS_4$ (vide infra). We conclude that aggregation of $TPPS_4$ is negligible for the evaluation of the LIOAS experiments. Thus, the volume changes observed at the higher temperatures in our study are definitively a property of the monomers and their environment.

Structural Volume Changes. Figure 8 summarizes the processes taking place after excitation of a molecule capable of generating $O_2(^1\Delta_g)$ by energy transfer.

The prompt component of the LIOAS signal for $TPPS_4$ consistently afforded a total contraction of ca. 6 mL/mol under air, under argon, and in deuterated solution (Table 1). Since the fluorescence lifetime of $TPPS_4$ is ca. 10 ns[13,15,44] in basic solution, all processes up to triplet formation are fast with respect to the observation time of the LIOAS experiment and thus contribute to the prompt component. Therefore, the contraction can be attributed to the triplet formation. Whether already the singlet excited state is contracted with respect to the ground state cannot be answered with the present experiments.

In the following discussion we use for each case (air, Ar, and H_2O-based and D_2O-based solutions) the average between the values determined by the ST and the TT methods. The expansion of 5.7 mL/mol determined for the combined decay of the second and third components in air-saturated aqueous buffer, albeit identical with the contraction observed for triplet formation, could not be unequivocally assigned to triplet decay on this basis only, since the value of τ_Δ is too short in this solvent and is similar to the value of the triplet-to-O_2 energy transfer time constant. Under argon atmosphere, where the triplet lifetime is very long with respect to our heat integration time, the triplet decay is not detectable. In this case, after the initial contraction of 6.1 mL/mol, the expansion occurs in a much longer time. In deuterated solution (τ_Δ of ca. 60 μs),[37] as in H_2O-buffer, the initial contraction (5.9 mL/mol) is similar to the expansion accompanied by a 1.5−1.9 μs decay (5.8 mL/mol). This indicates that the expansion (a) is produced in the process of triplet-to-O_2 energy transfer and not during the $O_2(^1\Delta_g)$ decay.

The structural volume change per photoconverted mole ($\Delta V_{R,i}$, Table 1) is obtained by dividing the total structural volume changes obtained so far by the respective triplet quantum yields (eq 3, with the assumption $\Phi_i = \Phi_T = 0.61$; see Calorimetric Calculations section). The absolute average $\Delta V_{R,i}$ value results in 10 mL per mole (17 Å^3 per molecule) of triplet produced. The assumption of the identity of the Φ_T value under all conditions (Ar, air, and H_2O-based and D_2O-based solutions) is a crude approximation, since there are indications in the literature of a larger triplet yield in D_2O-based solutions than in H_2O-based solutions.[45,46] Considering the long fluorescence lifetime, air might also alter the value of Φ_T. The differences in Φ_T should be held in part responsible for the different values of $\Phi_i\Delta V_{R,i}$ in the three types of solutions. The error implied in the above assumption has a relatively small influence in the calorimetric calculations performed (see corresponding section) using φ_1.

The values obtained for the volume changes are not related to the signs of the charged substituents on the porphyrin ring, i.e., the sulfonic groups, since we have preliminary data indicating that similar effects are observed for tetracationic porphyrins. Our data are also much larger than the values expected from the calculations by Bell et al.[47] These authors used semiempirical (AM1) calculations of the bond-length changes associated with excitation of free-base tetraphenylporphyrin (TPP). The calculated structural changes accorded satisfactorily with the observed changes in frequency on excitation of the vibrational modes of the compound, determined by time-resolved resonance Raman measurements.[48] The conclusion from the calculations was that the electron density at the H-bearing pyrrole ring (pyrrolenine) nitrogens in the triplet state is larger than in the ground state. Taking into account the fact that the resonance Raman shifts observed for excitation of free-base $TPPS_4$ follow the same trend as those for free-base TPP,[47] a similar argument can be made about the increase of electron density on the pyrrolenine ring nitrogens of triplet $TPPS_4$. It is then possible to speculate that the contraction we observe upon excitation is determined by the shortening of the hydrogen bonds of H_2O to the ring nitrogens with increased electron density in the triplet state, i.e., we have again the case of a specific solute−water interaction being responsible for the volume changes.[8]

General Conclusions from and Significance of the Results on Volume Changes. In conclusion, the production of the triplet state in basic solutions of $TPPS_4$ is accompanied by a contraction of 10 mL/(mol of triplet), which is reverted while the triplet decays (quenched or unquenched by O_2). We attribute this contraction to enhanced H-bonded water to the central N atoms in the triplet state.

The implications of the above conclusion for the study previously performed on the D1/D2-cyt *b*-559 reaction centers is that the volume expansion determined may be due to reorganization of the protein environment around the triplet state, i.e., after recombination of the initially formed radical pair and not necessarily at the moment of formation of the ion pair.[1] The time resolution of the LIOAS method used did not allow a differentiation of these two possibilities.

Calorimetric Calculations. The heat released by the various kinetically distinguishable components of the LIOAS signal is obtained by using the intercepts, α_i^m, of plots such as those in Figures 3−5 together with eq 3 (ST).

The last row in Table 2, α_i^m, shows the results obtained by kinetic analysis of the LIOAS data on the basis of consecutive steps, using the TRPD and the LIOAS values for the rate of triplet-to-O_2 energy transfer and for τ_Δ . The measured values α_i^m should be compared with those calculated, α_i^c, using eq 4 and the photophysical values. For the calculation a three-step sequential model was used, i.e., excited state → triplet state → $O_2(^1\Delta_g)$ → ground state, following the analysis by Rudzki-Small et al.[22] In H_2O-buffer/argon and in D_2O/air the three-step model was reduced to one and two steps, respectively. For the case of H_2O-buffer/air, a two-step model, i.e., excited state → (triplet state + $O_2(^1\Delta_g)$) → ground state was adopted, since the deconvolution was carried out with a fixed lifetime representing the average of τ_T and τ_Δ. Values of $\Phi_f = 0.060$, $E_f = 174$ kJ/mol, and $E_S = E_{0-0} = 186$ kJ mol^{-1}, obtained from the fluorescence spectra in this work, $\Phi_T = 0.7$ [14,44,49] and $E_T = 139$ kJ mol^{-1} from 77 K phophorescence measurements,[14] and $\Phi_\Delta = 0.62$ (this work) were used to calculate the heat release associated with the various processes.

The agreement between the values of α_i^c and α_i^m for the prompt heat release is good. Taking the average $\alpha_1^m = 0.58 \pm 0.05$ (lines 1, 5, 9, 10, 11, and 13 in Table 2) and using eq 4, we calculate $\Phi_T = 0.61 \pm 0.06$. This value is in very good agreement with the literature value of 0.7 (vide supra).

However, the measured values of α_2^m are too low (lines 4, 8, 12, and 14 in Table 2). Possible explanations for the discrepancy between α_2^m and α_2^c could be that (i) the relatively small contribution of ΔV_{th} to φ_2 and to φ_{2+3} makes difficult its determination, (ii) the structural volume change (the slopes of the lines of Figures 3, 4, and 5) is not constant with temperature, e.g., due to dimerization (this effect is more important in the ST-method), (iii) the inability of the deconvolution program to discriminate between triplet and $O_2(^1\Delta_g)$ decay leads to wrong amplitudes.

Use of Molecules with Long-Lived Transients as Calorimetric References. An additional conclusion derived from this work is that serious errors may be introduced in the values of photophysical parameters when obtained from photothermal methods if the structural volume changes contributing to the pressure wave are not taken into account. In particular, we have previously used $TPPS_4$ as a calorimetric LIOAS reference for the determination of the triplet yield of 4-thiouridine in argon-flushed 1 mM Tris-buffers (pH 7.4).[50] For $TPPS_4$ and $E_\lambda = 337$ kJ/mol (355 nm) a prompt heat released of 192 kJ mol^{-1} was calculated. The LIOAS signal amplitudes of $TPPS_4$ and 4-thiouridine were compared and a value $\Phi_T = 0.67$ was obtained from the prompt heat for 4-thiouridine and from its triplet energy. This value was distinctively lower than a previous one obtained by a comparative absorption flash photolysis study ($\Phi_T = 0.9$).[51] Since the measurements were only carried out at 23 °C and since we were not aware of a structural volume change concomitant with triplet formation, the analysis of the prompt pressure signal was incomplete. On the basis of the present studies, we expect that at 23 °C and an excitation at 355 nm the prompt negative structural volume change should account for ca. 40% of the prompt heat release signal. Thus, the value estimated of $\Phi_T = 0.90-0.95$ is in good agreement with the value from a previous optical study.[51] Although this correction does not question the main results of the study by Heihoff et al.,[50] it illustrates the necessity of considering the structural volume changes contributing to the pressure wave, especially in aqueous solutions.

An analogous discussion can be made with respect to the use of MB and RB as calorimetric references (see Figure 2). On the basis of similar studies with these dyes as those presented for $TPPS_4$, we assign the volume changes observed for MB and RB to triplet formation. So far, all LIOAS studies in aqueous solutions with molecules producing long-lived transients (longer than ca. 50 ns) show an important contribution of the structural volume changes to the pressure wave at room temperature.[5,8,9,52,53] Although these volume changes originate in various processes such as intrinsic bond-length changes and electrostriction effects, in these solutions specific solute−solvent interactions through H-bonding make the largest contribution to the volume changes.[8]

Thus, to select a substance as a calorimetric reference, it is not enough to know the photophysical parameters. Rather, a substance with rapid deactivation, i.e., $\alpha = 1$, should be used. In the meantime it has been shown that in aqueous solution there are several calorimetric references that fulfill this condition, e.g., bromocresol purple, bromocresol green, and Evans blue, depending on the excitation wavelength.[1,6]

During the reviewing process of this paper we learned of a recent report by Feitelson and Mauzerall[53] about structural volume changes upon photoexcitation of uroporphyrins. The contractions reported for the triplet formation are 0.5−1 mL/mol, much smaller than our values.

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Appendix

The equations needed for the evaluation of the data obtained with the time-resolved ST-method have been already published.[8,54] Here, we present again the equations needed for the calculations of enthalpies and structural volume changes with the time-resolved TT-method. The equations are similarly derived as those used with the TT-method for long-lived products, i.e., using the LIOAS signal amplitudes.[2,9]

$$\alpha_i = \varphi_i(T_{\beta\neq 0}) - \varphi_i(T_{\beta=0}) \tag{5}$$

$$\Phi_i \Delta V_{R,i} = \varphi_i(T_{\beta=0})\left(\frac{\beta}{c_p\rho}\right)_{T_{\beta\neq 0}} E_\lambda \tag{6}$$

The assumption behind these equations is the invariance of the compressibility in the small temperature range $T_{\beta=0} - T_{\beta\neq 0}$.

Abbreviations

LIOAS	laser-induced optoacoustic spectroscopy
$TPPS_4$	tetrakis(tetrasulfonated-phenyl)porphyrin
MB	methylene blue
RB	rose bengal
BCP	bromocresol purple
TPrPn	tetrapropyl porphycene
ST	several temperatures
TT	two temperatures
TRPD	time-resolved phosphorescence detection

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