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ARTICLES

Photoinduced Electron Transfer to Triplet Flavins. Correlation between the Volume Change-Normalized Entropic Term and the Marcus Reorganization Energy[†]

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The data obtained through the application of nanosecond laser-induced optoacoustic spectroscopy (LIOAS) to several electron donor−acceptor pairs in aqueous solution were analyzed together with the respective experimentally determined Marcus reorganization energy. Acceptors were the flavin mononucleotide and flavin-adenine dinucleotide triplet states (^{3}FMN and ^{3}FAD) and donors were tryptophan, tyrosine, histidine, triethanolamine, and ethylenediaminetetraacetic acid. The respective calculated Gibbs energy for electron transfer, $\Delta_{ET}G°$, was used together with the enthalpy change for the formation of free radicals, $\Delta_{FR}H$, obtained from the LIOAS data, to derive the entropy change for the formation of the radicals, $\Delta_{FR}S$. In all cases, variation of the monovalent cations, i.e., $[CH_3(CH_2)_3]_4N^+$, Li^+, $NH_4{}^+$, K^+, and Cs^+, resulted in variation of the enthalpy change, $\Delta_{FR}H$, and in the structural volume change, $\Delta_{FR}V$, for the free-radical production, both derived from LIOAS. $\Delta_{FR}H$ and $\Delta_{FR}V$ linearly correlated with each other within the cation series. From this correlation the respective entropic term $T\Delta_{FR}S$ was derived as well as the ratio $X = T\Delta_{FR}S/\Delta_{FR}V$ for each of the pairs. X linearly correlated with the respective total Marcus reorganization energy, λ, for all systems analyzed. This observation underlines the concept that both λ and $\Delta_{FR}V$ respond to the same phenomena. The correlation also offers an experimental approach for the understanding at a molecular level of the origin of the λ values as well as for their evaluation.

Introduction

In a recent publication we reported the thermodynamic parameters obtained by laser-induced optoacoustic spectroscopy (LIOAS) at various temperatures in aqueous solution, i.e., the enthalpy, structural volume, and entropy changes for the formation of free radicals, $\Delta_{FR}H$, $\Delta_{FR}V$, and $\Delta_{FR}S$, respectively, upon quenching of flavin mononucleotide triplet state, ^{3}FMN, by electron transfer from tryptophan, Trp, and tyrosine, Tyr.[1] We found that the $\Delta_{FR}V$ values obtained by LIOAS in solutions containing phosphate buffers of different monovalent cations, i.e., $[CH_3(CH_2)_3]_4N^+$, Li^+, $NH_4{}^+$, K^+, and Cs^+, quantitatively correlated with the respective $\Delta_{FR}S$ values calculated upon combining the calculated $\Delta_{FR}G°$ with the $\Delta_{FR}H$ values from the LIOAS measurements.

During these studies it was observed that the decrease in the ratio $X = T\Delta_{FR}S/\Delta_{FR}V = 55 \pm 9$ kJ cm^{-3} (constant for all cations) for Trp as ^{3}FMN quencher to a smaller $X = 83 \pm 9$ kJ cm^{-3} for Tyr as quencher correlated with a decrease in the value of the reorganization energy λ as derived using Marcus concepts for outer-sphere electron transfer.[2]

To verify the general validity of the above preliminary observation, we decided to extend the studies to the quenching of ^{3}FMN in the presence of the five monovalent cations, by other electron donors such as histidine (His), triethanolamine

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[†] Abbreviations: EDTA, ethylenediaminetetraacetic acid; ^{3}F, flavin triplet state; FAD, flavin-adenine dinucleotide; FMN, flavin mononucleotide; His, histidine; LIOAS, laser-induced optoacoustic spectroscopy; MB, methylene blue; ST, several-temperatures; TEA, triethanolamine; Trp, tryptophan; TT, two-temperatures; Tyr, tyrosine.

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(TEA), and ethylenediaminetetraacetic acid (EDTA) as well as to the quenching of the triplet flavin-adenine dinucleotide (FAD), ^{3}FAD, by the above-mentioned five electron donors, i.e., TEA, His, Trp, EDTA, and Tyr, also in the presence of the various cations.

Our present data confirm that there is a linear correlation between the respective $X = T\Delta_{FR}S/\Delta_{FR}V$ values and the Marcus reorganization energy λ for all electron donor−acceptor pairs studied.

Materials and Methods

Reagents. Lithium dihydrogen phosphate, lithium hydroxide, trilitium phosphate, ammonium phosphate dibasic, monobasic ammonium phosphate, cesium dihydrogen phosphate/cesium hydrogen phosphate, L-tryptophan (Trp), L-tyrosine (Tyr), triethanolamine (TEA), ethylenediaminetetraacetic acid (EDTA), flavin-adenine dinucleotide (FAD), methylene blue (MB), and new coccine were obtained from Sigma-Aldrich Chemie GmbH, Schnelldorf, Germany. Potassium dihydrogen phosphate and dipotassium hydrogen phosphate were from Merck, Darmstadt, Germany. Tetrabutylammonium phosphate buffer was from GFS Chemicals, Inc., and flavin mononucleotide (FMN) from Serva electrophoresis GmbH, Heidelberg, Germany.

Solutions. FMN and FAD solutions (7.5×10^{-3} M) were prepared by dissolving the respective flavin in 50 mL of a 50 mM phosphate buffer solution of each of the cations used. Stock solutions of Trp, Tyr, His, TEA, and EDTA were prepared by dissolving the electron donors in 25 mL of 50 mM of the respective phosphate buffer. Final solutions were prepared by dissolving the above-described flavin and electron-donors solutions in phosphate buffer to obtain a final 1.5×10^{-5} M flavin and appropriate concentrations of donors, 1.5×10^{-4} M Trp and Tyr, 0.025 M His, 7.5×10^{-3} M TEA, and EDTA, in a total volume of 5 mL. These concentrations corresponded to >95% quenching acording to the quenching constants determined (vide infra). All solutions had a final pH = 7. Phosphate solutions were kept cool in the dark when not in use to avoid deterioration by exposure to light and heat. Before the spectroscopic measurements, the solutions were purged during 15 min with Ar.

Methods. Absorption spectra were recorded with a UV-2102PC spectrophotometer (Shimadzu Germany, Duisburg, Germany).

Excitation at 450 nm for the LIOAS experiments was either from a Nd:YAG-pumped Beta Barium Borate Optical Parametric Oscillator (OPO-C-355, bandwidth 420−515 nm in the blue spectral region, Laser Technik Vertriebs GmbH, Ertestadt-Friesheim, Germany), as previously described,[1,3] or from an excimer laser-pumped dye laser with Coumarin-2 (Lambda-Physik, 15 ns pulse width). The beam was shaped by a 1 (w) × 6 (h) mm slit, allowing a time resolution of ca.100 ns by using deconvolution techniques.[4] The experiments were performed in the linear regime of amplitude versus laser fluence, which was up to 35 μJ/pulse. The total incident energy was typically <20 μJ/pulse (<25 μeinstein/m^2). Normally 100 shots were averaged for each waveform. A very small fraction of the sample was irradiated by the pulse (<2%). The slow repetition rate (1 Hz) ensured that the flavin concentration changes were negligible during the measurements. This was proven by continuously monitoring the transmitted light.

New coccine was the calorimetric reference.[5] The absorbance of sample and reference solutions at λ_{exc} was $A(450\text{ nm}) = 0.160 \pm 0.004$. The time evolution of the pressure wave was assumed to be a sum of two monoexponential functions. The convolution of the pressure-evolution function and the reference signal yielded the fractional amplitudes (φ_i) and the lifetimes (τ_i) of the transients (Sound Analysis 3000, Quantum Northwest Inc., Spokane, WA). The time window was between 100 ns and 5 μs. At a given temperature and for each resolved *i*th step the fractional amplitude φ_i is the sum of the fraction of absorbed energy released as heat (α_i) and the molar structural volume change ($\Delta_i V$), according to[6,7]

$$\phi_i = \alpha_i + \frac{\Phi_i \Delta_i V}{E_\lambda}\left(\frac{c_p \rho}{\beta}\right)_T \qquad (1)$$

where E_λ is the molar excitation energy, Φ_i is the quantum yield of the respective process, $\beta = (\partial V/\partial T)_p/V$ is the volume expansion coefficient, c_p is the specific heat capacity at constant pressure, and ρ is the mass density of the solvent, the three latter quantities at temperature T. Thus, by plotting φ_i vs $(c_p\rho/\beta)_T$, the values of α_i and the structural volume change per absorbed einstein, $\Phi_i\,\Delta_i V$, are obtained. This procedure is called the several-temperatures (ST) method. The assumption made when using this method is that the enthalpy change (contained in the intercept, α_i , eq 1) as well as the slope of the line represented by eq 1, i.e., the structural volume change per absorbed einstein, are independent of the temperature in the range studied.

The values of $(c_p\rho/\beta)_T$ were those determined in our previous publication.[1]

The two-temperatures (TT) method in LIOAS was also applied to separate the contribution to the signal of the enthalpy and the structural volume change.[1,8] A total of 100 traces was averaged for each measurement. Three independent measurements at each temperature were done for sample and reference and the signals were crossed for the deconvolution procedure. Nitrogen was flown over the closed cuvette during the measurements to avoid water condensation, especially at 5 °C.

Flash Photolysis Experiments. Flash photolysis experiments were performed with a simplified single-beam equipment as described previously.[9] Excitation was with 12 ns laser pulses at 308 nm from a XeCl excimer laser (EMG50, Lambda Physik, Göttingen, Germany). A methylene blue (MB) solution of matched absorbance to that of the sample at 308 nm, $A(308\text{ nm}) = 0.229 \pm 0.002$,was used as an actinometer with a triplet quantum yield $\Phi_T = 0.52 \pm 0.05$ and a triplet−triplet absorption coefficient at 420 nm, $\epsilon(420,\text{MB}) = 14\,400$ M^{-1} cm^{-1}.[10] Measurements were performed at 20 and 5 °C.

Results

Flash Photolysis Results. The formation quantum yields for the FMN and FAD triplet state (^{3}FMN and ^{3}FAD, respectively, generically called ^{3}F), $\Phi(^3\text{FMN}) = \Phi_T$ or $\Phi(^3\text{FAD}) = \Phi_T$ and for the neutral flavin semiquinone radical (FMNH$^\bullet$ and FADH$^\bullet$, respectively), $\Phi(\text{FMNH}^\bullet) = \Phi_{FR}$ or $\Phi(\text{FADH}^\bullet) = \Phi_{FR}$, i.e., for the radicals formation quantum yield upon ^{3}F quenching, respectively, by the electron donors, were determined by the comparative method in flash photolysis using eq 2[11] and the MB triplet state absorption, $\Delta_T A(420,\text{MB})$, as a reference. In eq 2, x is ^{3}FMN, FMNH$^\bullet$, ^{3}FAD, or FADH$^\bullet$.

$$\Phi(\text{x}) = \Phi_T(\text{MB})\frac{\Delta A(\text{x})}{\Delta_T A(420,\text{MB})}\,\frac{\epsilon(420,\text{MB})}{\epsilon(\text{x})} \qquad (2)$$

The values of $\Delta A(^3\text{FMN})$ and of $\Delta A(^3\text{FAD})$ were obtained immediately after the laser pulse. Φ_{FR} in the presence of the respective concentration of each donor quencher (see Materials and Methods) were determined from the transient absorption

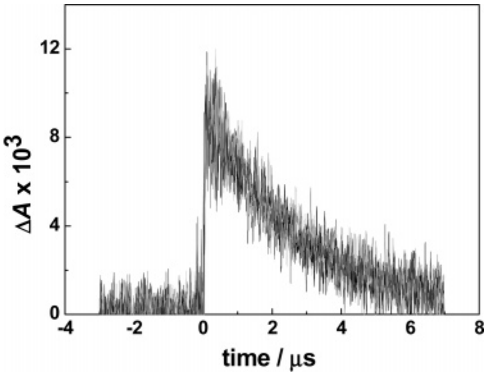

Figure 1. Transient absorbance decay observed at 510 nm upon excitation of 1.5×10^{-4} M FAD aqueous solutions with 15 ns laser pulses (1 mJ) at 308 nm in the presence of 0.025 M His and mono Cs^+, Li^+, NH_4^+, K^+, and $[CH_3(CH_2)_3]_4N^+$ phosphate salts. The traces essentially overlap each other. Each trace is the average of 100 decays at 25 °C and pH = 7.

TABLE 1: Quantum Yield for Free-Radical Formation, Φ_{FR}, upon Quenching of ^{3}FMN and ^{3}FAD by Various Electron Donors[a]

electron donor	FMN ± 0.04	FAD ± 0.04	lit. values [b]
Trp	0.52[1]	0.53	0.52[20]
Tyr	0.54[1]	0.54	0.48[c,20]
His	0.60	0.60	0.60[20]
EDTA	0.61	0.62	0.60[14]
TEA	0.60	0.61	0.66[14]

[a] Values independent of temperature (5−20 °C) and of cation nature ($[CH_3(CH_2)_3]_4N^+$, Li^+, NH_4^+, K^+, and Cs^+). Φ_{FR} values calculated from the decay traces at 510 nm in flash photolysis ($\lambda_{exc} = 308$ nm) with A_0 in eq 3, as an average of the values for Li^+ and Cs^+. [b] $\Phi_T = 0.7$,[20] the same for FAD and FMN. [c] Glycyl-L-tyrosine as electron donor.

decay at 510 nm, after the complete decay of ^{3}F, i.e., at 2 μs (see Figure 1).[12] In other words, each individual transient absorbance decay (ΔA, Figure 1) was analyzed with eq 3 and

$$\Delta A = A_0 + A_1 e^{-kt} \qquad (3)$$

the term A_0 together with the respective absorption coefficient of the radical [ϵ(510,FMNH$^\bullet$) = 4870 M^{-1} cm^{-1},[1] and ϵ(510,FADH$^\bullet$) = 4870 M^{-1} cm^{-1}][13] was used for the calculation of Φ_{FR} (Table 1). The amplitude at zero time after excitation, i.e., the sum ($A_1 + A_0$), together with $\epsilon(^3F) = 12\,500$ M^{-1} cm^{-1},[23] was used to calculate Φ_T.

The Φ_{FR} values were independent of the temperature in the range 5−20 °C and of the cation nature ($[CH_3(CH_2)_3]_4N^+$, Li^+, NH_4^+, K^+, and Cs^+) and were in good agreement with the literature values (Table 1).

Laser-Induced Optoacoustic Spectroscopy (LIOAS). Allowing all four parameters needed (eq 1 with $i = 1$ and 2; two lifetimes and two preexponential factors) to freely vary, in all cases the program found two well-separated times, i.e., $\tau_1 <$ 10 ns and τ_2 between 300 and 1200 ns, depending on the salt, quenchers, and temperature. The value of τ_1 only means that this process is faster than the time resolution of the experiment. Fixing τ_1 at any value <10 ns always resulted in a similar value of the associated amplitude (Table 2).

As already discussed,[1] upon laser excitation of flavins in deoxygenated solutions and in the absence of quenchers, only two consecutive processes occur, i.e., the formation of the triplet state, in our case ^{3}FMN or ^{3}FAD, followed by decay to the ground state. ^{3}F formation is always a prompt process and φ_1 is the measure of it, whereas in the presence of a quencher, φ_2 and the decay time τ_2 (see Table 3) are assigned to the formation of the free radicals.[1,15−17]

The values of k_q for the various electron donors (Table 4) were in agreement with those reported for the quenching of ^{3}F transient absorbance.[18−22] These quenching constants were independent of the nature of the salt present. Taking into account these values, a concentration was chosen for each electron donor for all further measurements that should quench >95% of ^{3}F (see Material and Methods).

Two different species for histidine are present at pH = 7, in view of its pK = 6.1. However, both protonated and neutral species efficiently act as electron donors.[22] In any case, we checked that the pH does not considerable change upon temperature changes. Different concentrations of histidine (0.01−0.035 M) were used to study possible changes in the rate constant with the concentration. Our quenching rate constant at pH 7 agrees with the value given by Heelis and Phillips at

TABLE 2: Prompt Heat, α_1 (eq 1 or eq 5), Associated with the Formation of ^{3}F in LIOAS after 450 nm Laser Pulse Excitation of FMN and FAD solutions in the presence of Trp, Tyr, His, TEA, and EDTA[a]

cation	FMN + His α_T[b] ± 0.04	FMN + EDTA α_T[b] ± 0.03	FMN + TEA α_T[b] ± 0.03	FAD + His α_T[b] ± 0.04	FAD + Tyr α_T[b] ± 0.04	FAD + Trp α_T[b] ± 0.03	FAD + EDTA α_T[b] ± 0.03	FAD + TEA α_T[b] ± 0.03
$[CH_3(CH_2)_3]_4N^+$	0.32	0.29	0.4	0.40	0.26	0.27	0.40	0.29
Li^+	0.34	0.43	0.38	0.32	0.33	0.29	0.39	0.38
NH_4^+	0.41	0.33	0.4	0.27	0.4	0.29	0.45	0.33
K^+	0.36	0.35	0.28	0.36	0.26	0.29	0.27	0.35
Cs^+	0.38	0.29	0.32	0.35	0.24	0.29	0.21	0.38

[a] For the values associated with ^{3}FMN formation in the presence of Trp and Tyr see ref 1. [b] The lifetime associated with the ^{3}F production was always <10 ns.

TABLE 3: Heat Dissipated upon Free Radical Formation, $\alpha_2 = \alpha_{FR}$ (eq 1 or eq 5) and Lifetimes Associated with the Formation of the Free Radicals in LIOAS (τ_2), at 5 °C for All Quenchers and at 7 °C for His, after 450 nm Laser Pulse Excitation of FMN and FAD Solutions with the Various Electron Donors As Indicated

	FMN[a]						FAD									
cation	His τ/ns	His α ± 0.03	EDTA τ/ns	EDTA α ± 0.03	TEA τ/ns	TEA α ± 0.03	Trp τ/ns	Trp α ± 0.04	Tyr τ/ns	Tyr α ± 0.03	His τ/ns	His α ± 0.03	EDTA τ/ns	EDTA α ± 0.04	TEA τ/ns	(TEA) α ± 0.02
$[CH_3(CH_2)_3]_4N^+$	548	0.21	410	0.34	825	0.26	830	0.26	400	0.31	550	0.33	678	0.33	990	0.29
Li^+	530	0.15	390	0.28	842	0.22	850	0.21	380	0.21	520	0.30	635	0.29	970	0.24
NH_4^+	540	0.12	385	0.24	825	0.18	820	0.21	380	0.16	550	0.29	654	0.27	950	0.19
K^+	556	0.06	412	0.19	890	0.08	860	0.15	400	0.11	576	0.24	667	0.25	925	0.09
Cs^+	520	0.04	395	0.18	860	0.03	850	0.10	390	0.10	530	0.21	692	0.20	893	0.03

[a] For the values associated with the formation of ^{3}FMN in the presence of Trp and Tyr see ref 1.

TABLE 4: Rate Constants at 20 °C Obtained from the LIOAS Data for Free-Radical Formation upon ^{3}FMN and ^{3}FAD Quenching by Various Donors As Indicated[a]

quencher	k_q (FMN) ± 1/M^{-1} s^{-1}	k_q (FAD) ± 1/M^{-1} s^{-1}
Trp	1.5×10^9 [b–d]	1.8×10^9 [c]
Tyr	1.3×10^9 [b–d]	1.3×10^9 [c]
His	4.8×10^7 [c,d]	5×10^7 [c]
TEA	4.2×10^8 [c]	4.4×10^8 [c]
EDTA	3.4×10^8 [c]	3.5×10^8 [c]

[a] Concentration range: Trp: 2×10^{-2} to 1×10^{-4} M, Tyr: 2×10^{-2} to 1×10^{-4} M, His: 0.01−0.035 M, TEA: 1×10^{-4} to 1×10^{-3} M, EDTA: 1×10^{-4} to 1×10^{-3} M. [b] From ref 1. [c] Identical to the data reported in ref 21. [d] Identical to the triplet quenching data reported in ref 22.

the same pH.[21] Our value also agrees with that reported by Tsentalovich et al.,[22] when considering the weighed rate constants of quenching of flavin by the two histidine species. These values are $k_{q1} = 3 \times 10^6$ M^{-1} s^{-1} for the neutral species and $k_{q2} = 2.5 \times 10^8$ M^{-1} s^{-1} for the protonated species and the relative concentrations of the two species at pH 7 are 90% of the neutral and 10% of the protonated species. Inasmuch as the quenching mechanism is the same for both the protonated and neutral histidine species, albeit with different rate constants, the most important consideration is that there was enough total histidine to quench >95% of ^{3}F.

By applying the two-temperatures (TT) method, deconvolution of the signals for the sample at $T_{\beta=0}$ and at $T_{\beta\neq0} = 5$ °C (with the exception of His for which $T_{\beta\neq0} = 7$ °C) with those for the reference at $T_{\beta\neq0}$ and eqs 4 and 5 afforded the values of α_T and $\Delta_T V$, and α_{FR} and $\Delta_{FR}V$ from φ_1 and φ_2, respectively (Table 2),[8]

$$\Phi_i \Delta_i V = \phi_i|_{T_{\beta=0}} E_\lambda \left(\frac{\beta}{c_p \rho}\right)_{T_{\beta>0}} \qquad (4)$$

$$\alpha_i = \phi_i|_{T_{\beta>0}} - \phi_i|_{T_{\beta=0}} \qquad (5)$$

With α_T and α_{FR}, the energy content of the triplet, E_T, and $\Delta_{FR}H$ are obtained, respectively. On the basis of simple energy balance considerations, the fraction of heat released during the production of ^{3}F, $\alpha_1 = \alpha_T$ (eq 5, Table 2) is related by eq 6 to E_T, Φ_T, and the flavin fluorescence quantum yield, Φ_F,

$$E_T = \left(1 - \alpha_T - \Phi_F \frac{E_F}{E_\lambda}\right) \frac{E_\lambda}{\Phi_T} \qquad (6)$$

where E_F is the average energy of the fluorescence emission (221 and 222 kJ mol^{-1} for FMN and FAD, respectively), $\Phi_F{}^{FMN} = 0.26$[23] and $\Phi_F{}^{FAD} = 0.03$,[24] $\Phi_T(\text{FMN}) = 0.62 \pm 0.02$ and $\Phi_T(\text{FAD}) = 0.63 \pm 0.01$, and $E_\lambda = 265.75$ kJ mol^{-1}.

With the average $\Phi_T(\text{FMN}) = 0.62 \pm 0.02$, $E_T(\text{FMN}) = (200 \pm 50)$ kJ mol^{-1} is obtained with eq 6, similar to the value determined in our previous paper (196 ± 120 kJ mol^{-1})[1] and by Losi et. al. using the same method (203 ± 34 kJ mol^{-1})[25] as well as to the value obtained by phosphorescence spectroscopy at 77 K (ca. 180 kJ mol^{-1}).[26] Similarly, with $\Phi_T(\text{FAD}) = 0.63 \pm 0.01$, $E_T(\text{FAD}) = 205 \pm 50$ kJ mol^{-1} was obtained. The use of average values for Φ_T and E_T permits decreasing the error of $\Delta_T V$ and of $\Delta_2 H = \Delta_{FR}H$.

In the presence of the five salts examined, the structural volume change for ^{3}F formation, $\Delta_1 V = \Delta_T V$, is small; the average values are $\Delta_T V(\text{FMN}) = -1.2 \pm 0.2$ mL mol^{-1} and $\Delta_T V(\text{FAD}) = -0.8 \pm 0.2$ mL mol^{-1}. The energy levels of the FMN and FAD state (singlet and triplet) should not change by the addition of salts.[1]

The enthalpy change associated with the electron-transfer reaction between both ^{3}FMN and ^{3}FAD and the various quenchers and the charge separation, protonation, and deprotonation steps (vide infra), $\Delta_2 H = \Delta_{FR}H$, is related by eq 7 to $\alpha_2 = \alpha_{FR}$ (Table 2), associated with the second decay, when considering the processes consecutive.

$$\Delta_{FR}H = E_T\{[\Phi_T/\Phi_{FR}] - 1\} - \alpha_{FR}E_\lambda/\Phi_{FR} \qquad (7)$$

In the presence of the appropriate concentration of donor, at least 95% of quenching occurs (vide supra) and the Φ_{FR} values are collected in Table 1.

$\Delta_2 V = \Delta_{FR}V$ and $\Delta_{FR}H$ changed in the different salts (Tables 5 and 6), and linearly correlated with each other (Figures 2 and 3). The slope of the correlation, however, was different for each electron donor. Data obtained from the application of the TT method and the three additional quenchers (EDTA, His, and TEA) to those previously used[1] are shown for FMN in Figure 2, whereas data obtained by the TT as well as the ST method for FAD and the five quenchers are in Figure 3. The two methods yield again in these cases the same results.

The values of the temperature-dependent τ_2, i.e., the time constant for the free-radical appearance upon electron-transfer quenching of ^{3}F were plotted using the semiclassical Marcus eq 8[27] (Figure 4). In eq 8, T is the absolute temperature, k_B is

$$\ln(k_q T^{1/2}) = \ln A - \Delta G^\ddagger/(k_B T) \qquad (8)$$

the Boltzmann constant, and $\Delta G^\ddagger$ is the Gibbs activation energy. The use of eq 8 is justified by the fact that the quenching constants for the five quenchers used are below the diffusional limit. A calculation for Trp and Tyr (the two more efficient quenchers) using Smoluchovski theory and Einstein and Stokes relations,[28] and the molecular radii for FMN (10 Å), Trp (2.4 Å),[29] and Tyr (2 Å) affords a value of $k_{diff} = 1.3 \times 10^{10}$ M^{-1} s^{-1} for both quenchers, higher than the experimental values (Table 3). The other quenching constants are all much lower than the possible diffusional limit.

The slope of a representation following eq 8 affords $\Delta G^\ddagger$, which, together with Marcus eq 9 for electron transfer, yields

TABLE 5: Structural Volume and Enthalpy Change for Free-Radical Formation, $\Delta_{FR}V$ and $\Delta_{FR}H$, Respectively, upon Reaction between ^{3}FMN and His, TEA, and EDTA[a]

cation	$\Delta S°$(cat)/ (J mol^{-1} K^{-1})	$\Delta_{FR}V$(His)/ (cm^3 mol^{-1}) ± 0.3	$\Delta_{FR}H$(His)/ (kJ mol^{-1}) ± 20	$\Delta_{FR}V$(TEA)/ (cm^3 mol^{-1}) ± 0.3	$\Delta_{FR}H$(TEA)/ (kJ mol^{-1}) ± 20	$\Delta_{FR}V$(EDTA)/ (cm^3 mol^{-1}) ± 0.3	$\Delta_{FR}H$(EDTA)/ (kJ mol^{-1}) ± 20
$[CH_3(CH_2)_3]_4N^+$	−125	2.8	−9	2.5	−30	0.7	−78
Li^+	−96	2.6	−19	1.8	−51	0.4	−81
$NH_4{}^+$	5	2.0	−49	1.4	−89	0.3	−106
K^+	20	1.3	−61	0.4	−106	0.2	−121
Cs^+	34	1.1	−90	0.1	−125	−0.1	−151

[a] Data derived from the TT method in LIOAS. $\Delta S°$(cat) is the tabulated water organization entropy.[30] For the values associated with the reaction of ^{3}FMN with Trp and Tyr see ref 1.

TABLE 6: Structural Volume and Enthalpy Change for Free-Radical Formation, $\Delta_{FR}V$ and $\Delta_{FR}H$, Respectively, upon Excitation of FAD in the Presence of Tyr, His, Trp, TEA, and EDTA[a]

cation	$\Delta S°$(cat)/ ($\text{J mol}^{-1}\text{ K}^{-1}$)	$\Delta_{FR}V$(His)/ ($\text{cm}^3\text{ mol}^{-1}$) ± 0.2		$\Delta_{FR}H$(His)/ (kJ mol^{-1}) ± 30		$\Delta_{FR}V$(Tyr)/ ($\text{cm}^3\text{ mol}^{-1}$) ± 0.2	$\Delta_{FR}H$(Tyr)/ (kJ mol^{-1}) ± 20	$\Delta_{FR}V$(Trp)/ ($\text{cm}^3\text{ mol}^{-1}$) ± 0.2	$\Delta_{FR}H$(Trp)/ (kJ mol−1) ± 22	$\Delta_{FR}V$(TEA)/ ($\text{cm}^3\text{ mol}^{-1}$) ± 0.2	$\Delta_{FR}H$(TEA)/ (kJ mol^{-1}) ± 22	$\Delta_{FR}V$(EDTA)/ ($\text{cm}^3\text{ mol}^{-1}$) ± 0.2		$\Delta_{FR}H$(EDTA)/ (kJ mol^{-1}) ± 25	
		TT	ST	TT	ST							TT	ST	TT	ST
$[CH_3(CH_2)_3]_4N^+$	−125	1.0	1.1	−12	−91	0.6	−19	0.4	−15	2.1	−33	0.6	0.7	−87	−91
Li^+	−96	0.9	0.9	−55	−105	0.4	−26	0.2	−42	1.5	−57	0.4	0.5	−90	−110
NH_4^+	5	0.4	0.5	−84	−124	0.3	−50	0.1	−72	1.2	−99	0.3	0.4	−117	−120
K^+	20	0.24	0.4	−120	−132	0.2	−73	0.1	−82	0.4	−118	0.1	0.2	−134	−130
Cs^+	34	0.23	0.3	−130	−144	0.1	−126	−0.1	−99	0.1	−139	−0.1	0.1	−168	−149

[a] Data derived with the ST and the TT method in LIOAS. $\Delta S°$(cat) is the tabulated water organization entropy.[30]

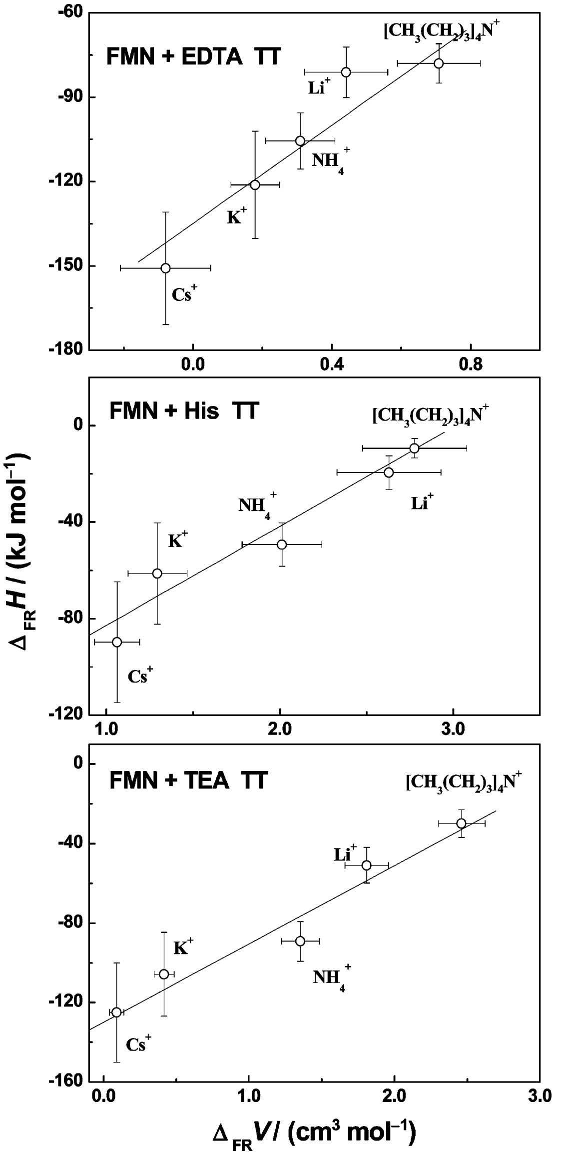

Figure 2. Enthalpy change vs structural volume change (LIOAS data, Table 5) for the formation of the free radicals ($\Delta_{FR}H$ and $\Delta_{FR}V$, respectively) upon electron-transfer quenching of ^{3}FMN by the various electron donors as indicated. The TT method in LIOAS was used. The error bars represent 67% confidence (one standard deviation).

the value of the reorganization energy λ. From the two possible

$$\Delta G^{\ddagger} = (\lambda + \Delta_{ET}G°)^2/4\lambda \qquad (9)$$

solutions to eq 9, the λ values were chosen such that $\Delta_{ET}G° < -\lambda$, because the reactions studied are in the normal Marcus region, i.e., where the reactions are activated and follow a normal Arrhenius behavior.

Discussion

Similar to the case discussed for the quenching of ^{3}FMN by Trp and Tyr,[1] all quenchers used in this publication, amino acids and amines (AH), react with the photoproduced triplet states (^{3}F) by a sequence of reactions leading to the formation of the free radicals $A^•$ and $FH^•$.[15,16,17] This is also the case for the protonated and deprotonated histidine.[22]

Similar to our previous report,[1] we find that the $\Delta_{FR}V$ values for the formation of $A^•$ and $FH^•$ in the five salts linearly correlate with the respective entropy change introduced by the cations on the water network structure, $\Delta S°$(cat)[30] (Figure 5A,B).The slopes

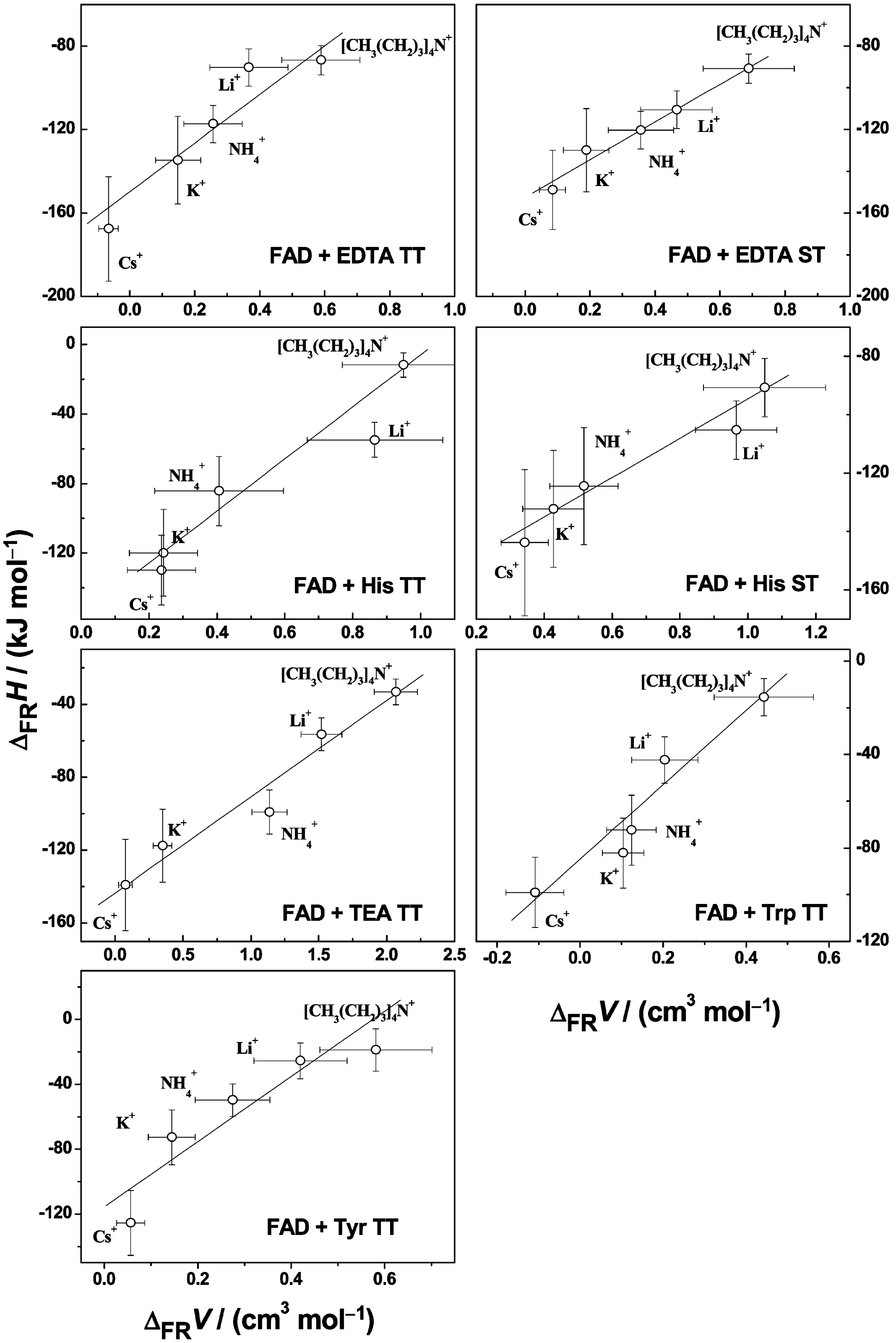

Figure 3. Enthalpy change vs structural volume change (LIOAS data, Table 6) for the formation of the free radicals ($\Delta_{FR}H$ vs $\Delta_{FR}V$, respectively) upon electron-transfer quenching of ^{3}FAD by the various electron donors as indicated. For His and EDTA the results of the ST and TT methods are separately plotted for reasons of clarity. The error bars represent 67% confidence (one standard deviation).

of $\Delta_{FR}V$ vs $\Delta S°$(cat)[30] present a good correlation, r^2, for all experiments. $\Delta_{FR}V$ increases as the structure-making properties of the salts increase, i.e., for more negative values of $\Delta S°$(cat).

The values of $\Delta_{FR}H$ are again larger when the reaction occurs in the presence of hydrogen bond organizing cations, e.g., $[CH_3(CH_2)_3]_4N^+$. and become smaller for structure breaking cations such Cs^+ (Figure 4). Essentially, the ions stabilization by solvation upon electron-transfer senses the water structure because the cation influences the number of hydrogen bonds perturbed upon formation of $A^•$ and $FH^•$.

The plots shown in Figures 2 and 3 are empirically described by

$$\Delta_{FR}H = C + X\Delta_{FR}V \tag{10}$$

which implies

$$C + X\Delta_{FR}V = \Delta_{FR}G + T\Delta_{FR}S \tag{11}$$

The respective value of Φ_{FR} upon ^{3}F quenching by amino acids and amines is constant for each quencher in the five

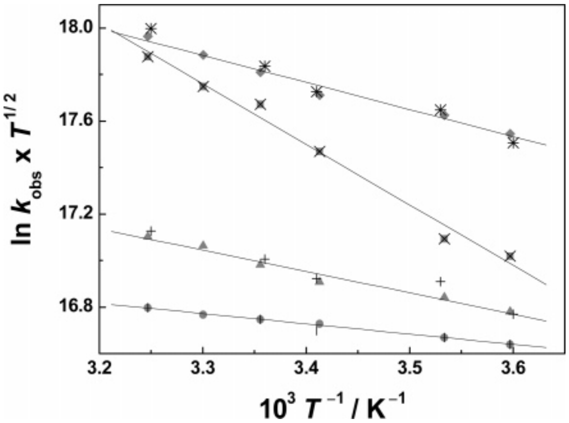

Figure 4. Modified Arrhenius plots (eq 8) for the quenching rate constant by Trp (▲),Tyr (◆), TEA (●), and EDTA (■) of ^{3}FMN, as well as for Trp (+), Tyr (∗), TEA (|), and EDTA (×) of ^{3}FAD.

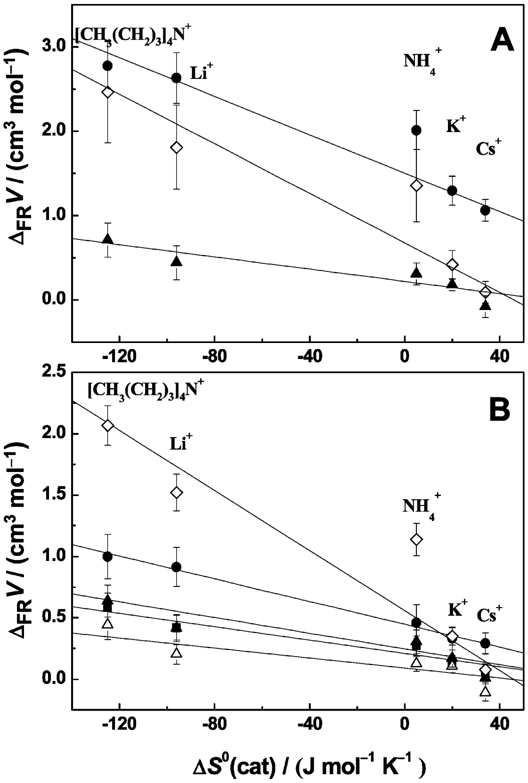

Figure 5. Structural volume change, $\Delta_{FR}V$, associated with the formation of free radicals after electron transfer from (●) His, (▲) EDTA, (◇) TEA, (■) Tyr, and (△) Trp to (A) ^{3}FMN and (B) ^{3}FAD, as a function of the structural standard-entropy change of the cations, $\Delta S°$(cat).[30] The data for the systems Trp-^{3}FMN and Tyr-^{3}FMN are in ref 1.

buffers. Then, it is possible to assume that the respective $\Delta_{FR}G$ value is invariant (see also ref 31).

Should $\Delta_{FR}G$ be constant (=C) in the five salts (as it seems to be, vide infra), eq 12 would apply:

$$X\Delta_{FR}V = T\Delta_{FR}S \qquad (12)$$

With the literature values for the standard electrode potentials $E°(FMN/FMN^-) = -238$ mV (pH 7),[32] $E°(FAD/FAD^-) = -238$ mV (pH 7),[33] $E°(Trp/Trp^-) = 1015$ mV, $E°(Tyr/Tyr^-) = 930$ mV,[34] $E°(His/His^-) = 1170$ mV,[35] $E°(TEA/TEA^-) = 820$ mV,[36] and $E°(EDTA/EDTA^-) = 85$ mV[37] (all vs SHE), as well as $E_T(FMN) = 200$ kJ mol^{-1} (average using also our previous values),[1] $E_T(FAD) = 205$ kJ mol^{-1}, and neglecting the Coulomb term, the calculated values for the Gibbs energies (Table 7) for the electron-transfer process are remarkably similar to the ordinate of the respective plot in Figures 2 and 3, with the exception of His. We have already outlined the problem of the presence of the two His species at pH 7 (vide supra).

Thus, we observe again in almost all cases that the Gibbs energy for the formation of the separated charges is very similar to that for the electron-transfer process. Most probably, the Coulombic effect compensates the stabilization of the separated charges, though this might not be the case for His.

The above similarity between $\Delta_{ET}G°$ and C strongly supports the use of eq 12. This conclusion is again underlined by the linear correlation between the values of $\Delta_{FR}V$ for the quenching by each AH in the presence of the five cations and the tabulated $\Delta S°$(cat) (Figure 5). Similar correlations have already been observed for the photoinduced intramolecular electron-transfer reaction of various Ru(II) bipyridine cyano complexes.[38] Clark and Hoffman[39] reported related correlations between the electron-transfer quenching constant of the excited states of ruthenium(II) diimine complexes and the tabulated Gibbs hydration energy for the counterions present.[30] Essentially, this means that the organization of the medium (perturbed by the counterions in the case of aqueous solutions) influences the values of the entropic and enthalpic terms.

Thus, the entropic term $T\Delta_{FR}S$ depends on the cation present. In other words, in the presence of water-organizing cations, the (very fast) reorganization of the solvent around the newly formed charged species drives the effective separation of the radical ions out of the cage.

Inasmuch as the $\Delta_{FR}V$ values and the Marcus λ values contain contributions from intrinsic changes and interactions with the solvent, there should be a relationship between both values. Taking into account the strong effect of the cations on $\Delta_{FR}H$

TABLE 7: Calculated Gibbs Energies for the Electron Transfer, $\Delta_{ET}G°$, C, and X Values Obtained from Figures 2 and 3 (Eq 3), Experimental Reorganization Energies, λ (eq 9), and Calculated outer-sphere Reorganization Energies, λ_{out}[27]

system	$\Delta_{ET}G°$/ kJ mol^{-1}	C/ kJ mol^{-1}	$X = T\,\Delta_{FR}S/\Delta_{FR}V$/ kJ cm^3	λ/ kJ mol^{-1} measured	λ_{out}/ kJ mol^{-1} calculated
^{3}FMN + Tyr	−102	−112 ± 27	83 ± 21	215	145
^{3}FMN + Trp	−89	−85 ± 14	55 ± 14	183	128
^{3}FMN + TEA	−115	−130 ± 21	38 ± 10	152	79
^{3}FMN + EDTA	−165	−135 ± 15	77 ± 19	203	57
^{3}FMN + His	−82	−124 ± 30	41 ± 10	160	134
^{3}FAD + Tyr	−110	−103 ± 14	107 ± 27	215	145
^{3}FAD + Trp	−95	−85 ± 9	67 ± 17	183	128
^{3}FAD + EDTA	−171	−150 ± 30	85 ± 27	204	57
^{3}FAD + TEA	−121	−143 ± 30	42 ± 20	156	79
^{3}FAD + His	−88	−140 ± 40	50 ± 37	160	134

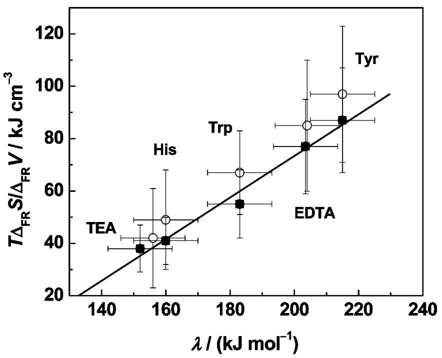

Figure 6. Marcus reorganization energy λ for the respective reaction, derived from plots as those in Figure 4 vs the slopes of the plots as derived from Figures 2 and 3 for (○) quenching of ^{3}FMN, (●) quenching of ^{3}FAD.

and $\Delta_{FR}V$ for the free-radical formation, the solvent should make a large contribution (vide infra).

The representation of λ derived from the plots in Figure 4 vs the slopes of the plots shown in Figures 2 and 3, i.e., $X = T\Delta_{FR}S/\Delta_{FR}V$ (eq 12), yields a straight line (Figure 6). In other words, λ for each of the five quenchers interacting with the two triplet electron donors correlates linearly with the respective value of the entropic term normalized by the reaction volume change for the formation of the free radicals.

The X vs λ correlation offers an experimental approach for the determination of λ without resorting to measurements in an extended temperature range as generally required for the application of eq 8.

The explanation of the linearity found between $X = T\Delta_{FR}S/\Delta_{FR}V$ (Figure 6) and λ does not seem to be straightforward. So far, it is an empirical observation which cannot be simply explained with the equations at hand such as eqs 9 and 11. We have some evidence indicating that this relationship includes also other systems undergoing a photoinduced electron-transfer reaction and extend the λ range shown in Figure 6.

The calculation of the solvent-reorganization contribution (λ_{out}) to the λ value using the equation derived by Marcus on the basis of the continuum model[2,39] affords the values listed in Table 7. Radii $r = 10$ Å for FMN,[40] 2 Å for Tyr, 2.40 Å for Trp, 2.31 Å for His,[29] 3.94 Å for TEA,[41] and 5.5 Å for EDTA[41] were used for the calculation. The calculation for the systems involving ^{3}FAD yields the same values because we assume that the approach of the two partners is through the aromatic ring side. The strong similarity in quenching rate constants for both triplet states supports this assumption. The radius for the complex was assumed to be the sum of the radii of the individual partners. The refractive index and the permittivity were assumed to be those of neat water at 25 °C. The calculated λ_{out} values are systematically smaller than those measured. Using the permittivity and the refractive index calculated for the salt solutions[42] made <1% difference in the calculated λ_{out} values.

Thus, there is an important contribution of the solvent-reorganization energy to the total λ value, albeit much smaller for the aliphatic amines (30% for EDTA and 50% for TEA) than for the amino acids used (70−80%). Undoubtely, the mechanism for the production of free radicals by electron-transfer quenching of the flavins is different for the amines than for the three amino acids as quenchers. This effect has already been noticed by previous authors, as discussed by Porcal et al.[43] The different behavior can also be visualized by a representation of log k_q vs $\Delta_{ET}G°$, which results in a plot with Trp, Tyr, and His nicely arranged and EDTA and TEA out of the correlation (plot not shown, data in Tables 4 and 7).

Notwithstanding the different mechanism, the structural value X, as a measure of the final changes in the intrinsic as well as solvent rearrangements, correlates with the total λ value (Figure 6) for all pairs studied.

Conclusions

We have found a strong correlation between the structural volume change-normalized entropic change, $X = T\Delta_{FR}S/\Delta_{FR}V$, and Marcus reorganization energy λ for the formation of free radicals upon electron transfer from amino acids and aliphatic amines to the triplet states of flavins. The value of X was derived from laser-induced optoacoustic studies in a series of cations for each of the electron-transfer pairs. Again in this paper we find that the entropic term for electron-transfer reactions taking place in hundreds of nanoseconds in aqueous medium is by no means negligible. The correlation between X and λ permits the derivation of λ using a relatively small temperature range. This should prove useful in the analysis of electron-transfer reactions in proteins, with which often a large temperature variation is not possible.

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