ORIGINAL PAPER

Induced Intersystem Crossing at the Fluorescence Quenching of Laser Dye 7-Amino-1,3-Naphthalenedisulfonic Acid by Paramagnetic Metal Ions

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Abstract The fluorescence and triplet state quenching of 7-amino-1,3-naphthalenesulfonic acid by paramagnetic metal ions have been investigated in an aqueous medium. The basic mechanism of the fluorescence quenching involves the static and dynamic electron transfer to the paramagnetic cation. The induced $S_1 \rightarrow T_1$ intersystem crossing at fluorescence quenching of the fluorophore by Cu^{2+} cation has been found. There is a correlation between triplet state quenching rate constants and values of the efficient paramagnetic susceptibility and spin of the cations. The rate constants for the quenching pathways have been calculated.

Keywords Fluorescence quenching · Paramagnetic metal ion · Amino-G-acid · Triplet-triplet absorption

Introduction

The fluorescence quenching of organic compounds by heavy atoms and paramagnetic metal ions is a well known phenomenon [1]. Nowadays it is generally concluded that the heavy-atom effect consists in the increase of the intersystem crossing efficiency due to the enhancement of spin-orbit interactions in a fluorophore molecule. The numerous publications are dedicated to this sort of quenching [2–7]. The fluorescence quenching by paramagnetic metal ions is far less studied. The major part of the publications deals with the intramolecular quenching of singlets and triplets of various lanthanide metallocomplexes. The proposed quenching mechanisms differ widely.

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So the significant enhancement of $T_1 \rightarrow S_0$ radiative and nonradiative deactivation constants in the chelates of benzoyltrifluoroacetone with paramagnetic Gd^{3+} ion (in comparison with diamagnetic La^{3+} and Lu^{3+}) ions was explained by the exchange interaction between ligand π-electrons and unpaired 4f electrons in the Gd^{3+} ion [8]. The enhancement of $S_1 \rightarrow T_1$ constant and significant decrease of τ_{fl} in chelates of methyl salicylate with Gd^{3+} ions in comparison with La^{3+} and Lu^{3+} was attributed to the above interaction [9]. The good linear correlation between the values of magnetic moments, τ_{fl}, and also $S_1 \rightarrow T_1$ constants was found for paramagnetic lanthanide(III) texaphyrin complexes. It was also noticed the decrease of ϕ_f, τ_{fl}, and τ_{ph} in paramagnetic vs diamagnetic complexes [10]. Some papers are dedicated to the intramolecular quenching in transition metal complexes. The linear Stern-Volmer fluorescence dependence of three anthrylazacrown ethers and the independence of τ_{fl} from concentration of Mn^{2+}, Co^{2+}, and Cu^{2+} cations at various pH indicated the static quenching mechanism (formation of chelates) [11]. The intersystem crossing by paramagnetic effect was mainly considered between excited ^{1}(Zn-porphyrin) and the [M(edta)] complex, M=Co^{2+}, Ni^{2+}, and Mn^{2+} [12]. There are literary data dealing with the intermolecular quenching by paramagnetic transition and rare-earth metals. In the early work [13] the quenching abilities of the lanthanides parallel with their polarographic reduction potentials in aqueous solutions suggests that quenching occurs through a mechanism involving electron transfer from the excited indole ring to the lanthanide. The fluorescence quenching of isoindole fluorescence probe OPA-Gly by paramagnetic Gd^{3+} ion in borate buffer was attributed to the heavy atom effect [14]. The efficient intersystem crossing in excited encounter complexes of pyrene and Ag^+, Tl^+, Ni^{2+}, Co^{2+}, Tb^{2+}, Sm^{3+}, and Dy^{3+} in sodium dodecyl sulfate micelle solutions was

proposed as the basic quenching pathway [15]. The analogous deactivation channel along with the electron and energy transfer is assumed to present in the quenching mechanism of zinc tetraphenylporphyrin (ZnTPP) by paramagnetic lanthanide(III) ions in MeCN and DMF [16]. A limited number of papers dealing with this field does not allow to make a certain conclusion on the contribution of ion inherent paramagnetism to the fluorescence quenching.

As a rule, to determine the actual fluorescence quenching mechanism it is necessary to monitor not only fluorescence quantum yield but also the triplet state yield of the fluorophore. The combination of these data allows to clarify the influence of ion inherent paramagnetism on $S_1 \rightarrow T_1$ intersystem crossing. In present work we have studied the fluorescence quenching of a model fluorophore—laser dye 7-amino-1,3-naphthalenedisulfonic acid (amino-G-acid) by paramagnetic metal ions and investigated their influence on the $S_1 \rightarrow T_1$ intersystem crossing.

Experimental

The absorption and fluorescence spectra of solutions were recorded on a Shimadzu UV-3100 spectrophotometer and a Perkin-Elmer LS-55 spectrofluorometer. The fluorescence quantum yields were calculated comparing the areas under corrected fluorescence spectra of a fluorophore in bidistilled water and quinine bisulfate in 1N H_2SO_4 (ϕ_f=0.546, [17]). The fluorescence lifetimes were calculated from the corresponding fluorescence kinetics recorded on a nanosecond SP-70 spectrometer (Applied Photophysics) by the time-correlated counting of single photons with excitation by air-filled flash-lamp radiation (excitation pulse duration 0.8 ns, registration channel width 0.0054 ns). The triplet-triplet absorption spectra were recorded on a flash-photolysis apparatus (excitation pulse duration 10 µs). The solutions were previously evacuated by five freeze-pump-thaw cycles to remove the oxygen. The values of optical densities of triplet-triplet absorption were averaged over five wavelengths. A spectroscopically pure 7-amino-1,3-naphthalenedisulfonic acid and reagent-grade salts: copper sulfate ($CuSO_4 \times 5\ H_2O$), nickel sulfate ($NiSO_4 \times 7\ H_2O$), cobalt chloride ($CoCl_2 \times 6\ H_2O$), manganese chloride ($MnCl_2 \times 4\ H_2O$), samarium chloride ($SmCl_3 \times 6\ H_2O$), and cerium nitrate ($Ce(NO_3)_3 \times 6\ H_2O$) were used as received.

Table 1 Fluorescence quantum yield (φ_f), fluorescence lifetime (τ_0), the sum of nonradiative deactivation and $T_1 \leftarrow S_1$ intersystem crossing rate constants $\left(k_d + k_{isc}^0\right)$, nonradiative deactivation rate constant of the triplet state of amino-G-acid $\left(k_d^T\right)$

| φ_f	τ_0, ns	$\left(k_d + k_{isc}^0\right) \times 10^{-7}$, s^{-1}	$k_d^T \times 10^{-3}$, s^{-1}
0.81	16.6	1.14	4.8
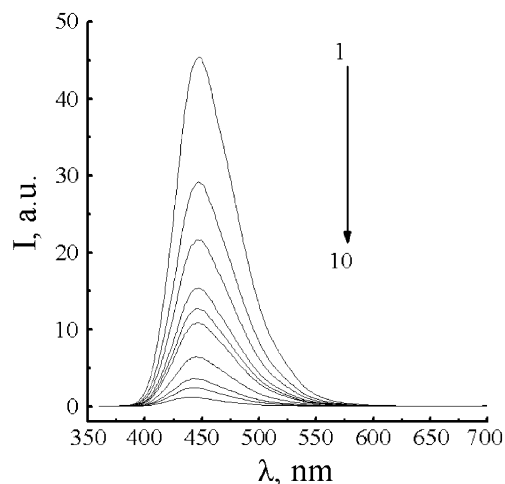
Fig. 1 The fluorescence spectra of amino-G-acid in water at various concentrations of CoCl$_2$: *1* (0); *2* (0.00133); *3* (0.0027); *4* (0.005); *5* (0.0067); *6* (0.0083); *7* (0.0167); *8* (0.0333); *9* (0.05), and *10* (0.1M). λ_{exc}=350 nm

All measurements were carried out in a bidistilled water at 20°C.

Results and discussion

The water-soluble laser dye amino-G-acid was chosen as a fluorophore due to its large intrinsic fluorescence lifetime that gives a possibility to calculate the rate constant of dynamic fluorescence quenching. The photophysical parameters of amino-G-acid are given in Table 1. The paramagnetic ions Cu^{2+}, Ni^{2+}, Co^{2+}, Mn^{2+}, Sm^{3+}, and Ce^{3+} were used as quenchers of the fluorescence of aqueous amino-G-acid solutions. In aqueous solutions the protolysis
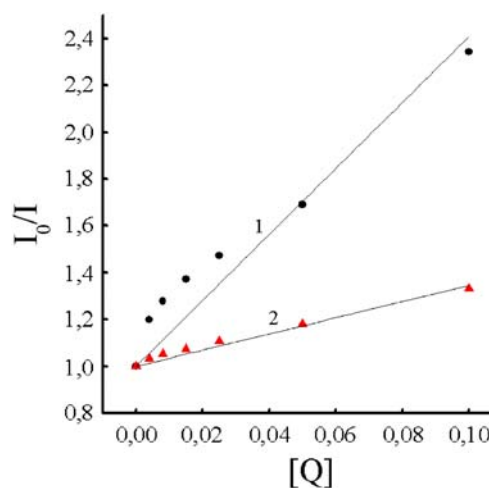
Fig. 2 Stern-Volmer plots for the fluorescence quenching of amino-G-acid by SmCl$_3$ (*1*) and Ce(NO$_3$)$_3$ (*2*) registered at 500 nm; λ_{exc}= 350 nm
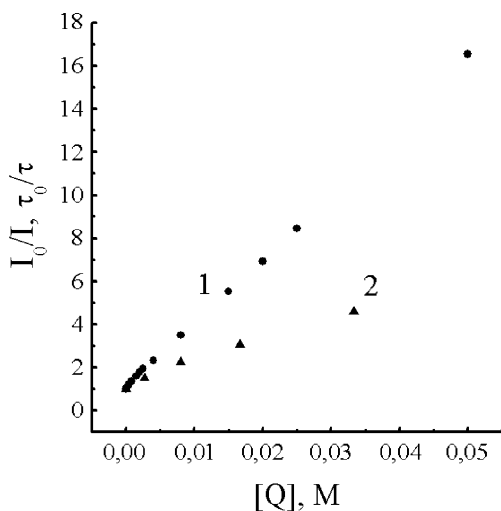
Fig. 3 Stern-Volmer plots for total (I_0/I) (1) and dynamic (τ_0/τ) (2) fluorescence quenching of amino-G-acid by CuSO$_4$ ([Q])

of hydrated cations gives the additional quencher—H$_3$O$^+$ ion. Since at the increasing of salts concentration up to 0.1M the corresponding pH values are in a range 4.2–5.6, we have neglected this very insignificant quenching pathway. At the addition of acetic acid up to pH2 under excitation at the isosbestic point the fluorescence quenching of amino-G-acid was not observed.

The addition of the salts of these cations does not affect the position and shape of the absorption spectrum of a fluorophore. The spectral overlap between corresponding absorption spectra of amino-G-acid and some salts (NiSO$_4$, CuSO$_4$, and SmCl$_3$) at excitation wavelength was taken into account if necessary. The increase of the salt concentration in the solution is accompanied by the fluorescence quenching without any spectral shifts (Fig. 1). Because of the partial overlap of amino-G-acid fluorescence and absorption spectra of some salts, the corresponding fluorescence quenching rate constants were calculated using not values of ϕ_f, but fluorescence intensities at wavelengths, where the overlap is absent. In all cases, except Sm^{3+} and Ce^{3+}, the I_0/I dependence vs [Q] is slightly convex down. The initial section of the dependence for Sm^{3+} and Ce^{3+} is convex up. This convexity vanishes at higher salt concentrations (Fig. 2).

The similar behavior was observed in the studies of the fluorescence quenching of zinc tetraphenylporphyrin (ZnTPP) by trivalent lanthanide ions (Gd^{3+}, Sm^{3+}, and Er^{3+}) in acetonitrile [16]. The fluorescence lifetime of amino-G-acid decreases in parallel to the addition of a quencher. In all cases we observe a linear τ_0/τ dependence vs [Q], proving the dynamic fluorescence quenching. However, the slopes of the lines are less than those obtained for I_0/I - [Q] dependence (Fig. 3). This slopes difference testifies the existence of the static fluorescence quenching pathway. The dynamic fluorescence quenching is characterized by the rate constant (k_q); the static one—by the equilibrium constant (K_{ST}) according to the equations:

$$\frac{I_0}{I} = (1 + k_q \tau_0 [Q])(1 + K_{ST}[Q]) \tag{1}$$
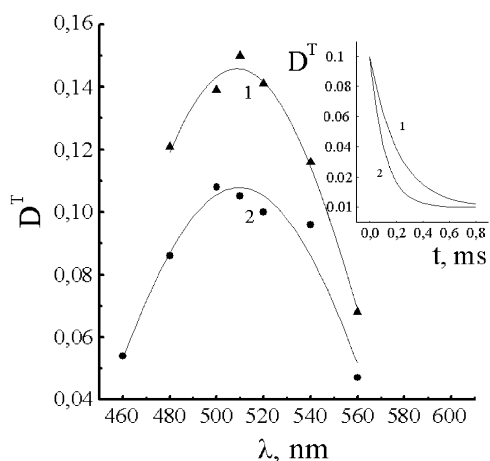
$$\frac{\tau_0}{\tau} = 1 + k_q \tau_0 [Q] \tag{2}$$

$$\frac{I_0 \tau}{I \tau_0} = 1 + K_{ST}[Q] \tag{3}$$

The constants of the static and dynamic fluorescence quenching calculated from Eqs. 2 and 3 are given in Table 2. It is seen that there is no correlation between the fluorescence quenching constants and the values of magnetic susceptibility of ions characterizing their paramagnetism. It can be concluded that the paramagnetic effect of cations used on the fluorescence quenching of amino-G-acid is not determining one. On the other hand, the linear correlation between logarithms of the static and dynamic fluorescence quenching constants and polarographic reduction potentials of paramagnetic ions is observed (Fig. 4). Apparently, the basic fluorescence quenching mechanism of the system consists in the electron transfer from the excited molecule of a fluorophore to the metal ion. The similar conclusion was used at the explanation of the analogous correlation obtained at indole fluorescence quenching by lanthanide ions in aqueous solution [13]. The fluorescence quenching by electron transfer may take place both in the

Table 2 The constants of static (K_{st}) and dynamic (k_q) fluorescence quenching, rate constant of amino-G-acid triplet state quenching (k_q^T) by paramagnetic metal ions, magnetic susceptibility (μ_{eff}) and spin value (S) of cations

The error of k_q measurement ~10%.

| Cation	K_{st}, M^{-1}	$k_q \times 10^{-9}$, M^{-1}s^{-1}	$k_q^T \times 10^{-7}$, M^{-1}s^{-1}	μ_{eff}, mB [18]	S
Cu^{2+}	47.2	6.8	0.014	1.83	1/2
Co^{2+}	24.3	11.2	100	4.97	3/2
Sm^{3+}	6.4	0.54	<0.001	1.5	5/2
Ni^{2+}	0.89	0.43	–	3.11	1
Mn^{2+}	0.2	0.09	260	5.9	5/2
Ce^{3+}	0.13	0.23	–	2.5	1/2

Fig. 4 The triplet-triplet absorption spectra of amino-G-acid in water without a quencher (1) and in the presence of 0.01 M CuSO$_4$ (2). Upper inset: triplet-triplet absorption kinetics registered at 520 nm

singlet excited state and triplet state. However, the efficiency of the process in the latter case is less.

To justify the effect of paramagnetic ions on the intersystem crossing efficiency (S$_1$→T$_1$ and T$_1$→S$_0$) the triplet-triplet absorption spectra of amino-G-acid were registered in the presence and in the absence of a quencher. The flash photolysis experiments were carried out for the solutions containing Cu^{2+}, Co^{2+}, Mn^{2+}, and Sm^{3+} cations (Fig. 5). The Ni^{2+} and Ce^{3+} cations were not studied because of the significant overlap of the absorption spectra of amino-G-acid and NiSO$_4$ and Ce(NO$_3$)$_3$. The data on the quenching of a triplet state of the fluorophore are given in Table 2. It can be seen that there is a qualitative correlation between the quenching rate constant of the triplet state, magnetic susceptibility of cations, and the spin value of the
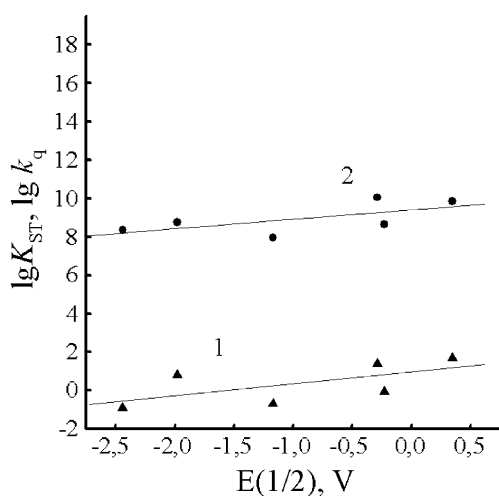
Fig. 5 The dependence of static (1) and dynamic (2) fluorescence quenching constants of amino-G-acid on the polarographic reduction potential, [19]

ion. According to the data obtained we may conclude that the paramagnetism of a quencher affect stronger T$_1$→S$_0$ than S$_1$→T$_1$ intersystem crossing. Since both Co^{2+} and Mn^{2+} ions are efficient quenchers of the triplet state of amino-G-acid, the study of their influence on S$_1$→T$_1$ intersystem crossing was impossible due to the absence of the triplet-triplet absorption already in the presence of negligible concentrations of the quencher.

The interesting results were obtained on the influence of Cu^{2+} ion on S$_1$→T$_1$ intersystem crossing (Table 3). As it is seen from the Table, the ratio of optical densities of triplet-triplet absorption (D^T/D^0) in the presence and in the absence of Cu^{2+} ion exceeds the ratio of fluorescence lifetimes (τ/τ_0) at the same concentration of a quencher. It points out that the efficiency of the S$_1$→T$_1$ intersystem crossing increases at the interaction between the excited molecule of amino-G-acid and paramagnetic Cu^{2+} ion. Let us consider the following scheme including all pathways taking place in the system studied:
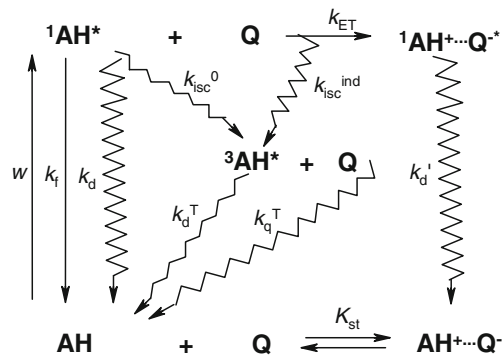
From the scheme we can get Stern-Volmer equation for the fluorescence quenching:

$$\frac{[^1AH_0^*]}{[^1AH^*]} = \left(1 + \left(k_{ET} + k_{isc}^{ind}\right)\tau_0[Q]\right)\left(1 + K_{ST}[Q]\right) \quad (4)$$

Where $k_{ET} + k_{isc}^{ind} = k_q$ is the rate constant of the dynamic fluorescence quenching, K$_{ST}$—equilibrium constant, [Q]—quencher concentration. Considering the triplet state of the fluorophore under photostationary conditions it is easy to

Table 3 Relative fluorescence lifetime (τ/τ_0) and optical density at the maximum of the triplet-triplet absorption spectra (D^T/D^0), induced intersystem crossing rate constant $\left(k_{isc}^{ind}\right)$, and its fraction in the dynamic fluorescence quenching $\left(k_{isc}^{ind}/k_{isc}^{ind} + k_{ET}\right)$ of amino-G-acid at two concentrations of CuSO$_4$ ([Q])

[Q], M	τ/τ_0	D^T/D^0	$k_{isc}^{ind} \times 10^{-9}$, M^{-1}s^{-1}	$\frac{k_{isc}^{ind}}{k_{isc}^{ind}+k_{ET}}$, %
0.01	0.47	0.75	2.3	34
0.03	0.23	0.772	2.1	30

The error of measurements ~10%

obtain the ratio of the singlet and triplet state concentrations:

$$\frac{[^3AH^*][^1AH_0^*]}{[^3AH_0^*][^1AH^*]} = \frac{1 + \frac{k_{isc}^{ind}}{k_{isc}^0}[Q]}{1 + \frac{k_q^T}{k_d^T}[Q]} \qquad (5)$$

Where k_{ET}, k_{isc}^0, k_{isc}^{ind} are the rate constant of dynamic electron transfer, the rate constants of intrinsic and induced intersystem crossing, respectively; k_q^T, k_d^T—rate constants of the quenching and nonradiative deactivation of the triplet state. The Eq. 6 was used for calculations.

$$\frac{[D^T][\tau_0]}{[D_0^T][\tau]} = \frac{1 + \frac{k_{isc}^{ind}}{k_{isc}^0}[Q]}{1 + \frac{k_q^T}{k_d^T}[Q]} \qquad (6)$$

Where D_0^T, τ_0; D^T, τ are the values of optical densities registered at the maximum of triplet-triplet absorption spectra and fluorescence lifetimes in the presence and in the absence of a quencher, respectively. Assuming $k_d = 0$, we get $k_{isc}^0 = 1.14 \times 10^7 \text{s}^{-1}$ and $k_{isc}^{ind} = 2.2 \times 10^9 \text{M}^{-1}\text{s}^{-1}$ (Table 3). Therefore, a fraction of the intersystem crossing component in the dynamic fluorescence quenching of amino-G-acid by Cu^{2+} cation comprises at least 30%.

Thus, paramagnetic ions quench the fluorescence of amino-G-acid. The dynamic and static fluorescence quenching constants do not correlate with magnetic susceptibility of cations, but show a linear correlation with their polarographic reduction potentials. It points out that the basic mechanism of fluorescence quenching is the electron transfer from the excited molecule to the cation. The fluorescence quenching of amino-G-acid by Cu^{2+} cation induces $S_1 \rightarrow T_1$ intersystem crossing. The paramagnetic ions studied quench efficiently the triplet states of amino-G-acid. The corresponding rate constants of triplet quenching rise up as the spin and magnetic susceptibility of a quencher increase.

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