ELSEVIER

Journal of Photochemistry and Photobiology A: Chemistry 110 (1997) 75–78

Journal of PHOTOCHEMISTRY AND PHOTOBIOLOGY A: CHEMISTRY

Fluorescence spectroscopic analysis of indocyanine green J aggregates in water

F. Rotermund, R. Weigand [1], W. Holzer, M. Wittmann [2], A. Penzkofer *

Institut II - Experimentelle und Angewandte Physik, Universität Regensburg, D-93040 Regensburg, Germany

Accepted 15 May 1997

Abstract

Indocyanine green in water forms J aggregates, which are observed as a red-shifted narrow absorption band at 890 nm. The fluorescence quantum distribution of the indocyanine green J aggregates was studied by continuous wave laser excitation at 834 nm. A fluorescence quantum yield of $\phi_F \approx 3 \times 10^{-4}$ was measured. The fluorescence peak coincides with the absorption peak (no Stokes shift). © 1997 Elsevier Science S.A.

Keywords: Fluorescence quantum distribution; Fluorescence quantum yield; Indocyanine green; J aggregates; Stimulated emission cross-section

1. Introduction

Indocyanine green (ICG) dissolved in dimethylsulphoxide (DMSO) is used as an organic near-IR laser dye under the name IR-125 [1–3] (laser tuning range, 890–960 nm). The fluorescence quantum yield of IR-125 in DMSO is $\phi_F \approx 0.11$ [4]. The sodium iodide salt of ICG (ICG-NaI) in aqueous solution is widely used in medical diagnosis [5,6], and has potential applications in photodynamic therapy [7,8].

At high concentrations ($C \gtrsim 10^{-3}$ mol dm^{-3}), ICG in water forms a J aggregate-like [9,10], red-shifted narrow absorption band at 890 nm after about 2 weeks [11,12]. The formation of J aggregates is enhanced considerably at elevated temperature [13]. The ICG-NaI J aggregate formation and absorption behaviour have been studied previously [13].

J aggregation is a familiar phenomenon of various concentrated aqueous cyanine dye solutions [9,10,14,15], and has been most widely studied for 1,1′-diethyl-2,2′-quinocyanine halides (pseudo-isocyanine halides) [16–21]. For pseudo-isocyanine halide J aggregates, high resonance fluorescence quantum yields have been reported [17,18,22–25] and a strong excitation intensity-dependent fluorescence quenching is observed [17,23,25].

In this paper, the fluorescence behaviour of ICG-NaI J aggregates is studied. A very weak fluorescence emission is observed, which has no Stokes shift between the absorption and emission peaks. A fluorescence quantum yield of $\phi_F \approx 3 \times 10^{-4}$ is determined.

2. Experimental details

The dye ICG-NaI was purchased from Pulsion Medizintechnik, Munich [26]. It was used without further purification. The structural formula of ICG-NaI is shown in Fig. 1(b).

The J aggregates were formed by preparing a 1.5×10^{-3} M aqueous solution of ICG-NaI and heating it to 65 °C for a period of 32 h. The solution was then stored at room temperature. The J aggregates formed are very stable. They remain unchanged over several months. Before fluorescence measurements, the samples were diluted to a concentration of 3×10^{-5} mol dm^{-3}. At this concentration, the J aggregates formed remain stable over about 1 day before they detach to monomers, dimers and small oligomers [13].

The fluorescence quantum yield measurements were carried out using a self-assembled fluorometer [27]. The fluorescence signal in the backward direction was collected (front face illumination technique). An argon ion laser-pumped continuous wave Ti:sapphire laser was used for excitation.

* Corresponding author. Tel.: +49 941 9432107; fax: +49 941 9432754; e-mail: alfons.penzkofer@physik.uni-regensburg.de

[1] On leave from the Departamento de Optica, Facultad de Ciencias Fisicas, Universidad Complutense, 28040 Madrid, Spain.

[2] Present address: Max-Born-Institut, Rudower Chaussee 6, D-12489 Berlin-Adlershof, Germany.

PII S1010-6030(97)00167-6

The laser wavelength was set to $\lambda_{exc} = 834$ nm. The laser pump power was $P_{exc} = 10$ mW and the cross-sectional area was approximately 0.1 mm^2, giving an excitation photon flux density of 4×10^{19} cm^{-2} s^{-1}. The excitation laser was vertically polarized. A polarizer sheet was placed in the fluorescence path to pass either vertically or horizontally polarized fluorescence light.

The excitation wavelength had to be shifted to the short-wavelength shoulder of the J aggregate absorption band because the fluorescence emission peak coincides with the absorption peak, the fluorescence quantum yield is very weak and the sample scatters strongly [28].

For the absolute fluorescence quantum distribution and quantum yield measurements, the Rayleigh scattering of Ludox PG (aqueous colloidal suspension of silicon dioxide (particle diameter, 22 nm) from DuPont [29]) was used as reference. The Rayleigh scattering of Ludox PG at 543 nm (green line of an He–Ne laser) was calibrated to the fluorescence emission of rhodamine 101 in methanol (fluorescence quantum yield $\phi_F = 0.98$ [30]). The wavelength dependence of the Rayleigh scattering ($S(\lambda) \propto \lambda^{-4}$; S, scattering signal; λ, wavelength [31,32]) was taken into account. In the data analysis, the fluorescence re-absorption in the absorption region was accounted for by following the procedure described in Ref. [27]. The scattered light in the fluorescence spectral region was subtracted by comparison with the Rayleigh scattering signals of the Ludox PG sample.

3. Results

In Fig. 1(a), the parallel polarized fluorescence quantum distribution contribution ($E_{F,\parallel}(\lambda)$) (proportional to the fluorescence signal $S_{F,\parallel}(\lambda)$ polarized parallel to the excitation light) is displayed. In Fig. 1(b), the perpendicular polarized fluorescence quantum distribution contribution ($E_{F,\perp}(\lambda)$) (proportional to the fluorescence signal $S_{F,\perp}(\lambda)$ polarized perpendicular to the excitation light) is shown. The fluorescence quantum distribution, $E_F(\lambda) = [E_{F,\parallel}(\lambda) + 2E_{F,\perp}(\lambda)]/3$, is depicted by the broken curve in Fig. 2(a). The fluorescence quantum yield is given by $\phi_F = \int_{em} E_F(\lambda) d\lambda$ (integration of $E_F(\lambda)$ over S_1–S_0 fluorescence emission wavelength region). The value obtained is $\phi_F = (3 \pm 1) \times 10^{-4}$.

The absorption cross-section spectrum, $\sigma_{a,J}(\lambda) = -\ln(T)/N_m l$, of the ICG-NaI J aggregates in water is shown in Fig. 2(a) (from Ref. [13]). $\sigma_{a,J}(\lambda)$ is the absorption cross-section per molecule in the aggregate, T is the transmission, N_m is the molecule number density and l is the sample length. It can be seen that the fluorescence emission peak coincides with the absorption cross-section peak. There is no Stokes shift between the absorption peak and the emission peak. However, it should be noted that excitation occurs at $\lambda_{exc} = 834$ nm and the fluorescence emission peak is observed at $\lambda_{F,max} = 890$ nm. The coincidence of the absorption peak and the emission peak and the narrow absorption and emission

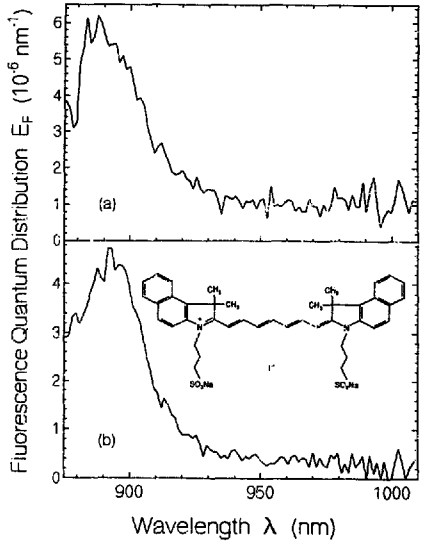

Fig. 1. (a) Fluorescence quantum distribution ($E_{F,\parallel}(\lambda)$) of emission polarized parallel to excitation light. (b) Fluorescence quantum distribution ($E_{F,\perp}(\lambda)$) of emission polarized perpendicular to excitation light. Structural formula of ICG-NaI is included. The fluorescence is excited with continuous wave laser light at 834 nm.

linewidths indicate the Frenkel excitonic nature of the J band [33,34]. The shoulder in the absorption spectrum at 670 nm is thought to be due to an impurity, which is observed as a fluorescence peak at 690 nm when excited below 680 nm [35].

The radiative S_1–S_0 exciton lifetime τ_{rad} can be calculated using the Strickler–Berg formula [36,37]

$$\frac{1}{\tau_{rad}} = \frac{8\pi c_0 n_F^3}{n_A} \frac{\int_{em} E_F(\lambda) d\lambda}{\int_{em} E_F(\lambda) \lambda^3 d\lambda^{abs}} \int \frac{\sigma_{a,chr}(\lambda)}{\lambda} d\lambda \qquad (1)$$

where c_0 is the speed of light in vacuum and n_F and n_A are the average refractive indices of the dye solution in the fluorescence region and absorption region respectively. The integrals extend over the $S_1 \rightarrow S_0$ exciton fluorescence band (em) and over the $S_0 \rightarrow S_1$ exciton absorption band (abs). $\sigma_{a,chr}(\lambda) = n_{chr}\sigma_{a,J}(\lambda)$ is the absorption cross-section of the chromophore (absorbing entity, coherent extension of Frenkel exciton). n_{chr} is the number of molecules forming a chromophore. In saturable absorption studies [38], we found for the ICG-NaI J aggregates a value of $n_{chr} = 16 \pm 8$. Our analysis gives $\tau_{rad} = 5.5 \pm 0.5$ ns$/n_{chr} \approx 340 \pm 100$ ps.

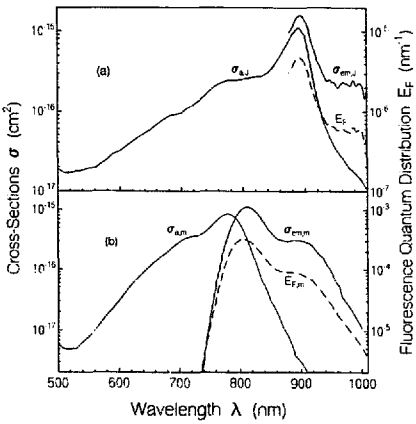

Fig. 2. (a) Fluorescence quantum distribution $E_F(\lambda)$, stimulated emission cross-section spectrum $\sigma_{em,J}(\lambda)$ and ground state absorption cross-section spectrum $\sigma_{a,J}(\lambda)$ of ICG-NaI J aggregates in water. $\sigma_{em,J}$ and $\sigma_{a,J}$ are the cross-sections per molecule. (b) Fluorescence quantum distribution $E_{F,m}(\lambda)$, stimulated emission cross-section spectrum $\sigma_{em,m}(\lambda)$ and ground state absorption cross-section spectrum $\sigma_{a,m}(\lambda)$ of ICG-NaI monomers in water (from Ref. [35]).

The fluorescence lifetime τ_F is given by $\tau_F = \phi_F \tau_{rad}$. Using the determined values of ϕ_F and τ_{rad}, we find a fluorescence lifetime of 1.7 ± 0.7 ps/$n_{chr} = 110 \pm 60$ fs.

The stimulated emission cross-section spectrum per chromophore, $\sigma_{em,chr}(\lambda) = n_{chr}\sigma_{em,J}(\lambda)$, where $\sigma_{em,J}(\lambda)$ is the stimulated emission cross-section per molecule, is derived from the fluorescence quantum distribution and the fluorescence lifetime by [39]

$$\sigma_{em,chr}(\lambda) = \frac{\lambda^2 E_F(\lambda)}{8\pi c_0 n_F^2 \tau_F} \tag{2}$$

The stimulated emission cross-section spectrum per molecule, $\sigma_{em,J}(\lambda)$, is included in Fig. 2(a).

The degree of fluorescence polarization P_F [40] is calculated from $E_{F,\parallel}(\lambda)$ and $E_{F,\perp}(\lambda)$ using the relation

$$P_F = \frac{\int_{em} E_{F,\parallel}(\lambda)\mathrm{d}\lambda - \int_{em} E_{F,\perp}(\lambda)\mathrm{d}\lambda}{\int_{em} E_{F,\parallel}(\lambda)\mathrm{d}\lambda + \int_{em} E_{F,\perp}(\lambda)\mathrm{d}\lambda} \tag{3}$$

Using the $E_{F,\parallel}(\lambda)$ and $E_{F,\perp}(\lambda)$ curves of Figs. 1(a) and 1(b), we obtain $P_F = 0.24 \pm 0.05$. From the degree of fluorescence polarization, the reorientation time of the S_1–S_0 exciton transition dipole moments may be estimated using the relation [41–43]

$$\tau_{or} = \frac{1/P_0 - 1/3}{1 - P_F/P_0} P_F \tau_F \tag{4}$$

where $P_0 = 0.5$. A value of $\tau_{or} = 1.3 \pm 0.5$ ps/$n_{chr} = 90 \pm 50$ fs is calculated. The short reorientation time indicates a fast excitation energy transfer in the J aggregates [44].

4. Discussion

The determined fluorescence spectroscopic parameters of ICG-NaI in water are collected in Table 1. For comparison with the J aggregate absorption and emission spectra (Fig. 2(a)), the monomer absorption and emission cross-section spectra and monomer fluorescence quantum distribution are displayed in Fig. 2(b) (from Ref. [35]).

The fluorescence quantum yield of $\phi_F \approx 3 \times 10^{-4}$ is very low. For monomeric ICG-NaI in water (concentration $C = 10^{-7}$ mol dm^{-3}), a fluorescence quantum yield of $\phi_F = 0.02$ was reported [35], whereas for oligomeric ICG-NaI in water (freshly prepared solution, $C = 10^{-2}$ mol dm^{-3}), a fluorescence quantum yield of $\phi_F \approx 2 \times 10^{-5}$ was found [35]. For the J aggregates, there is an increase in the fluorescence quantum yield by a factor of ten compared with the oligomers, but the increase occurs at a very low level.

For pseudo-isocyanine J aggregates, a fluorescence quantum yield of nearly 100% was reported at low photon excitation flux (less than 10^{20} cm^{-2} s^{-1} for continuous wave excitation [17], less than 5×10^{21} cm^{-2} s^{-1} for 20 ps pulse excitation [23,25]). At high excitation intensities, a fluorescence quantum yield reduction due to singlet–singlet annihilation was observed [12,23,25]. In our experiments, the photon excitation flux was 4×10^{19} cm^{-2} s^{-1} (continuous wave pump intensity $I_P = 10$ W cm^{-2}).

The fraction of excited chromophores $N_{chr,exc}/N_{chr,0}$ is given by [45]

$$\frac{N_{chr,exc}}{N_{chr,0}} = \frac{I_P/I_S}{1 + I_P/I_S} \tag{5}$$

where $I_S = h\nu_P/(\sigma_{P,chr}\tau_F)$ is the saturation intensity. Using our experimental data ($I_P = 10$ W cm^{-2}, $\lambda_P = c_0/\nu_P = 834$ nm, $\sigma_{P,chr} = 3 \times 10^{-16}$ cm$^2 \times n_{chr}$, $\tau_F = 1.7$ ps/n_{chr}), we estimate $N_{chr,exc}/N_{chr,0} = 10^{-8}$. This small fraction of excited chromophores indicates that singlet–singlet annihilation plays no role for ICG-NaI in water at room temperature.

The long-wavelength fluorescence tail is thought to be due to J aggregates, since practically all molecules are incorporated in J aggregates [13] and the oligomers have an even

Table 1
Spectroscopic data of ICG-NaI J aggregates in water at room temperature

Parameter	Value	Comments
ϕ_F	$(3 \pm 1) \times 10^{-4}$	This work
n_{chr}	16 ± 8	[38]
$n_{chr}\tau_{rad}$ (ns)	5.5 ± 0.5	This work
$n_{chr}\tau_F$ (ps)	1.7 ± 0.7	This work
P_F	0.24 ± 0.05	This work
$n_{chr}\tau_{or}$ (ps)	1.3 ± 0.7	This work

smaller fluorescence quantum yield than J aggregates [35]. For pseudo-isocyanine J aggregates, the long-wavelength absorption tail is also considered to be due to J aggregate emission [46].

5. Conclusions

The fluorescence behaviour of ICG-NaI J aggregates in water was studied. The weak fluorescence quantum yield ($\phi_F \approx 3 \times 10^{-4}$) and the strong light scattering of J aggregates make accurate measurements difficult. Nevertheless, the fluorescence quantum distribution, fluorescence quantum yield, stimulated emission cross-section spectrum and degree of fluorescence polarization could be determined. Within the limited resolution, the absorption cross-section and stimulated emission cross-section spectra are mirror images. No Stokes shift occurs between the absorption peak and emission peak, as expected for Frenkel excitons coherently extending over adjacent molecules.

References

[1] C. Decker, Appl. Phys. Lett. 27 (1973) 607.
[2] J. Webb, F. Webster, B. Plourde, IEEE J. Quantum Electron. 11 (1975) 114.
[3] B. Pierce, R. Birge, IEEE J. Quantum Electron. 18 (1982) 1164.
[4] S. Reindl, A. Penzkofer, S.-H. Gong, M. Landthaler, R.M. Szeimies, C. Abels, W. Bäumler, J. Photochem. Photobiol. A: Chem. 105 (1997) 65.
[5] I.J. Fox, E.H. Wood, Mayo Clin. Proc. 35 (1966) 732.
[6] T. Nahmimisa, Tokai J. Exp. Clin. Med. 7 (1982) 419.
[7] Y. Gu, J.-H. Li, Z.-H. Gou, SPIE 1616 (1991) 266.
[8] S. Fickweiler, R.M. Szeimies, W. Bäumler, S. Karrer, A.E. Goetz, C. Abels, F. Hofstädter, M. Landthaler, J. Photochem. Photobiol. B: Biol. to be published.
[9] G. Scheibe, Angew. Chem. 50 (1937) 51.
[10] E.E. Jelley, Nature 138 (1936) 1009.
[11] M.L.J. Landsman, G. Kwant, G.A. Mook, W.G. Zijlstra, J. Appl. Physiol. 40 (1976) 575.
[12] J. Baker, Proc. Soc. Exp. Biol. Med. 122 (1966) 957.
[13] F. Rotermund, R. Weigand, A. Penzkofer, J-Aggregation and disaggregation of indocyanine green in water, Chem. Phys., in press.
[14] A.H. Herz, Adv. Colloid Interface Sci. 8 (1977) 237.
[15] D. Möbius, Adv. Mater. 7 (1995) 437.
[16] S.K. Rentsch, R.V. Danielius, R.A. Gadonas, A. Piskarskas, Chem. Phys. Lett. 84 (1981) 446.
[17] H. Stiel, S. Daehne, K. Teuchner, J. Lumin. 39 (1988) 351.
[18] S. De Boer, D.A. Wiersma, Chem. Phys. 131 (1989) 135.
[19] B. Kopainsky, W. Kaiser, Chem. Phys. Lett. 88 (1982) 357.
[20] R. Gagel, R. Gadonas, A. Laubereau, Chem. Phys. Lett. 217 (1994) 228.
[21] K. Minoshima, M. Taiji, K. Misawa, T. Kobayashi, Chem. Phys. Lett. 218 (1994) 67.
[22] H.-P. Dorn, A. Müller, Appl. Phys. B 43 (1987) 167.
[23] V. Sundström, T. Gillbro, R.A. Gadonas, A. Piskarskas, J. Chem. Phys. 89 (1988) 2754.
[24] F. Fink, E. Klose, K. Teuchner, S. Dähne, Chem. Phys. Lett. 45 (1977) 548.
[25] R. Gadonas, R. Danielius, A. Pelakauskas, V. Sundström, T. Gilbro, in: A. Piskarskas (Ed.), Lasers and Ultrafast Processes, vol. 4, Vilnius University Press, Vilnius, 1991, p. 230.
[26] Data Sheet ICG-Pulsion, Pulsion Medizintechnik, Kirchenstrasse 88, D-81675 München, March, 1994.
[27] A. Penzkofer, W. Leupacher, J. Lumin. 37 (1992) 297.
[28] R. Weigand, F. Rotermund, A. Penzkofer, Degree of aggregation of indocyanine green in aqueous solutions determined by Mie scattering, Chem. Phys., in press.
[29] Data Sheet of DuPont Chemical and Pigments Department, Genf, Switzerland, 1990.
[30] T. Kuhin, K. Kobs, J. Chem. Phys. 84 (1980) 1871.
[31] H.C. van de Hulst, Light Scattering by Small Particles, Dover, New York, 1957.
[32] H. Gratz, A. Penzkofer, P. Weidner, J. Non-Cryst. Solids 189 (1995) 50.
[33] E.W. Knapp, Chem. Phys. 85 (1984) 73.
[34] J. Knoester, J. Chem. Phys. 99 (1993) 8466.
[35] R. Philip, A. Penzkofer, W. Bäumler, R.M. Szeimies, C. Abels, J. Photochem. Photobiol. A: Chem. 96 (1996) 137.
[36] S.J. Strickler, R.A. Berg, J. Chem. Phys. 37 (1962) 814.
[37] J.B. Birks, D.J. Dyson, Proc. R. Soc. London, Ser. A 275 (1963) 135.
[38] M. Wittmann, F. Rotermund, R. Weigand, A. Penzkofer, Saturable absorption and absorption recovery of indocyanine green J-aggregates in water, Appl. Phys. B, to be published.
[39] O.G. Peterson, J.P. Webb, W.C. McColgin, J.M. Eberly, J. Appl. Phys. 42 (1971) 1917.
[40] J.R. Lakowicz, Principles of Fluorescence Spectroscopy, Plenum, New York, 1983.
[41] C.A. Parker, Photoluminescence of Solutions, Elsevier, Amsterdam, 1968.
[42] G. Weber, in: D.M. Hercules et al.), Fluorescence and Phosphorescence Analysis, Principles and Applications, Interscience, New York, 1966, p. 217.
[43] P. Weidner, A. Penzkofer, Chem. Phys. 191 (1995) 303.
[44] Th. Förster, in: O. Sinanglu (Ed.), Modern Quantum Chemistry, vol. 3, Academic Press, New York, 1968, p. 93.
[45] A. Penzkofer, W. Blau, Opt. Quantum Electron. 15 (1983) 325.
[46] H.-P. Dorn, A. Müller, Chem. Phys. Lett. 130 (1986) 426.