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Association of nucleosides and their 5′-monophosphates with a tryptophan containing tripeptide, Trp–Leu–Glu: The source of an overestimation by fluorescence spectroscopy

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Abstract

Association of 7-methylguanosine 5′-monophosphate with a tryptophan containing tripeptide, Trp–Leu–Glu, has been studied by fluorescence titration using two different geometries of detection, viz. right angle and front surface geometry. The applicability of these two techniques to determine the stability constant of the nucleotide–peptide adduct is discussed. Evidence is presented that fluorescence titration based on right angle detection may lead to considerable overestimation of the strength of interaction.

Keywords: Association of nucleosides; Fluorescence titration; Right angle detection; Tryptophan containing tripeptide; Adduct stability constant

1. Introduction

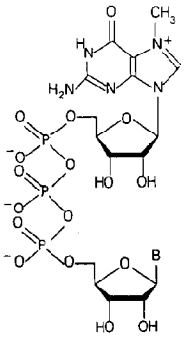
The 5′-end of eukaryotic mRNAs consists of a modified nucleoside, 7-methylguanosine, linked by a 5′,5′-triphosphate bridge to the next nucleoside [1]. This *cap* structure (**1**, see Scheme 1) enhances the splicing of pre-mRNA in nucleus [2], protects mRNA against the action of 5′-exonucleases [3], and markedly facilitates the attachment of mRNA to ribosomes [4]. The latter process is mediated by several proteins, of which the so-called 24 kDa *cap*-binding protein (CBP-I) directly interacts with the *cap* structure [5]. The molecular basis of this nucleic acid–protein interaction has become one of the central subjects of the studies aimed at elucidating the biochemical significance of the 5′-*cap*.

The CBP-I proteins from different sources exhibit two common features: an exceptionally high content of tryptophan residues and a conserved sequence Lys–Xaa–Xaa–Xaa–Glu–Xaa–Baa–Trp–Ala–Leu (Xaa; any amino acid; Baa a basic amino acid) [5]. The Trp-residue of the conserved sequence has been shown to be essential for the *cap* binding [6]. Furthermore, it has been demonstrated that titration of CBPs with *cap* analogues

results in quenching of the fluorescence emission of the Trp-residue [7]. These findings, together with the fact that 7-methylguanine ring stacks rather efficiently with the indole ring of tryptophan [8,9], have led to the conclusion that stacking interaction between the 7-methylguanosine and tryptophan residues plays a major role in binding of *cap* to CBP-I [6,7].

To elucidate further the mode of interaction between the *cap* structure and CBPs, association of *cap* analogues with small peptides, mimicking the conserved sequence of CBPs has been studied. The experimental techniques applied include fluorescence spectroscopy [10–12], ^{1}H NMR spectroscopy [8,10–12] and phase distribution [11]. However, the values obtained for the stability constant of the 1:1 adduct appear to be highly dependent on the technique used, the fluorescence quenching measurements suggesting a much stronger interaction than the other approaches. For example, the stability constants determined by fluorescence titration for the adducts of nucleoside monophosphates with tryptophan containing tripeptides range from 2×10^3 to 6×10^3 M^{-1} (aqueous solution, pH 7.5, 20°C), whereas phase distribution measurements gave values varying from 2 to 13 M^{-1} [11]. Comparison to the ^{1}H NMR data is not quite straightforward, since the NMR measurements have usually been carried out in DMSO-d_6. The results referring to aqueous conditions are limited to association of P^1-(adenosine-5′) P^3-(7-methylguanosine-5′) triphosphate (**1**, B = Adenine) with Trp–Leu–Glu [8]. The stability constant observed for this adduct is low (7.6 M^{-1}, pD 6.7, 20°C) [8], consistent with the results of phase distribution and those reported earlier for the association of tryptophan with various derivatives of adenine [13]. If this kind of small peptides really associate with nucleotides as effectively as suggested by the results of fluorescence titration, they are excellent model compounds for CBPs; a stability constant of 1.95×10^4 M^{-1} has been reported for the adduct of 7-methylguanosine 5′-monophosphate (7-Me-GMP; **2**) with Trp–Glu–Asp–Glu [12], while the values obtained by the same technique with the adducts of **2** with various CBIs are of the order 10^5 M^{-1} [6,14,15].

The present paper is aimed at clarifying why the fluorescence titration method applied previously (right angle geometry) gives exceptionally high stability constants for the adducts of *cap* analogues with small tryptophan containing peptides. For this purpose some of the measurements



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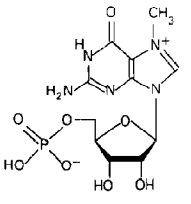

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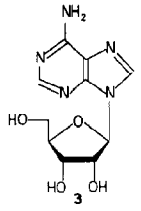

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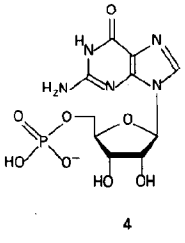

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Scheme 1.

described in literature have been repeated, and the results are compared to those obtained by a front surface method. The reasons for the marked discrepancy between the results of these two approaches are discussed.

2. Experimental

The fluorescence spectra were recorded on a Perkin Elmer LS 50 Luminescence Spectrometer when the right angle detection (1 cm × 1 cm cell) was applied. The titration was carried out by adding 3 μl portions of the nucleoside or nucleotide solution (2 mM) to 3 ml of 5 μM solution of peptide in a phosphate buffer (0.07 M, pH 5.0). The set-up used in front surface measurements has been described previously [20,21]. The solutions were prepared by mixing the stock solutions of peptide or indole (1–2 mM) and 7-Me-GMP (**2**) in acetate buffer (0.2 M, pH 5.0). The fluorescence intensity of the tripeptide (Trp–Leu–Glu), or indole, was obtained by integrating the whole fluorescence spectrum. When the solutions contained 7-Me-guanosine or 7-Me-GMP, the contributions of the fluorescence of these compounds were subtracted by least-squares fitting. Absorption spectra were recorded on a Zeiss Specord M-40 Spectrophotometer. All the measuremets were performed at 293.2 K. The temperature was measured directly from the cell with a thermoelement.

Self-stacking of nucleosides and nucleotides were neglected in calculations. On applying the right angle detection the concentration of nucleosides and nucleotides was less than 10 μM, and under such conditions self-stacking may well be ignored [22]. The front surface measurements refer to higher concentrations ([Q] < 12 mM). However, these measurements were carried out only with 7-Me-Guo and 7-Me-GMP at pH 5. Under such conditions the 7-methylguanine base is positively charged [23], which prevents marked self-stacking.

The phase distribution measurements were carried out as described previously [9].

3. Results and discussion

It is generally known that stacking of a fluorescent aromatic molecule with another aromatic ring (quencher, Q) results in quenching of the fluorescence. When the life-time of the excited state is short and the concentrations of the interacting molecules are low, the quenching may be described by the static part of the well known Stern–Volmer equation (1) [16,17].

$$\eta_0/\eta = 1 + K_S[Q] \quad (1)$$

Here η_0 and η are the quantum yields of a fluorescent probe in the absence and presence of the quencher, and K_S is the association constant of the fluorescent probe and the quencher [16,17]. In studies dealing with association of *cap* analogs with tryptophan containing peptides, the quantum yields have repeatedly been replaced by fluorescent intensities (F_0/F) [8,10–12,18]. However, η_0/η is equal to F_0/F only as long as the excitation intensity remains constant. This is the case when the quencher (*cap* analogue) does not absorb radiation at the wavelength of excitation. Otherwise the effective excitation intensity is reduced as a function of [Q], and hence the ratio F_0/F increases with [Q], even if the quencher is not associated with the fluorescent probe. This problem is a real one on studying the stacking of tryptophan residues with nucleic acid bases, since the absorption spectra of these species extensively overlap.

To estimate how seriously the absorption of Q may affect the values of F_0/F of the indole ring, the interaction of adenosine (**3**) with a tryptophan containing tripeptide, Trp–Leu–Glu, was studied by fluorescence titration, applying right angle geometry of detection at four different wavelengths of excitation, viz. 260, 270, 280 and 290 nm. The absorptivity of adenosine is maximal at 260 nm and diminishes gradually on going to 290 nm. The measurements were carried out in a 1 cm × 1 cm cell depicted in Fig. 1. The beam of excitation enters the cell through the centre of phase A and hits at right angles a mirror on the opposite phase, B. The beam of fluorescence emission leaves the cell perpendicular to the beam of excitation, as indicated in Fig. 1. Table 1 records

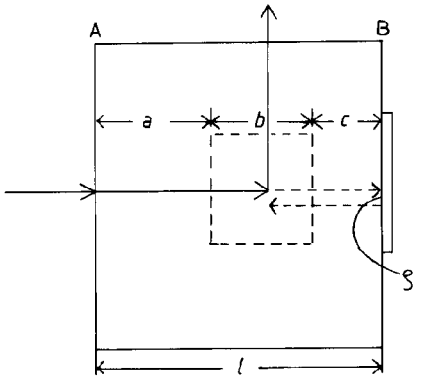

Fig. 1. Geometry of the cell employed in fluorescence measurements when the right angle detection was applied. Notation: $a = 0.40$ cm, $b = 0.25$ cm, $c = 0.35$ cm, and $l = 1.00$ cm; ρ stands for the reflection coefficient of the mirror (0.73 at 280 nm).

the results obtained. As seen at 260 nm the absorbance of the reaction mixture is increased to 6-fold with increasing the concentration of adenosine from 0 to 6 μM, whereas at 290 nm the total absorbance is practically independent of presence of adenosine. If η_0/η in eq. (1) is replaced by F_0/F and the latter values are not corrected with respect of the absorption of adenosine, the values obtained for K_S are $(3.60 \pm 0.02) \times 10^4\ M^{-1}$ (excited at 260 nm), $(2.33 \pm 0.01) \times 10^4\ M^{-1}$ (270 nm), $(5.58 \pm 0.03) \times 10^3\ M^{-1}$ (280 nm) and $0\ M^{-1}$ (290 nm). Accordingly, the value of the stability constant strongly depends on the excitation wavelength, and is clearly an artifact. It should be noted that all series of measurements were carried out at $A < 0.1$, i.e. under conditions where the correction with respect to the absorption of the nucleoside component has usually been neglected.

The absorption spectra of guanosine 5′-monophosphate (GMP; **4**) and 7-Me-GMP (**2**) overlap so severely with that of Trp–Leu–Glu that it is impossible to find an excitation wavelength where the absorption of these nucleotides would not interfere. Excitation at 280 nm gave $K_s = (1.99 \pm 0.01 \times 10^4\ M^{-1}$ and $(2.63 \pm 0.01) \times 10^4\ M^{-1}$ with GMP and 7-Me-GMP, respectively, when uncorrected values of F_0/F were employed (Table 2). However, as discussed above, these stability constants are undoubtedly artifacts, resulting from the fact that absorption of the nucleotide reduces the effective excitation intensity.

As mentioned above, in the Stern–Volmer equation (eq. 1), quantum yields, η, should be used instead of fluorescent intensities, F. F is related to η by eq. (2)

$$F = I_0(1 - 10^{-A})\eta\ , \qquad (2)$$

Table 1

The fluorescence emission of Trp–Leu–Glu in a phosphate buffer (0.07 M, pH 5.0, $T = 293.2$ K) at various concentrations of adenosine, when excited at 260, 270, 280 and 290 nm using a right angle geometry

[Ado] (μm)	Absorbance of the mixture at (nm)				Fluorescence intensity of the mixture when excited at (nm)			
	260	270	280	290	260	270	280	290
0	0.0190	0.0253	0.0262	0.0211	1841	2409	2572	1602
0.600	0.0284	0.0322	0.0293	0.0214	1798	2372	2560	1600
1.20	0.0389	0.0398	0.0305	0.0216	1765	2344	2556	1607
1.80	0.0479	0.0463	0.0327	0.0217	1729	2309	2541	1602
2.40	0.0580	0.0538	0.0347	0.0219	1695	2280	2535	1607
3.00	0.0664	0.0602	0.0360	0.0221	1663	2248	2523	1602
3.60	0.0764	0.0679	0.0382	0.0223	1630	2221	2519	1609
4.20	0.0865	0.0747	0.0403	0.0225	1600	2194	2504	1603
4.80	0.0959	0.0818	0.0422	0.0228	1569	2165	2500	1608
5.40	0.1052	0.0891	0.0453	0.0230	1543	2139	2497	1603
6.00	0.1170	0.0965	0.0476	0.0230	1511	2112	2493	1608

Table 2

The fluorescence emission of Trp–Leu–Glu in a phosphate buffer (0.07 M, pH 5.0, T = 293.2 K) at various concentrations of guanosine 5′-monophosphate (GMP) and 7-methylguanosine 5′-monophosphate (7-Me-GMP, 2), when excited at 280 nm using a right angle geometry

[GMP] (μM)	A(280 nm)	F	[7-Me-GMP] (μM)	A(280 nm)	F
0	0.0203	3148	0	0.0228	3328
0.600	0.0249	3105	0.600	0.0294	3271
1.20	0.0303	3076	1.20	0.0342	3212
1.80	0.0353	3039	1.80	0.0403	3169
2.40	0.0396	3009	2.40	0.0465	3124
3.00	0.0442	2972	3.00	0.0514	3075
3.60	0.0485	2935	3.60	0.0569	3033
4.20	0.0551	2902	4.20	0.0631	2990
4.80	0.0588	2872	4.80	0.0681	2948
5.40	0.0634	2841	5.40	0.0756	2910
6.00	0.0688	2811	6.00	0.0797	2871
6.60	0.0740	2784	6.60	0.0850	2827
7.20	0.0785	2756	7.20	0.0912	2795
7.80	0.0834	2722	7.80	0.0958	2757
8.40	0.0869	2697			
9.00	0.0914	2670			

where I_0 is the excitation radiation intensity and A is the absorbance of the sample [16]. It should be noted, however, that owing to absorption, the excitation intensity is in the centre of the cell smaller than at front surface. When the quencher also absorbs at the excitation wavelength, the excitation intensity becomes dependent on [Q]. It has been estimated [19] that when the total absorbance is 0.1, excitation intensity in the center of a 1 cm × 1 cm cell is $0.88I_0$. Owing to reflection from the mirror at phase B, the diminution of the excitation intensity is even greater, As seen from Tables 1 and 2, in those series of measurements where F significantly decreases as a function of [Q], the change in total absorbance is about 0.1 units. This means that the 15% diminution observed in the value of F on going from [Q] = 0 to [Q] = 9 μM, may be almost entirely attributed to lowering of the excitation intensity due to absorption of Q, and hence η_0/η is practically independent of [Q] over the concentration range employed. Estimation of the values of η_0/η from eq. (3), referring to the detection geometry depicted in Fig. 1, yields a similar result. In this equation only single reflection from the mirror has been taken into account.

$$\frac{F_0}{F} = \left[\eta_0(1-10^{-A_0(b/l)})10^{-A_0(a/l)} \times\left(1+\rho 10^{-A_0[(b+2c)/l]}\right)\right] \times\left[\eta(A_0/A)(1-10^{-A(b/l)})10^{-A(a/l)} \times\left(1+\rho 10^{-A[(b+2c)/l]}\right)\right]^{-1} \quad (3)$$

Table 3

Estimation of quantum yields, η, from fluorescence intensities, F, for the mixtures of 7-Me-GMP (2) and Trp-Leu-Glu, when excited at 280 nm using a right angle geometry (see Fig. 1) [a]

[7-Me-GMP] (μM)	A(280 nm)	F_0/F	P_1	P_2	P_3	η_0/η
0	0.0228	1	1	1	1	1
0.60	0.0294	1.017	1.003	1.006	1.005	1.003
1.20	0.0342	1.036	1.005	1.011	1.009	1.012
1.80	0.0403	1.050	1.007	1.016	1.014	1.012
2.40	0.0465	1.065	1.010	1.022	1.019	1.013
3.00	0.0514	1.082	1.012	1.027	1.023	1.019
3.60	0.0569	1.097	1.014	1.032	1.027	1.020
4.20	0.0631	1.113	1.016	1.038	1.032	1.022
4.80	0.0681	1.129	1.018	1.043	1.036	1.026
5.40	0.0756	1.144	1.021	1.050	1.042	1.024
6.00	0.0797	1.159	1.023	1.054	1.045	1.028
6.60	0.0850	1.177	1.025	1.059	1.049	1.033
7.20	0.0912	1.191	1.028	1.065	1.054	1.032
7.80	0.0958	1.207	1.030	1.070	1.058	1.036

[a] $P_1 = (1-10^{-A_0(b/l)})/[(A_0/A)(1-10^{-A(b/l)})]$, $P_2 = 10^{-A_0(a/l)}/10^{-A(a/l)}$, and $P_3 = (1+\rho 10^{-A_0[(b+2c)/l]})/(1+\rho 10^{-A[(b+2c)/l]})$.

Since the reflection from the wall A is much weaker than the one from mirror B, the subsequent reflections are of minor importance. The correction factors and the values of η_0/η for the 7-Me-GMP/peptide system are given in Table 3 as an illustrative example. Although F_0/F is markedly increased with [Q], η_0/η remains practically unchanged. As mentioned above, when the excitation is carried out at a wavelength where Q does not absorb (see the data at 290 nm in Table 1), no change in F can be observed.

In order to find out how stable the adduct between 7-Me-GMP and Trp–Leu–Glu really is, a method based on front surface geometry was applied to the fluorescence titration. The measurements were carried out in a 0.1 mm thick cell, which was excited frontally as shown in Fig. 2. With this approach much higher concentrations of solutes may be used than in the right angle technique. Under such conditions the dynamic quenching must also be taken into account, and hence the dependence of η_0/η on [Q] must be expressed by the complete Stern–Volmer equation (eq. 4)

$$\eta_0/\eta = 1 + (K_D + K_S)[Q] + K_D K_S [Q]^2, \qquad (4)$$

where the parameter K_D refers to dynamic quenching. Table 4 summarizes the results obtained. Also in this case the absorption of the quencher (7-Me-GMP) contributes markedly to the observed total absorbance. In other words, the excitation intensity, and hence F, is again decreased with increasing concentration of 7-Me-GMP. However, in the present case the estimation of η_0/η is quite straightforward. At surface the dependence of η_0/η on F_0/F may be expressed by eq. (5).

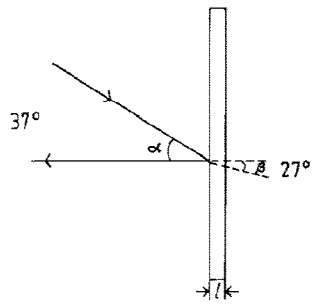

Fig. 2. Geometry of the cell employed in fluorescence measurements when the front surface detection was applied. Notation: $l = 0.1$ mm.

Table 4

The fluorescence emission of Trp–Leu–Glu in an acetate buffer (0.2 M, pH 5.0,T = 293.2 K) at various concentrations of 7-Me-GMP (**2**), when excited at 290 nm using a front surface geometry

[7-Me-GMP] (mM)	A(290 nm) [a]	F_0/F	η_0/η [b]
0	0.075	1	1
0.640	0.1176	1.11	1.06
1.287	0.1606	1.22	1.11
2.573	0.2465	1.49	1.24
3.327	0.2960	1.70	1.34
4.118	0.3490	1.89	1.42
5.353	0.4302	2.27	1.57
6.424	0.5023	2.60	1.69
7.067	0.5450	2.85	1.79
7.708	0.5880	3.09	1.86
8.400	0.6341	3.40	1.95
9.250	0.6892	3.77	2.06
10.41	0.7670	4.34	2.22
11.10	0.8120	4.75	2.34
12.02	0.8740	5.24	2.46

[a] Total absorbance of the mixture containing the peptide (~ 1.5 mM) and 7-Me-GMP.

[b] Calculated by eq. (5).

$$\frac{\eta_0}{\eta} = \frac{F_0 A_0 (1 - 10^{-A/\cos\beta})}{FA(1 - 10^{-A_0/\cos\beta})} \qquad (5)$$

Here A_0 and A are the total absorbances in the absence and presence of the quencher. Application of this eqn. to the values of F_0/F gives the values of η_0/η listed in Table 4. Least-squares fitting to eq. (4) results in values of 35 M^{-1} and 60 M^{-1} for the parameters of Stern–Volmer equation. Determination of the dynamic quenching constant on the basis of the fluorescence life-time data (eq. 6) enabled to assign the former value as K_S.

$$\tau_0/\tau = 1 + K_D[Q] \qquad (6)$$

We have previously [9] determined the association constant of 7-methylguanosine and indole by the method of phase distribution. The value obtained was $K_S = 15\ M^{-1}$ at pH 5.3 and $T = 298.2$

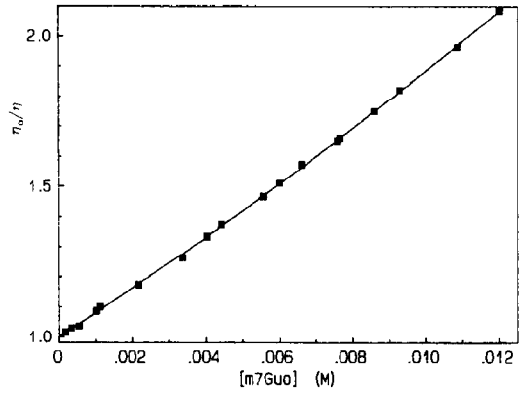

Fig. 3. Stern–Volmer plot for quenching of indole with 7-methylguanosine at pH 5.0 (0.2 *M* acetate buffer) and 298.2 K. Excitation wavelength is 294 nm.

K. To evaluate the reliability of our fluorescence approach, we determined the same association constant by the front surface technique described above. Figure 3 shows the observed dependence of η_0/η on the concentration of 7-methylguanosine. Fitting to eq. (4) gave $K_S = (13.3 \pm 0.9)\ M^{-1}$ ($K_D = 65.1\ M^{-1}$), in good agreement with the value obtained by phase distribution.

Association of 7-Me-GMP with indole was also studied by both fluorescence and phase distribution measurements. Figure 4 shows the results of fluorescent measurements. Again the stability constants obtained agree well: $K_S = (18.7 \pm 0.8)\ M^{-1}$ by fluorescence (pH 5.0; $T = 298.2$ K; $K_D =$ 61.5 M^{-1}) and $K_S = 15.4\ M^{-1}$ (pH 5.3; $T = 298.2$ K) by phase distribution.

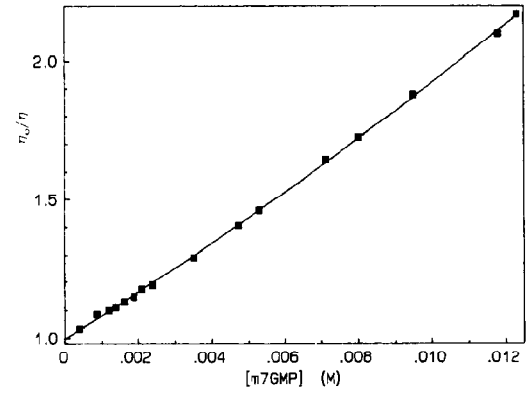

Fig. 4. Stern–Volmer plot for quenching of indole with 7-Me-GMP at pH 5.0 (0.2 *M* acetate buffer) and 298.2 K. Excitation wavelength is 294 nm.

In summary, the preceding discussion strongly suggests that fluorescence titration based on right angle geometry of detection may easily lead to overestimation of the stability constant of nucleotide–peptide adducts.

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References

1 R.E. Rhoads, in: Progress in molecular and subcellular biology, eds. F.E. Hahn, D.J. Kopecho and W.E. Muller (Springer, Berlin, 1985) vol. 9, p. 104.
2 M.M. Konarska, R.A. Padgett and P.A. Sharp, Cell 36 (1984) 731.
3 M.R. Green, T. Maniatis and D.A. Melton, Cell 32 (1983) 681.
4 A. Shatkin, Cell 40 (1985) 223.
5 N. Sonenberg, Progr. Nucleic Acids, Res. Mol. Biol. 35 (1988) 175.
6 H. Ueda, M. Iyo, M. Doi, M. Inoue, T. Ishida, H. Morioka, T. Tanaka, S. Nishikawa and S. Uesugi, FEBS Lett. 280 (1991) 207.
7 S.E. Carberry, R.E. Rhoads and D.J. Goss, Biochemistry 28 (1989) 8078.
8 H. Ueda, M. Doi, M. Inoue, T. Ishida, T. Tanaka and S. Uesugi, Biochem. Biophys. Res. Commun. 154 (1988) 199, and references therein.
9 E. Darzynkiewicz and H. Lönnberg, Biophys. Chem. 33 (1989) 289.
10 H. Iyo, H. Ueda, Y. Usami, Y. Kafuku, M. Doi, M. Inoue and T. Ishida, Chem. Pharm. Bull. 39 (1991) 2483.
11 Y. Kafuku, Y. Matsui, J. Ohtani, Y. Usami, H. Ueda, M. Doi, M. Inoue and T. Ishida, Chem. Pharm. Bull. 39 (1991) 2487.
12 H. Ueda, H. Iyo, M. Doi, M. Inoue and T. Ishida, Biochem. Biophys. Acta 1075 (1991) 181.
13 H. Sigel, Chimia 41 (1987) 11.
14 H. Ueda, H. Maruyama, M. Doi, M. Inoue, T. Ishida, H. Morioka, T. Tanaka, S. Nishikawa and S. Uesugi, J. Biochem. 109 (1991) 882.
15 S.E. Carberry, E. Darzynkiewicz, J. Stepinski, S.M. Tahara, R.E. Rhoads and D.J. Goss, Biochemistry 29 (1990) 3337.
16 C.A. Parker, Photoluminescence of solutions (Elsevier, Amsterdam, 1968) Ch. 1 and 2.

17 J.R. Lakowicz, Principles of fluorescence spectroscopy (Plenum Press, New York, 1983) Ch. 9.
18 M. Tarui, H. Furumura, Y. Kafuku, T. Ishida and M. Inoue, Biochem. Biophys. Res. Commun. 183 (1992) 577.
19 C.A. Parker, Photoluminescence of solutions (Elsevier, Amsterdam, 1968) Ch. 1 and 2. Ch. 2.
20 B. Smyk and R. Drabent, Analyst 114 (1989) 723.
21 Z. Wieczorek, E. Darzynkiewicz, J. Stepinski and H. Lönnberg, Collect. Czech. Chem. Commun. 55 (1990) 2765.
22 H. Lönnberg, J. Ylikoski and A. Vesala, J. Chem. Soc. Faraday trans. 1 80 (1984) 2439, and references therein.
23 E. Darzynkiewicz, I. Labadi, D. Haber, K. Burger and H. Lönnberg, Acta Chem. Scand. B42 (1988) 86.