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Influence of alkyl group on amide nitrogen atom on fluorescence quenching of tyrosine amide and *N*-acetyltyrosine amide

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Abstract

The steady-state and time-resolved fluorescence spectroscopy was applied to determine the influence of an alkyl substituent(s) (methyl or ethyl, *n*-propyl, iso-propyl, *n*-butyl, sec-butyl, or *t*-butyl) on amide nitrogen atom on photophysical properties of tyrosine and *N*-acetyltyrosine amides in water. Generally, the amide group strongly quenches the fluorescence of tyrosine, however, the size and number of substituents on amide nitrogen atom modify the quenching process only in small degree. The fluorescence intensity decays of all amides studied are bi-exponential. The contribution of both components (α_i) to the fluorescence decay undergoes irregular change. An introduction of alkyl substituent on amide nitrogen atom causes an increase of the fluorescence lifetime of tyrosine derivative compared to the unsubstituted amide for both *N*-acetyltyrosine and tyrosine with the protonated amino group. Calculated, basing on the fluorescence quantum yield (QY) and average lifetime, the radiative rate constants (k_f) are similar, which indicates that the substituent(s) does not have substantial influence on radiative process of the deactivation of the excited state of the phenol chromophore for all compounds studied regardless the amino group status as well as the number and type of substituent (linear or branched). The comparison of the ground-state rotamer populations of tyrosine amides and N-acetyltyrosine amides with different alkyl substituent on amide nitrogen atom obtained from ^{1}H NMR with the value of pre-exponential factors indicates that not the rotamer populations, but specific hydration of a whole molecule of the amino acid including chromophore and amino acid moiety, seems to be the main reason of the heterogenous fluorescence intensity decay of tyrosine derivatives.

Keywords: Tyrosine; Flourescence; Rotamers

1. Introduction

The fluorescence of aromatic amino acids (phenylalanine, tyrosine and tryptophan) and their residues incorporated into a peptide or protein chain is a subject of extensive studies because of their use as internal probes in conformational analysis [1–5]. In the case of the tyrosine zwitterion and tyrosine derivatives with the ionized α-carboxyl group, mono-exponential fluorescence decays were observed [3–8]. The conversion of the α-carboxyl group into the corresponding amide or its protonation results in a complex fluorescence decay [1–25]. An explanation of this behaviour was offered by ground-state rotamer model which assumes the existence of well-defined rotamers about the $C^{\alpha}-C^{\beta}$ bond, where interconversion time is considerably longer than the excited-state lifetimes of the rotamers [6]. The different lifetimes of the rotamers arise from the interaction between the phenol fluorophore and the quenching groups. Cowgill [26,27], Tournon et al. [28] and Feitelson [10] suggested that the fluorescence quenching of an aromatic amino acid by the peptide (amide) group occurred by a charge transfer between the excited aromatic chromophore, as a donor, and electrophilic units in the amino acid backbone (the carbonyl of the amide group), as an acceptor. Such mechanism of the fluorescence quenching of tyrosine by the amide group was further supported by the dependence of quenching efficiency on the distance between the phenol of tyrosine residue and the amide group and type of the substituent on the amide nitrogen atom

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[9,24], as well as on the ionization potential of aromatic amino acids [7]. In the case of tyrosine analogues and derivatives, the rotamer model showed that the rotamer in which the phenol ring can come into the closest contact with the carbonyl group had the shortest fluorescence lifetime. Since the slow-exchange rotamer model predicts that the fluorescence intensity decay should be described by the sum of three exponents, for those tyrosine compounds exhibiting a single or double exponential decay, it is assumed that the three or two rotamers have similar, unresolved fluorescence lifetimes or rotamer interconversion is fast, averaging the emission [3]. However, basing on the ground-state rotamer model, the photophysical properties of simple derivatives of tyrosine analogues could not be fully explained [11–15].

In order to better recognize the influence of steric hindrance on amide nitrogen atom on rotamer population and fluorescence quenching of tyrosine by the amide group in this paper, we present spectroscopic and photophysical properties of tyrosine and *N*-acetyltyrosine derivatives possessing a different alkyl substituent(s) on nitrogen amide atom.

2. Materials and methods

2.1. Synthesis

Triethylamine (TEA), methylamine hydrochloride, dimethylamine hydrochloride, ethylamine, *N*-Ac-L-tyrosinamide and isobutyl chloroformate were purchased from Aldrich. Diethylamine, propylamine, di-*n*-propylamine, 2-butylamine, *tert*-butylamine, di-*n*-butylamine, diisobutylamine, *N*,*N*′ -dicyclohexylcarbodiimide (DCC), *O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*′ ,*N*′ -tetramethyluroniumtetrafluoroborate (TBTU) and *N*-hydroxysuccinimide (HOSu) were purchased from Lancaster, whereas isopropylamine and butylamine were from Sigma. *N*-Boc-(2-bromo-*Z*)-L-tyrosine was purchased from Bachem, whereas *N*-Ac-L-tyrosine and *N*-ethyldiisopropylamine (DIEA) were from Fluka.

2.1.1. Synthesis of tyrosine derivatives

All tyrosinamides were synthesized from *N*-Boc-protected Tyr(2-bromo-*Z*)-OH (3 mmol) and corresponding amine (6 mmol) as substrates in anhydrous THF in the presence of TEA (3.3 mmol) at 0 °C via the intermediate mixed anhydride obtained using isobutyl chloroformate (3.3 mmol). After a few hours, the solvent was evaporated, the residue was treated with AcOEt, and solution was washed with saturated NaCl solution, 5% $NaHCO_3$ solution, 0.1 M $KHSO_4$ solution and dried over anhydrous $MgSO_4$. The product was isolated by means of column chromatography (Macherey Nagel, Silica gel 60, 0.063–0.2 mm) using as an eluent a mixture of CH_2Cl_2/EtOH (9:1, v/v).

The Boc removal was performed using about 10 ml of 6 M HCl in dioxan. After about 3 h, the solvent was evaporated and obtained oily residue was crystallized from the mixture of EtOH/Et_2O. The 2-BrZ removal was performed using catalytic hydrogenation in MeOH (H_2, Pd/C). After about 3 h, the catalyst was filtered off, the solvent was evaporated and the residue was crystallized from the mixture of MeOH/Et_2O or the product was isolated by means of semi-preparative RP-HPLC (Kromasil column, C-8, 5 μm, 250 mm long, i.d. = 20 mm). The progress of all the reactions and the purity of the products was checked by means of TLC (CH_2Cl_2/EtOH 9:1 (v/v), Merck plates, Kieselgel 60 F254) and RP-HPLC (Kromasil column, C-18, 5 μm, 250 mm long, i.d. = 4.5 mm). The mobile phase was a gradient running from 100% A (0.1% aqueous solution of trifluoroacetic acid (TFA)) to 100% B (80% aqueous solution of acetonitrile with addition of 0.08% TFA) in 60 min with detection at 223 or 275 nm. The identification of desired product was based on the ^1H NMR spectrum recorded on Varian, Mercury-400 BB spectrometer (400 MHz) in D_2O.

2.1.2. Synthesis of N-acetyltyrosine derivatives

All *N*-acetyltyrosine derivatives were synthesized from *N*-Ac-protected Tyr(OH)-OH as a substrate using different coupling methods.

2.1.2.1. DCC/HOSu/TEA. *N*-acetyltyrosine 0.2 g (0.89 mmol) was dissolved in dioxan/DMF mixture in the presence of 124 μl (0.89 mmol) TEA. Afterwards, 1.78 mmol of the appropriate amine (ethylamine, *n*-butylamine or 2-butylamine, or *tert*-butylamine, di-*n*-butylamine or diisobutylamine), 205 mg (1.78 mmol) of HOSu and 202 mg (0.98 mmol) of DCC were added. After a few hours, 8.6 μl of acetic acid was added. The progress of the reaction was checked by means of TLC (CH_2Cl_2/EtOH 9:1, v/v). The obtained carbamide was filtered and the mixture of solvents was evaporated. The residue was treated with AcOEt, and the solution was washed with water, 1 M HCl solution, saturated NaCl solution, 5% $NaHCO_3$ solution, saturated NaCl solution and dried ($MgSO_4$). The products were purified either by crystallization from the mixture of AcOEt/petroleum ether or using RP-HPLC method.

2.1.2.2. TBTU/DIEA. *N*-acetyltyrosine 0.2 g (0.89 mmol) was dissolved in DMF and 285.7 mg (0.89 mmol) of TBTU was added. The mixture was activated for 2 min and after that, 152.4 μl (0.89 mmol) of DIEA and 1.78 mmol of the appropriate amine (*N*,*N*-diethylamine, propylamine, iso-propylamine or *N*,*N*-dipropylamine) were added. The progress of the reaction was checked by means of TLC (CH_2Cl_2/MeOH/AcOH 100:10:1, v/v). After a few hours, the solvent was evaporated and the residue was treated with AcOEt, and the solution was washed with water, 1 M HCl solution, saturated NaCl solution, 5% $NaHCO_3$ solution, saturated NaCl solution and dried over anhydrous $MgSO_4$. The products were purified either by crystallization from a mixture of AcOEt/petroleum ether or using RP-HPLC.

2.1.2.3. By acetylation of Tyr(OH)NHMe and Tyr(OH)NMe$_2$. *N*-Ac-Tyr(OH)NHMe and *N*-Ac-Tyr(OH)NMe$_2$ were obtained from Boc-Tyr(2-bromo-*Z*)NHMe and Boc-Tyr(2-bromo-*Z*)NMe$_2$, which were synthesized according to the procedure described previously (Section 2.1.1). After the Boc removal, the amino group was acetylated and 2-bromo-*Z* group was removed. Obtained products were work-up as described previously.

3. Spectroscopic measurements

Fluorescence spectra were recorded using a Perkin-Elmer LS-50B spectrofluorimeter with 4.0 nm bandwidth for excitation and emission. Fluorescence quantum yields (QYs) were determined at room temperature using L-tyrosine (QY = 0.14) as a reference [29].

Fluorescence intensity decays were collected using a time-correlated single-photon counting apparatus using excitation wavelength $\lambda = 275$ nm (the picosecond/femtosecond laser system, Ti:Shaphire 'Tsunami' laser pumped with an argon ion laser 'BeamLok' and thermoelectrically cooled MCP-PTM R3809U-05) at the Laboratory of Ultrafast Laser Spectroscopy, Adam Mickiewicz University, Poznań, Poland [30]. The emission wavelength $\lambda = 315$ nm was selected by means of monochromator (7.5 nm bandwidth). The fluorescence intensity decays were recorded at 20 °C with a polarizer set up at a magic angle. The Ludox solution was used as a reference. The fluorescence intensity decays were collected at 1×10^4 counts at maximum, whereas the instrument response function (half-width about 35 ps) at 4×10^4 counts at maximum. Fluorescence decay data were fitted by the iterative convolution to the sum of exponents:

$$I(t) = \sum_i \alpha_i \exp(-t/\tau_i) \quad (1)$$

where α_i and τ_i are the pre-exponential factor and fluorescence lifetime, respectively.

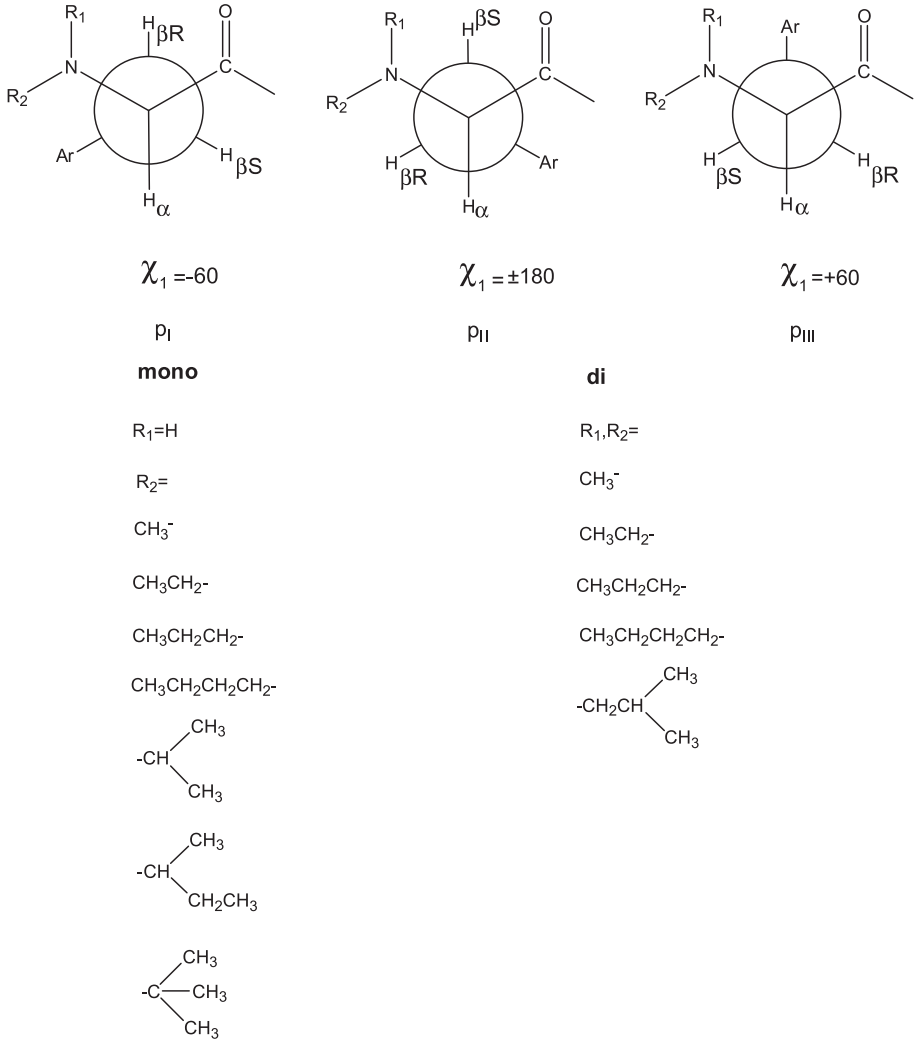

Fig. 1. The Newman projection of the rotamers about the $C^{\alpha}-C^{\beta}$ bond.

Table 1
The fluorescence lifetimes (τ_i), the pre-exponential factors (α_i), the fluorescence quantum yield (QY), the average fluorescence lifetime, $\langle\tau\rangle_T$ and the radiative (k_f) and non-radiative constants (k_{nr}) of tyrosine amides in water

Compound	τ_1 [ns]	α_1	f_1	τ_2 [ns]	α_2	f_2	$\langle\tau\rangle_T$ [ns]	QY	k_f=QY/$\langle\tau\rangle_T$ $\times 10^{-7}$ [s^{-1}]	k_{nr}=(1 − QY)/$\langle\tau\rangle_T$ $\times 10^{-8}$ [s^{-1}]
Tyr	3.36	1.00	1.00	–	–	–	3.36	0.140	4.17	2.56
$TyrNH_2$	1.24	0.60	0.87	0.27	0.40	0.13	1.12	0.045	4.03	8.54
TyrNHMe	1.54	0.81	0.92	0.59	0.19	0.08	1.46	0.055	3.76	6.47
TyrNHEt	1.79	0.51	0.66	0.95	0.49	0.34	1.51	0.068	4.52	6.19
$TyrNHPr^n$	1.81	0.61	0.75	0.95	0.39	0.25	1.60	0.058	3.63	5.89
$TyrNHPr^i$	1.77	0.65	0.77	0.96	0.35	0.23	1.59	0.048	3.02	5.99
$TyrNHBut^n$	1.74	0.75	0.86	0.89	0.25	0.14	1.62	0.061	3.76	5.79
$TyrNHBut^s$	1.83	0.82	0.91	0.79	0.18	0.09	1.74	0.052	2.99	5.45
$TyrN(Me)_2$	0.98	0.57	0.82	0.28	0.43	0.18	0.85	0.025	2.93	11.40
$TyrN(Et)_2$	1.36	0.48	0.68	0.59	0.52	0.32	1.11	0.026	2.34	8.77
$TyrN(Pr^n)_2$	1.33	0.43	0.68	0.50	0.58	0.32	1.07	0.036	3.37	9.04
$TyrN(But^n)_2$	1.52	0.35	0.58	0.58	0.65	0.42	1.12	0.033	2.94	8.60
$TyrN(But^i)_2$	1.47	0.72	0.89	0.46	0.28	0.11	1.36	0.032	2.35	7.10

The contribution of each decay time to the steady-state intensity f_i as well as the average fluorescence lifetimes $\langle\tau\rangle_T$ were calculated using an equation:

$$f_i = \alpha_i\tau_i / \sum \alpha_i\tau_i \quad (2)$$

$$\langle\tau\rangle_T = \sum_i f_i\tau_i \quad (3)$$

3.1. ^1H NMR measurements

^1H NMR spectra of tyrosine and its derivatives were recorded using a Varian Mercury 400 BB instrument (400 MHz) in D_2O for tyrosine amides and in DMSO-d_6 for *N*-Ac-tyrosine amides (because of their low solubility in water). All ^1H NMR spectra were obtained at 20 °C in the pulse and Fourier transform mode and coupling constants were obtained with an estimated accuracy of ± 0.2 Hz. The fractional populations p_I, p_{II} and p_{III} of rotamers I, II and III, respectively, connected with the rotation about the $C^\alpha-C^\beta$ bond (the X_1 rotamers assigned according to Laws et al. [5]) were calculated from the coupling constants between vicinal H^α and $H^{\beta R}$ ($H^{\beta S}$) using the following equations:

$$p_I = [{}^3J(H^\alpha - H^{\beta R}) - {}^3J_g]/\Delta^3J \quad (5)$$

$$p_{II} = [{}^3J(H^\alpha - H^{\beta S}) - {}^3J_g]/\Delta^3J$$

$$p_{III} = 1 - p_I - p_{II}$$

where $\Delta^3J = {}^3J_t{}^3 - J_g$ and ${}^3J_t$ = 13.56 Hz and ${}^3J_g$ = 2.6 Hz are the nominal values of coupling constants for vicinal protons in trans and gauche conformations, respectively [5] (Fig. 1).

Table 2
The fluorescence lifetimes (τ_i), the pre-exponential factors (α_i), the fluorescence quantum yield (QY), the average fluorescence lifetime $\langle\tau\rangle_T$ and the radiative (k_f) and non-radiative constants (k_{nr}) of *N*-acetyltyrosine amides in water

Compound	τ_1 [ns]	α_1	f_1	τ_2 [ns]	α_2	f_2	$\langle\tau\rangle_T$	QY	k_f=QY/$\langle\tau\rangle_T$ $\times 10^{-7}$ [s^{-1}]	k_{nr}=(1 − QY)/$\langle\tau\rangle_T$ $\times 10^{-8}$ [s^{-1}]
AcTyr	3.31	0.90	0.95	1.45	0.10	0.05	3.22	0.134	4.17	2.69
$AcTyrNH_2$	1.66	0.71	0.91	0.41	0.29	0.09	1.54	0.057	3.69	6.11
AcTyrNHMe	2.60	0.73	0.80	1.77	0.27	0.20	2.43	0.095	3.91	3.73
AcTyrNHEt	2.44	0.91	0.95	1.25	0.09	0.05	2.38	0.087	3.66	3.84
$AcTyrNHPr^n$	2.54	0.95	0.99	0.46	0.05	0.01	2.51	0.085	3.38	3.64
$AcTyrNHPr^i$	2.49	0.95	0.99	0.53	0.05	0.01	2.47	0.086	3.48	3.7
$AcTyrNHBut^n$	2.40	0.95	0.99	0.64	0.05	0.01	2.38	0.097	4.08	3.8
$AcTyrNHBut^s$	2.85	0.72	0.85	1.30	0.28	0.15	2.62	0.108	4.12	3.4
$AcTyrNHBut^t$	3.02	0.60	0.69	2.05	0.40	0.31	2.72	0.102	3.75	3.3
$AcTyrN(Me)_2$	1.45	0.69	0.86	0.51	0.31	0.14	1.32	0.048	3.65	7.23
$AcTyrN(Et)_2$	1.85	0.55	0.69	1.02	0.45	0.31	1.59	0.056	3.52	5.93
$AcTyrN(Pr^n)_2$	1.70	0.76	0.88	0.72	0.24	0.12	1.59	0.061	3.84	5.91
$AcTyrN(But^n)_2$	1.66	0.73	0.85	0.82	0.27	0.15	1.53	0.055	3.59	6.18
$AcTyrN(But^i)_2$	1.82	0.77	0.91	0.60	0.23	0.09	1.71	0.059	3.45	5.5

4. Results and discussion

4.1. Steady-state fluorescence

All tyrosine and *N*-acetyltyrosine amides studied possess the absorption and fluorescence spectra (both position and shape) similar to the parent molecule (data not shown) and consistent with published data [3–5,7–9,11–16,21–23,29]. However, the substituent(s) on amide nitrogen atom modifies the fluorescence quantum yield of the phenol fluorophore of tyrosine. The highest fluorescence quantum yield possesses tyrosine (0.14 [29]) as well as *N*-acetyltyrosine (0.135) in which small quenching by the acetyl group is observed [15]. Generally, the amide group strongly

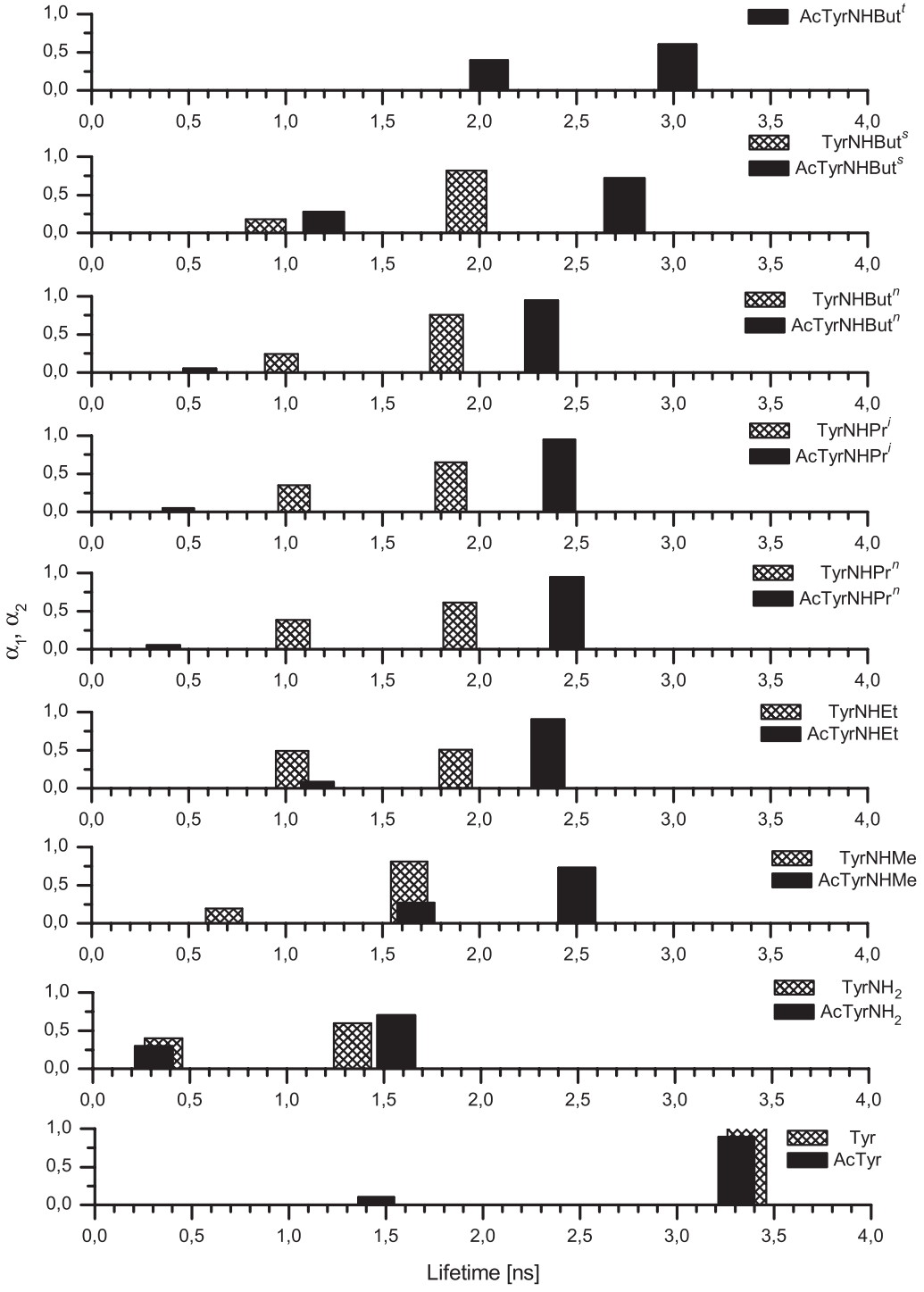

Fig. 2. The fluorescence lifetimes of tyrosine and *N*-acetyltyrosine for mono-substituted amides. The height of the post is proportional to the value of the pre-exponential factor (α).

quenches the fluorescence of tyrosine, however, the size and number of substituents on amide nitrogen atom only in small degree modify the quenching process (Table 1). For unsubstituted amide, the fluorescence quantum yield is about 0.045, similarity to the previously published result [7] (Table 1). τ_i is the lowest value of the fluorescence quantum yield among the mono-substituted amides studied. Moreover, the di-*N*,*N*-substituted amides of tyrosine possess about two times lower and less diversified fluorescence quantum yield (in the range from 0.025 to 0.032, Table 1) than mono-substituted amides. The fluorescence quantum yield of *N*-acetyltyrosine derivatives are generally about two times higher (except unsubstituted amide of *N*-acetyltyrosine) than observed for tyrosine derivatives with the protonated amino group which confirms previous information on the enhanced quenching process of the tyrosine fluorescence by the amide group in the presence of the protonated amino group [3,7,8,14]. In the case of *N*-acetyl tyrosine derivatives similarly to tyrosine derivatives, the di-*N*,*N*-substituted amides possess lower fluorescence quantum yields than the mono-substituted ones (Table 2).

4.2. Time-resolved fluorescence

The fluorescence intensity decay of tyrosine is mono-exponential with the lifetime compatible to the published

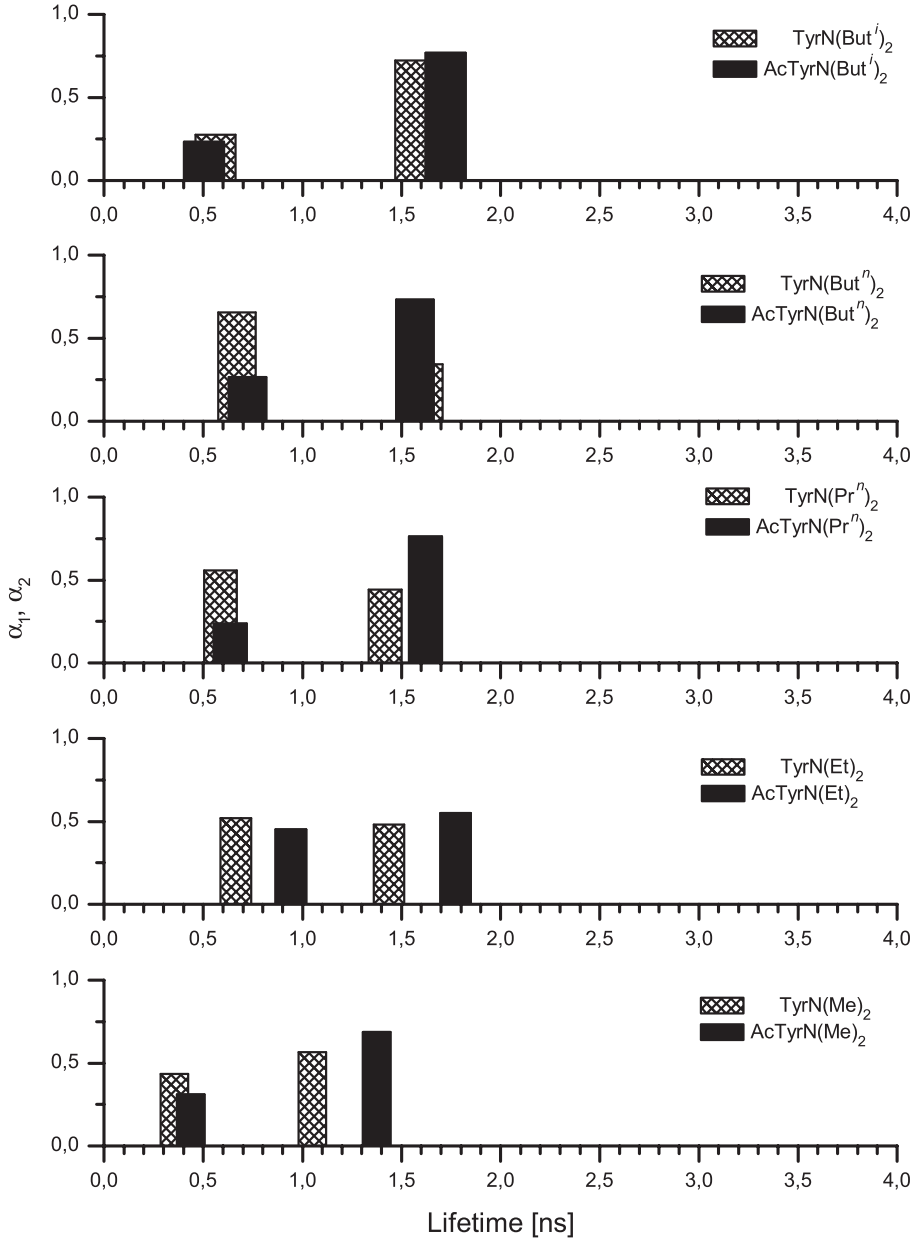

Fig. 3. The fluorescence lifetimes of tyrosine and *N*-acetyltyrosine for di-substituted amides. The height of the post is proportional to the value of the pre-exponential factor (α).

results [3,5–8,15,16,18,20]. Introduction of the acetyl group on the tyrosine amino group made the fluorescence intensity decay heterogenous. The decay becomes bi-exponential with a small contribution of the short component as was previously observed [15]. Also, the fluorescence intensity decays of tyrosine amide and *N*-acetyltyrosine amide are bi-exponential and the fluorescence lifetimes (shown in Tables 1 and 2 for comparison) are in agreement with results published by others [3,5–7,15–18,22]. An introduction of alkyl substituent on amide nitrogen atom causes an increase of the fluorescence lifetime of tyrosine derivative compared to the unsubstituted amide for both *N*-acetyltyrosine and tyrosine with the protonated amino group (Figs. 2 and 3). For tyrosine with protonated amino group, an increase of an alkyl chain length causes the increase of the both long and short fluorescence lifetimes from 1.24 and 0.27 ns for tyrosine amide to 1.81 ns and about 1 ns for its derivative possessing ethyl group on amide nitrogen atom (Tables 1 and 2). A further increase of an alkyl chain length does not result in the increase of the fluorescence lifetime. For the remaining tyrosine amides studied, regardless whether substituent is linear or branched, the fluorescence lifetimes are, in the experimental error range, the same. The contribution of both components (α_i) to the fluorescence decay undergoes irregular change. The highest values of the short-lifetime component of the contribution, comparable to the value for unsubstituted amide, are observed for derivatives containing the ethyl or propyl substituent.

Substituents on the amide nitrogen atom of acetyl tyrosine amides change the fluorescence lifetimes similarly as in the case of tyrosine amides, however, they are much longer (about 2.5 ns), especially for more branched substituents such as s-butyl (2.8 ns) and *t*-butyl (about 3 ns) (Table 2). More differentiated changes are observed for the short fluorescence lifetime component. For *N*-acetyltyrosine amide conataining methyl substituent, it increases to the value of 1.8 ns, and then for ethyl derivative, it decreases (together with its contribution) to the value of 1.25 ns and for propyl derivatives to about 0.5 ns. Moreover, for iso-propyl and *n*-butyl derivatives, it remains almost unchanged, while it substantially increases to the value of 1.3 ns for s-butyl and 2.05 ns for *t*-butyl derivative. The contribution of this lifetime (α) to the total fluorescence decay also increases from a value of a few percent observed for propyl and *n*-butyl derivatives to a value of about 40% for *t*-butyl derivative. The unsubstituted amide for both tyrosine and *N*-acetyltyrosine has the shortest average fluorescence lifetime calculated from Eqs. (2) and (3). For remaining mono-substituted amides, the average fluorescence lifetime is about 1.6 ns for tyrosine with the protonated amino group and about 2.6 ns for *N*-acetyltyrosine (Tables 1 and 2). Calculated, basing on the fluorescence quantum yield and average lifetime, the radiative rate constants (k_f) are similar (higher diversification than the average fluorescence lifetimes are made by dividing a small fluorescence quantum yield by average lifetime both burden with error). This indicates that the substituent(s) does not have a substantial influence on radiative process of the deactivation of the excited state of the phenol chromophore for all compounds studied regardless the amino group status as well as the number and type of substituents (linear or branched) (Tables 1 and 2). The non-radiative rate constants are more diversified and higher for tyrosine amide with the protonated amino group than for *N*-acetyl derivative. This confirms previous observation on the enhancement of quenching properties of the amide group by the protonated amino group because of its electro-accepting character and hydration properties [3,7,8,14,15]. Contrary to the radiative rate constant, the non-radiative ones (k_{nr}) are higher for unsubstituted amide than for mono-substituted amide for which the substituent size does not have the influence on the k_{nr}. Di-substituted amides of both tyrosine and *N*-acetyltyrosine possess shorter average fluorescence lifetimes than mono-substituted ones. Moreover, *N*-acetyl derivatives have longer lifetimes (about 1.6 ns) than derivatives with the protonated amino group (about 1 ns) (Tables 1 and 2). The shorter average fluorescence lifetimes and lower fluorescence quantum yields observed for di-substituted amides result in the higher non-radiative rate constant (Tables 1 and

Table 3
^{1}H NMR coupling constants and rotamer populations calculated from Eq. (5) for Tyr derivatives in D_2O and Ac-Tyr derivatives in DMSO-d_6

Compound	$^3J(H^{\alpha}-H^{\beta R})$	$^3J(H^{\alpha}-H^{\beta S})$	p_I	p_{II}	p_{III}
D_2O					
Tyr	7.82	5.37	0.48	0.25	0.28
$TyrNH_2$	7.53	6.74	0.45	0.38	0.17
TyrNHMe	7.68	6.58	0.46	0.37	0.17
TyrNHEt	8.3	6.35	0.52	0.34	0.14
$TyrNHPr^n$	8.75	6.28	0.56	0.34	0.1
$TyrNHPr^i$	9.16	5.94	0.6	0.3	0.1
$TyrNHBut^n$	9.22	5.93	0.6	0.3	0.1
$TyrNHBut^s$	8.91	6.69	0.58	0.37	0.05
$TyrN(Me)_2$	7.9	6.59	0.49	0.36	0.15
$TyrN(Et)_2$	7.32	6.34	0.43	0.34	0.23
$TyrN(Pr^n)_2$	9.4	5.94	0.62	0.3	0.08
$TyrN(Pr^i)_2$	9.41	5.94	0.62	0.31	0.07
$TyrN(But^n)_2$	8.92	5.79	0.58	0.29	0.13
$TyrN(But^i)_2$	9.4	5.94	0.62	0.3	0.08
DMSO-d_6					
AcTyrOH	9.52	5.13	0.63	0.23	0.14
$AcTyrNH_2$	9.52	4.88	0.63	0.21	0.16
AcTyrNHMe	9.52	5.31	0.63	0.25	0.12
AcTyrNHEt	8.97	5.49	0.58	0.26	0.16
$AcTyrNHPr^n$	8.97	5.49	0.58	0.26	0.16
$AcTyrNHPr^i$	8.79	5.86	0.56	0.3	0.14
$AcTyrNHBut^n$	8.81	5.61	0.57	0.27	0.16
$AcTyrNHBut^s$	9.34	4.76	0.61	0.2	0.19
$AcTyrNHBut^t$	8.55	5.86	0.54	0.3	0.16
$AcTyrN(Me)_2$	7.61	7.61	0.46	0.45	0.09
$AcTyrN(Et)_2$	7.51	7.32	0.45	0.43	0.12
$AcTyrN(Pr^n)_2$	7.51	7.32	0.45	0.43	0.12
$AcTyrN(But^n)_2$	7.32	7.32	0.43	0.43	0.14
$AcTyrN(But^i)_2$	8.06	6.59	0.5	0.36	0.14

2). The values of k_{nr} practically do not depend on the kind of substituent (linear or branched), except for di-methyl one for which k_{nr} has a little higher value than for the remaining ones.

4.3. The rotamer population

The fractional population of ground-state rotamers connected with the rotation around the $C^{\alpha}-C^{\beta}$ bond (X_1 rotamers assigned according to Laws et al. [5]) calculated based on the ^{1}H NMR measurements in water (for tyrosine amides) and DMSO (for *N*-acetyl tyrosine derivatives) are presented in Table 3. For mono-substituted amides of tyrosine, the population of the p_{III} rotamer decreases with the increase of a substituent size, whereas the population of the p_I increases, especially for small substituents and p_{II} rotamers do not show a regular change. Also, for di-substituted tyrosine amides, influence of the substituent size on rotamer population was not observed. Because the solvent has influence on the rotamer population [31,32], the relative rotamer population for *N*-acetyltyrosine amides cannot be directly compared with that for tyrosine amides. These data are presented to show the tendency of changes caused by the substituent(s). As can bee seen, the substituent size does not have influence on the rotamers population (Table 3). For di-substituted *N*-acetyltyrosine amide, a very weak influence of the substituent size on the p_I and p_{II} rotamers is observed and the lack of influence on the rotamer p_{III}.

5. Conclusion

The photophysical and rotamer population data presented in this work indicate that there is no direct correlation between the ground-state rotamer population and pre-exponential factor of the fluorescence decay. A higher pre-exponential factors (α_2), (Tables 1 and 2) obtained from the fluorescence intensity decay for di-substituted amides for both tyrosine and tyrosine with the blocked amino group are caused by a change of the whole molecule hydration rather the rotamers population. A lack of possibility to form a strong hydrogen bond network by the di-substituted amide group compared to the unsubstituted and mono-substituted ones resulted in a modification of the photophysical properties of the phenol chromophore of tyrosine (shortening of the average fluorescence lifetime and fluorescence quantum yield and thereby increase of the non-radiative rate constant). Thus, not rotamer population, but specific hydration of a whole molecule of the amino acid including chromophore and amino acid moiety, seems to be the main reason of the heterogenous fluorescence intensity decay of tyrosine derivatives. Such a conclusion is also supported by the lack of the quenching of the tyrosine fluorescence by the amide group in the organic solvent unable to form a strong hydrogen bond [15].

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