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Photophysical properties of (*p*-hydroxy)phenylglycine

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Abstract

Photophysical properties of (*p*-hydroxy)phenylglycine [Phg(OH)] (an analogue of tyrosine) and its derivatives with the unmodified hydroxyl group: Ac–Phg(OH)–OH, Phg(OH)–$NHCH_3$, Ac–Phg(OH)–$NHCH_3$, and the derivatives with the methylated phenolic hydroxyl group: Phg(OCH_3), Ac–Phg(OCH_3)–OH, Phg(OCH_3)–$NHCH_3$, Ac–Phg(OCH_3)$NHCH_3$ were the subject of our investigations. The synthesized compounds were used to study the influence of (i) the conversion of the carboxylic group into the amide, (ii) the *N*-acetylation of the amino group and (iii) the *O*-methylation of the phenolic oxygen on the photophysical properties of the tyrosine analogue. All prepared derivatives displayed monoexponential fluorescence decays. The *N*-acetylation and amidation decreased the fluorescence lifetime of the resulting compounds. The data obtained are discussed on the basis of the rotamer theory and compared with theoretical calculations results.

Keywords: (p-Hydroxy)phenylglycine; Rotamer theory; Fluorescence

1. Introduction

(*p*-Hydroxy)phenylglycine[Phg(OH)] is a non-proteinogenic aromatic amino acid, and a very important component of the β-lactam antibiotic *amoxycicillinum*. Introduction of this amino acid as the acylating moiety of the amino group of 6-aminopenicillic acid improved bioavailability of the resulting antibiotic, mostly due to its enhanced stability at acidic pH (the half-lifetime at pH = 1.5 and temperature 37 °C is around 17 h) [1].

On the other hand, (*p*-hydroxy)phenylglycine is an analogue of tyrosine, the amino acid that displays an interesting photophysical behavior after incorporation in a peptide or a protein chain. Tyrosine is often the only aromatic amino acid in a studied molecule and knowledge of its photophysical properties is essential. The photophysical parameters of the chromophore (fluorescence lifetime and quantum yield) are strongly dependent on the structure of the compound studied.

The fluorescence of tyrosine, tyrosine derivatives and tyrosine residue in peptides and proteins is the subject of extensive investigations. Both the tyrosine zwitterion and its derivatives with an ionized α-carboxyl group exhibit monoexponential decay kinetics. A conversion of the α-carboxyl group to the corresponding amide or its protonation results in complex fluorescence decay [2–5]. Several explanations for the complex fluorescence kinetics of tryptophan and tyrosine have been forwarded, including involvement of the ^1L_a and ^1L_b states [6–8], an excited state reaction [9–11], and different lifetimes for the side-chain rotamers about the C^α–C^β bond [2,4,5,12–23]. In the rotamer model [2,4,5,12–23], multiexponential decay kinetics are proposed to be the result of the presence of a number of ground-state rotamers, some of which do not interconvert within the fluorescence time scale (typically 3–5 ns). The individual rotamers are assumed to exhibit monoexponential decay kinetics. This model, introduced by Gauduchon and Wahl [4], suggests a charge transfer reaction between the excited aromatic chromophore (indole or phenol ring) as the donor and electrophilic units in the amino acid backbone (the carbonyl or protonated amino group [16–18,21,22]) as the acceptor. As shown by Laws et al. [2,22] the shorter fluorescence lifetime was associated with the protonated carboxyl group, while the longer lifetime was associated with the ionized carboxylate. Applying the global and the linked-function analysis Laws et al. were able to show that the rotamer model can be used to explain the complex fluorescence decay of tyrosine analogues [2,21], as well as tyrosine [21,22], and tryptophan residue [18] in small peptides. The rotamer model for fluorescence

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quenching in tyrosine or tryptophan derivatives implies that the shorter lifetime components will come from those rotamers in which the aromatic ring is at the shortest distance from carbonyl group(s). An analysis of these results, makes clear that the rotamer interconversion about C^{α}–C^{β} bond in tyrosine is slower than the lifetime of the excited state. Unfortunately, for tyrosine components exhibiting a single-exponential decay, Laws et al. [2] were unable to establish whether (i) the slow-exchange rotamer model is the accurate description, but the three rotamers have similar unresolved fluorescence lifetimes or (ii) the rotamers interconversion is fast enough averaging the emission. The rotamer model has also been used to explain acrylamide quenching of tyrosineamide [23]. The differential quenching of rotamers has also been supported by the suggestion of Tournon et al. [24], that the carbonyl groups can quench the fluorescence of aromatics efficiently by a charge transfer mechanism. Direct interaction between the peptide carbonyl or amide group and the phenol ring is responsible for the quenching of the tyrosine fluorescence in peptides and proteins as previously suggested by Cowgill [25–27] and Feitelson [28,29]. The charge-transfer reaction between the electrophilic unit in the amino acid backbone and the excited aromatic phenol subunit, leads to the bi-exponential fluorescence decay of tyrosine in an acidic aqueous solution. It is also reasonable that quenching is more efficient with protonated carboxyl groups since ionization would diminish the electron-accepting capability of the carbonyl function. This phenomenon was investigated by Kungl [30]. Based on the dynamics of tyrosine and the peptide Gly–Tyr–Gly in vacuo, and in water, calculated with classical molecular dynamics and with stochastic computer simulations, he concluded that, since the rotamers frequently interconvert within the fluorescence lifetime of tyrosine, their contribution to the non-exponential fluorescence decay should be negligible.

In this paper we present the results of our studies of the photophysical properties of (*p*-hydroxy)phenylglycine[Phg(OH)] (an analogue of tyrosine) and its derivatives with the unmodified hydroxyl group: Ac–Phg(OH)–OH, Phg(OH)–$NHCH_3$, Ac–Phg(OH)–$NHCH_3$, and the derivatives with the methylated phenolic hydroxyl group: Phg(OCH_3), Ac–Phg(OCH_3)–OH, Phg(OCH_3)–$NHCH_3$, Ac–Phg(OCH_3)–$NHCH_3$. They were used to study the influence of (i) the conversion of the carboxylic group into the amide, (ii) the *N*-acetylation of the amino group and (iii) the *O*-methylation of the phenolic oxygen on the photophysical properties of the tyrosine analogue. The data obtained are discussed on the basis of the rotamer theory.

2. Experimental details

2.1. Materials

p-(Hydroxy)phenylglycine was purchased from Aldrich. The following derivatives were synthesized in our laboratory:

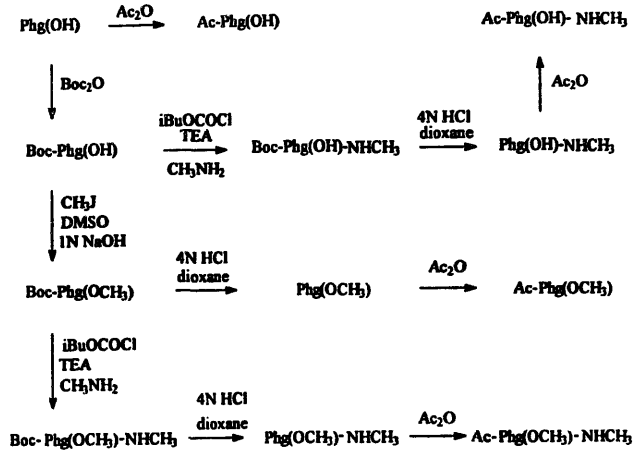

Fig. 1. Synthetic scheme.

Ac–Phg(OH)–OH, Phg(OH)–$NHCH_3$, Ac–Phg(OH)–$NHCH_3$, Phg(OCH_3), Ac–Phg(OCH_3)–OH, Phg(OCH_3)–$NHCH_3$, Ac–Phg(OCH_3)–$NHCH_3$ according to the scheme shown in Fig. 1. The *t*-butyloxycarbonyl group as a transient protection was used for all analogues except Ac–Phg(OH). The *O*-methylation of Boc–Phg(OH) was accomplished by the action of CH_3I in DMSO/H_2O in the presence of 3 equivalents of NaOH at 0 °C [31]. The *N*-methyl amides were synthesized in THF using a solution of 33% CH_3NH_2 in EtOH and *iso*-butyl chloroformate as a coupling reagent. Removal of *t*-butyloxycarbonyl group was performed by acidolysis with a solution of 4 N HCl in dioxane. Acetylation of the amino group was realized by reaction with acetic anhydride in THF/H_2O. After purification, by means of column chromatography, the chemical homogeneity of compounds (in a form of lyophilized powder) was checked by analytical RP-HPLC (a linear 60 minutes gradient from 0 to 80% CH_3CN in 0.1% TFA in H_2O at a flow rate of 1 ml min^{-1} on a Kromasil C-8 column, 4.6×250 mm, 5 μm) and by mass spectrometry: Ac–Phg(OH)–OH (R_t = 14.80 min; M^+ = 209 (FDMS)); Phg(OH)–$NHCH_3$ (R_t = 11.03 min; M^+ = 180 (FDMS)); Ac–Phg(OH)–$NHCH_3$ (R_t = 17.23 min; M^+ = 222 (FDMS)); Phg(OCH_3)–OH (R_t = 12.26 min; M^+ = 181 (FDMS)); Ac–Phg(OCH_3)–OH (R_t = 18.91 min; M^+ = 223 (FDMS)); Phg(OCH_3)–$NHCH_3$ (R_t = 14.40 min; M^+ = 194 (FDMS)); Ac–Phg(OCH_3)–$NHCH_3$ (R_t = 23.10 min; M^+ = 236 (FDMS)).

2.2. Methods

Fluorescence decays were collected by time-resolved single photon counting technique on an Edinburgh Analytical Instrument type CD-900 fluorimeter interfaced with an IBM PC AT. The excitation source was a flash lamp filled with 0.5 atm hydrogen, operated at 40 kHz with about 6.5 kV across a 1 mm electrode gap. The half width of the instrument response was 1.2 ns. The excitation (270 nm) and emission wavelengths (315 nm) were selected by means of monochromators (about 10 nm bandwidth).

Fluorescence decays from sample and the reference (Ludox, observation wavelength 315 nm) were measured to 10^4 counts at the peak. The counting rate did not exceed 2% of the lamp repetition rate. The decay curves were stored in 1024 channels of the 0.054 ns per channel. Fluorescence decay data were fitted by the iterative convolution to the sum of exponents:

$$I(\lambda, t) = \sum_i \alpha_i(\lambda) \exp\left(-\frac{t}{\tau_i}\right) \quad (1)$$

where τ_i is the decay time of the ith components and $\alpha_i(\lambda)$ is its pre-exponential factor at emission wavelength λ.

The adequacy of the exponential decay fitting was judged by an inspection of the plots of weighted residuals and by the statistical parameters χ^2 and the shape of the autocorrelation function of the weighted residuals and the serial variance ratio (SVR).

The steady-state spectra were obtained on a Perkin-Elmer LS-50B spectrofluorimeter with a 2.5 nm bandwidth for the excitation and the emission. The excitation wavelength was 270 nm. The quantum yields were measured relative to the value of 0.14 for tyrosine in water at room temperature [32]. A sample concentration was about 5×10^{-5} M in the steady-state measurement and 1×10^{-4} M in the time-resolved experiment. All measurements were performed in doubly deionized water, pH = 7.0 at room temperature.

Absorption spectra were measured on a Beckman model DU-600 spectrophotometer.

A systematic conformational search was performed on two model compounds, (1) Ac–Phg(OH)–OH and (2) Phg(OH)–$NHCH_3$, using the AMBER 4.1 program [33]. The potential energy surfaces plotted against dihedral angles for compounds (1) and (2), were generated maintaining those dihedrals as or close to fixed values and minimizing the energy while allowing all other variables to change freely. This method—called adiabatic relaxation—was employed with AMBER using CONS option in the PARM block. A harmonic constraining potential with the force constant of 400 kcal mol^{-1} was used to maintain the values of selected dihedrals within 0.5° of desired values. Values of energy were computed at every point of a two-dimensional grid on which two selected dihedrals (ϕ = C′–N–C^α–C, χ_1 = N–C^α–C^β–C^γ for Ac–Phg(OH) and ϕ = N–C^α–C–N′, χ_1 = N–C^α–C^β–C^γ for Phg(OH)–$NHCH_3$) were varied between −180° and 180° at 15° intervals. Calculations were performed using all-atom parameters on isolated molecule without solvent molecules; the solvation effect was approximated by distance-dependent dielectric constant.

3. Results and discussion

3.1. Steady-state fluorescence

Fluorescence and excitation spectra (*p*-hydroxy)-phenylglycine measured in H_2O at pH = 7.0 and appropriate spectra of tyrosine (for comparison) are shown in Fig. 2. Fluorescence spectra of all compounds were measured using the wavelength λ_{ex} = 270 nm for excitation. Excitation spectra were recorded at wavelength λ_{em} = 315 nm. The excitation spectrum of Phg(OH) displayed a blue shift (≈ 2 nm) when compared with the appropriate spectrum of tyrosine. Similar blue shifts in excitation and absorption spectra were observed for all derivatives of (*p*-hydroxy)phenylglycine studied. Emission spectra of Phg(OH) and its derivatives are also blue shifted (~ 2 nm) in comparison with the spectrum of tyrosine, whereas the shape of the fluorescence spectra remain almost the same. Similar changes in the emission and excitation spectra were observed for *O*-methylated derivatives of (*p*-hydroxy)phenylglycine. The quantitative characteristics of steady-state fluorescence measurements are collected in Table 1.

Analyzing the fluorescence measurements data obtained one can easily conclude that the quantum yield of the parent compound (Phg(OH)) is significantly higher (QY = 0.21) than that of tyrosine (QY = 0.14). The methylation of the phenolic oxygen of Phg(OH) increased the fluorescence quantum yield even more up to the value of QY = 0.29.

An introduction of substituents protecting the amino (acetyl) and the carboxyl group (*N*-methyl amide) generally

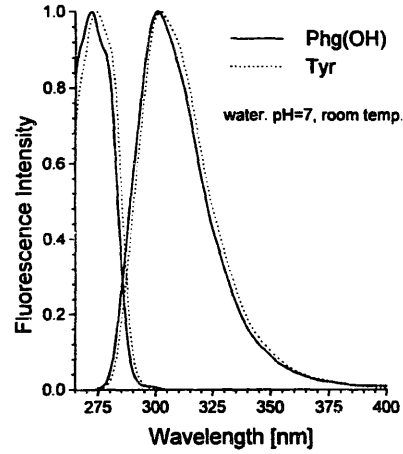

Fig. 2. Emission and fluorescence excitation spectra of Phg(OH) (solid line) and Tyr (dotted line) recorded in water at pH 7.

Table 1
Photophysical properties of Phg(OH) derivatives measured in water, pH = 7.0 at room temperature

Compound	τ (ns)	Quantum yield	χ^2_R
Phg(OH)	2.89	0.210	1.09
Ac–Phg(OH)	2.43	0.180	0.99
Ac–Phg(OH)–NHMe	1.50	0.112	0.95
Phg(OH)–NHMe	1.22	0.096	1.15
Phg(OCH_3)	4.07	0.290	1.12
Ac–Phg(OCH_3	3.14	0.223	1.07
Ac–Phg(OCH_3)–NHMe	2.69	0.194	1.10
Phg(OCH_3)–NHMe	2.96	0.212	1.04

decreased the quantum yields, both for Phg(OH) and Phg(OCH_3) derivatives but different groups quenched the fluorescence with different strength. *N*-Acetylation of Phg(OCH_3) caused the quenching of fluorescence by 23% in comparison with the parent compound, whereas the *C*-amidation resulted in quenching of the fluorescence by 27%. A fluorescence quantum yield for Ac–Phg(OCH_3)–$NHCH_3$ is about 67% of the quantum yield of the precursor (Phg(OCH_3)). This data suggests a superposition of the both substituents in the process of the fluorescence quenching of the excited derivative of Phg(OCH_3).

In the case of derivatives of Phg(OH) (without protection on the phenolic oxygen) the influence of the amino and the carboxylate substituents is not so regular as was observed for the appropriate derivative of Phg(OCH_3). The *N*-acetylation of Phg(OH) caused a 17% decrease of the fluorescence, whereas the introduction of the methyl amide moiety decreased the fluorescence quantum yield more than two times (see Table 1). The acetylation of Phg(OH)–$NHCH_3$ did not enhance the fluorescence quenching effect of the amide as was observed in the case of Ac–Phg(OCH_3)–$NHCH_3$; Ac–Phg(OH)–$NHCH_3$ had an even higher fluorescence quantum yield than the non-acetylated compound. The change of the efficiency of the fluorescence quenching by acetyl and/or the amide in *O*-methylated and in the non-methylated derivatives of (*p*-hydroxy)phenylglycine indicates the contribution of the phenolic hydroxyl to a fluorescence quenching process.

3.2. Time-decay of emission intensity

Time-decay curves of emission intensity for Phg(OH) and its derivatives with a protected or an unprotected amino and carboxyl group are shown in Figs. 3 and 4, whereas decay times are collected in Table 1. The fluorescence decays are monoexponential for all measured compound with χ^2_R value close to 1. The deviations between the measured and calculated fluorescence decay curves oscillate symmetrically around zero and no systematic deviations are observed. The obtained fluorescence decay time for Phg(OH) (τ=2.89 ns) is shorter than the one for tyrosine (τ=3.38 [4], 3.76 ns, [2]). This shorter decay time and the enhancement of the fluorescence quantum yield observed for Phg(OH) (at the same time) is the reason for the higher fluorescence rate constant (k_f) for Phg(OH) ($k_f=\phi/\tau=7.5\times10^7\ s^{-1}$) than for tyrosine ($k_f=3.5\times10^7\ s^{-1}$). The methylation of the phe-

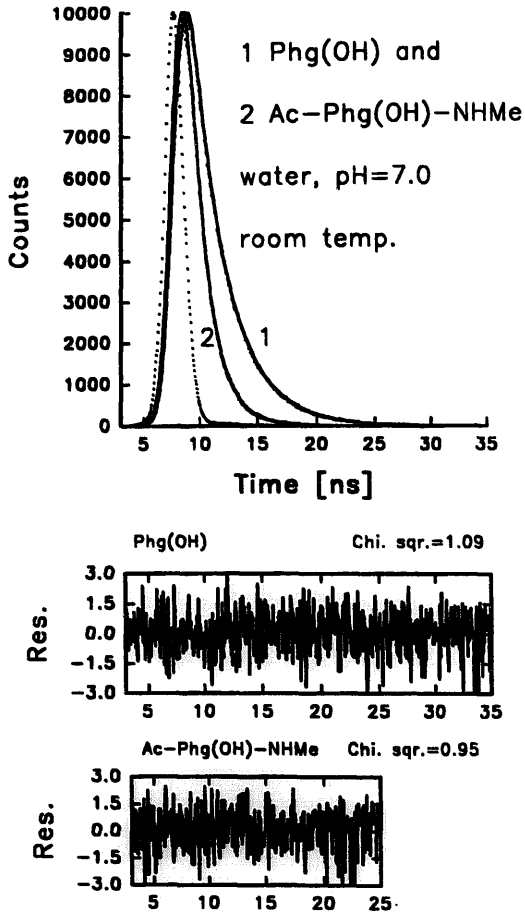

Fig. 3. Upper part: fluorescence decay curves for Phg(OH) and Ac–Phg(OH)–NHMe (solid lines) recorded in water at pH 7 and lamp profile (dotted line). Lower part: weighted residuals.

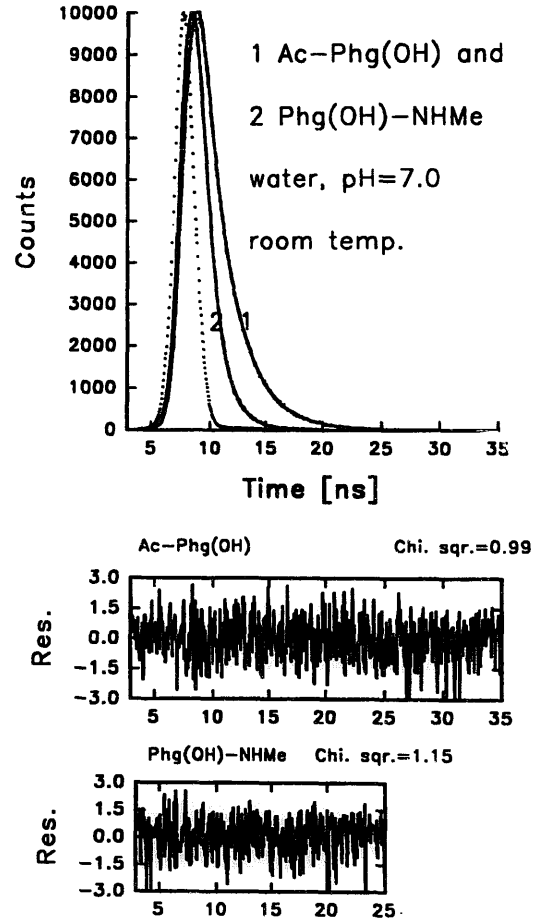

Fig. 4. Upper part: fluorescence decay curves for Ac–Phg(OH) and Phg(OH)–NHMe (solid lines) recorded in water at pH 7 and lamp profile (dotted line). Lower part: weighted residuals.

nolic oxygen of Phg(OH) increased the fluorescence decay time (τ=4.07 ns) but the fluorescence rate constant for this derivative remains almost unchanged ($k_f = 7.1 \times 10^7\ s^{-1}$). The same effect of the *O*-methylation was observed for tyrosine (the fluorescence decay times for Tyr(OCH_3) and for Tyr were τ=5.06 ns and τ=3.76 ns, respectively [2]). An interesting fact is that ratio of the decay times for the methylated and non-methylated (*p*-hydroxy)phenylglycine and tyrosine: $\tau_{Phg(OMe)}/\tau_{Phg(OH)} = 1.41$ and $\tau_{Tyr(OMe)}/\tau_{Tyr} = 1.49$ is similar. The fluorescence decay times obtained for the acetylated and amidated derivatives of (*p*-hydroxy)-phenylglycine vary as the quantum yield. In the case of Ac–Phg(OH) and Ac–Phg(OCH_3) the fluorescence quenching caused by acetyl is more effective than for the corresponding derivatives of tyrosine (Table 1). The decay times (τ) obtained for Ac–Tyr and Tyr by Laws et al. [2] were 3.6 ns and 3.76 ns, respectively, whereas Gauduchon and Wahl [4] obtained shorter decay times (3.2 ns for Ac–Tyr and 3.38 ns for Tyr), but their measurements were carried out in similar conditions to ours (λ_{ex}=270 nm, λ_{em}=315 nm, room temp.). The acetylation of Phg(OH)–$NHCH_3$ increases both the quantum yield and the fluorescence lifetime—contrary to the effects of the acetylation observed for Phg(OCH_3)–$NHCH_3$. Because of the *N*-acetylation of the amino group an increased fluorescence decay time was also observed for Tyr–NH_2 [2,4]. According to Laws et al. [2], the fluorescence decay time for Ac–Tyr–NH_2 is $\langle\tau\rangle_I$=2.26 ns and according to Gauduchon and Wahl [4] $\langle\tau\rangle_I$=1.61 ns, which is significantly longer than the decay time observed for Tyr–NH_2: 1.75 ns [2] and 1.11 ns [4]. Unfortunately there are no fluorescence data (decay times) for *O*-methylated tyrosine derivatives, so a comparison with our *O*-methylated (*p*-hydroxy)phenylglycine derivatives cannot be carried out. The fluorescence rate constants (k_f) for all derivatives of Phg(OCH_3) vary slightly around value $k_f = 7.1 \times 10^7\ s^{-1}$ and value $k_f = 7.5 \times 10^7\ s^{-1}$ for all derivatives of Phg(OH). These data suggest that the fluorescence quenching of the studied derivatives of Phg(OH) caused by acetyl or the amide substituent increased radiationless rate constant of a deactivation of the excited state.

4. Conclusions

The results obtained support Cowgill's [27] suggestion that (i) the interaction between peptidic carbonyl and the phenolic chromophore is responsible for tyrosine fluorescence quenching regardless of whether the carbonyl is located in a peptide bond or terminal group, and that (ii) the quenching is an intramolecular process requiring a hydrated carbonyl that facilitates charge transfer and has strict spatial requirements [34]. These data confirm the rotamer model introduced by Laws et al. [2] for the fluorescence decay of an aromatic amino acid. This model predicts that the amplitude of the kinetic components corresponds to the ground-state rotamer population, and the rotamer in which the phenol ring can come into closest contact with the carbonyl group has the shortest fluorescence lifetime. In our case theoretical calculations showed that for Ac–Phg(OH) and Phg(OH)–$NHCH_3$ and for Ac–Phg(OH)–$NHCH_3$ in ground state only one conformer is present (for Ac–Phg(OH) $\phi = -168°$ and $\chi_1 = 60 \pm 180°$; for Phg(OH)–$NHCH_3$ $\phi = -180°$ and $\chi_1 = 68 \pm 180°$). So, for all the derivatives of Phg(OH) and Phg(OMe) investigated single exponential fluorescence decay was obtained.

In the case of the *N*-acetyl derivative of Phg(OH) and Phg(OCH_3) the fluorescence quenching caused by acetyl is more efficient than for the corresponding derivatives of tyrosine. This higher efficiency of the fluorescence quenching of the derivatives of (*p*-hydroxy)phenylglycine by acetyl group could be explained as a result of the shorter distance between the chromophore (phenol ring) and the quenching group (acetyl) in (*p*-hydroxy)phenylglycine (the amino group in tyrosine is further from the chromophore by one $-CH_2-$ moiety).

More complex is the quenching of the fluorescence of the phenolic chromophore by a carboxyamide group. The *N*-methyl amide quenches the fluorescence of the derivatives of Phg(OCH_3) only slightly more efficiently than the acetyl group—the carbonyl of the carboxyamide group is closer to the chromophore than the acetyl. In the case of the derivatives of Phg(OH) the quenching of the fluorescence by the carboxyamide group is significantly more pronounced than for the *O*-methylated compounds, and thus the Phg(OH) derivatives are more similar to tyrosine, as far as their photophysical behavior is concerned. A literature survey of tyrosineamide (Tyr–NH_2) decay time data revealed that the averaged intensity lifetime is 1.75 ns [2] or 1.11 ns [4] (depending on the data used in the calculations) and it is almost three times shorter than for the parent tyrosine. Despite the fact, that the amide group in Tyr–NH_2 is further from the chromophore than the *N*-methyl amide group in Phg(OH)–$NHCH_3$ the efficiency of the fluorescence quenching in the tyrosine derivative is higher. This phenomenon can be partially explained as the result of the difference in acceptor characteristics of $-CO-NH_2$ and $-CO-NHCH_3$ groups. The donor properties of substituents at the nitrogen of the carboxyamide group can influence acceptor abilities of the carbonyl group, due to modification of charge distribution on its oxygen and carbon. The donor properties of *N*-substituents for the carboxyamides discussed vary in the order $H < -CH_3 < -CH_2COO^-$, although the acceptor properties of the resulting amides should influence their fluorescence quenching abilities. The most efficient quencher of fluorescence of the phenolic chromophore is the $-CO-NH_2$ group followed by primary and secondary amides. Confirmation of these quenching properties of the amides came from the studies of average fluorescence lifetimes for the dipeptide Tyr–Gly and Tyr–NH_2 performed by Laws [2], and Gauduchon and Wahl [4]. They found that the average fluorescence lifetime of Tyr–Gly is 20% longer than that of Tyr–NH_2. Such particular influence of *N*-substituents on the acceptor properties of the

carboxyamide carbonyl proved that quenching of the fluorescence of the phenolic chromophore occurs via a charge transfer mechanism. It seems that in the case of the amide containing derivatives of (*p*-hydroxy)phenylglycine in which steric hindrance is higher than in tyrosine derivatives, also the level of hydration of the carbonyl and its space orientation has to be considered.

Comparison of the fluorescence data obtained for the derivatives of (*p*-hydroxy)phenylglycine (*O*-methylated and non-methylated, as well) containing the methylamide moiety and with/without acetylated amino group indicates clearly the importance of phenol hydroxyl group in the quenching process. This is particularly evident for compounds with the unprotected amino group [Phg(OH)–$NHCH_3$ and (Phg(OCH_3)–$NHCH_3$]. Even a thorough analysis of the data obtained did not solve the problem of the importance of the hydroxyl group in the quenching process, but it is known that the excited state changes the charge distribution in the phenol ring so that the acidity of the hydroxyl is significantly increased. On the other hand the excited-state proton transfer reaction from tyrosine to water is too slow to compete effectively with other deactivation pathways [34,35].

The quenching properties of the ammonium group in tryptophan and tyrosine has been discussed in terms of its electrostatic effect on the quenching efficiency of the carbonyl group and it is not attributed directly to a straight quenching process [4,14,36,37]. The α-ammonium substituent quenches the fluorescence of tryptophan by either a proton transfer [38], or an electron transfer mechanism [39,40]. In our studies of (*p*-hydroxy)phenylglycine derivatives the univocal mechanism of fluorescence quenching by the ammonium group cannot be established. The electrostatic field produced by the ammonium group in the derivatives of tyrosine with protected carboxylate can change the polarization of the surrounding water molecules which will lead to higher proton accepting properties of water and finally to a higher possibility of ionization of the phenolic hydroxyl. Low quantum yield of fluorescence of tyrosine with ionized hydroxyl and its appearance at longer wavelength (λ_{max} = 345 nm) [34] can cause an apparent quenching effect of tyrosine fluorescence.

The fluorescence quenching of tyrosine and analogues, and their derivatives is a highly complex process. All functional groups in the molecule (phenolic hydroxyl, amino group and carboxylate) or their derivatives (acylated amino group, alkylated hydroxyl, carboxyamine) contribute to the quenching. The functional groups sometimes quench the fluorescence of the phenolic chromophore, enhancing the effect of the other groups and sometimes not. Our fluorescence data of the derivatives of *p*-hydroxy)phenylglycine generally support the rotamer theory, but to establish a universal mechanism of fluorescence quenching synthesis of sterically constrained analogues of tyrosine with a lower possibility of rotation followed by their fluorescence studies will be required.

Acknowledgements

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