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Acidity of carboxyl group of tyrosine and its analogues and derivatives studied by steady-state fluorescence spectroscopy

Mariusz Szabelski, Katarzyna Guzow, Alicja Rzeska, Joanna Malicka, Monika Przyborowska, Wiesław Wiczk*

Faculty of Chemistry, University of Gdańsk, Sobieskiego 18, 80-952 Gdańsk, Poland

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Abstract

The acidity of the carboxyl group of tyrosine and its derivatives and analogues was studied by means of fluorimetric titration using a steady-state fluorescence method. The pK_a value of carboxyl group of tyrosine, its analogues and derivatives with blocked amino or hydroxyl group or both determined from the fluorimetric titration curve indicates that the methylation of hydroxyl group of phenolic ring, as well as the position of carboxyl group with respect to the phenol ring (Tyr, β-Tyr, β-Hty, Phg, Tic(OH)) have a minor influence on the value of pK_a. The conversion of a protonated amino group of tyrosine or its analogues to the *N*-acetyl derivatives or its removal result in major lowering of the acidity of carboxyl group. The introduction of an additional hydroxyl group into a phenolic ring (Dopa) slightly increased acidity of the carboxyl group compared to tyrosine, whereas the replacement of α-hydrogen atom in Dopa by α-methyl group caused increase of pK_a to the value observed for tyrosine. The pK_a values of acetyl group of amino acid studied are in the range from 0.3 to 0.6.

Keywords: Steady-state fluorescence spectroscopy; *N*-acetyl derivatives; Carboxyl group of tyrosine

1. Introduction

Tyrosine is one of three naturally occurring amino acids, besides phenylalanine and tryptophan, that contributes to ultraviolet fluorescence [1–4]. The emission of tyrosine in water occurs at 303 nm and is relatively insensitive to a solvent polarity. Tyrosine is often regarded as a simple fluorophore. In the case of the tyrosine zwitterion and tyrosine derivatives with an ionized α-carboxyl group mono-exponential fluorescence decay was observed, with a fluorescence lifetime about 3.3–3.4 ns [5–8] and their fluorescence quantum yield is equal to 0.14 [9]. However, under some circumstances, tyrosine can display complex spectral properties [5]. After incorporation of tyrosine into a peptide chain, its fluorescence quantum yield decreases, whereas the fluorescence intensity decay becomes heterogeneous [4,5]. Also, conversion of the α-carboxyl group of tyrosine or its analogues to the corresponding amide causes the quenching of their fluorescence. The influence of the conversion of carboxyl group into an amide group or the amino group into the *N*-acetyl group of tyrosine or its analogues on their photophysical properties was widely investigated [10–16]. Both, Cowgill [17,18] and Tournon et al. [19] suggested that the fluorescence quenching of aromatic amino acid by peptide (amide) group was caused by a charge transfer between the excited aromatic chromophore (phenolic ring), as a donor, and electrophilic units in the amino acid backbone (carbonyl of amide bond), as an acceptor. This mechanism of fluorescence quenching of tyrosine derivatives was further supported by the dependence of quenching efficiency on the distance between the phenol of tyrosine residue and amide group [15,16], as well as by the dependence of electron transfer rate constant on the ionization potential of the excited fluorophore in a series of amidated tyrosine analogues and derivatives [14]. The acetylation of the amino group of tyrosine or its analogues causes moderate decrease of the fluorescence quantum yield and the fluorescence lifetime, but the fluorescence intensity decay is still mono-exponential [5]. The methylation of the hydroxyl group of phenolic ring of tyrosine increases both, the fluorescence quantum yield and the fluorescence lifetime as compared to tyrosine, but does not change the character of the fluorescence intensity decay of tyrosine itself as well as the amide or *N*-acetyl derivative [5,16]. It is known that the protonation of tyrosine carboxyl group decreases the fluorescence quantum yield [5,17,18,20] and the fluorescence lifetime [7–9,21–24]. In the pH range 4–8,

* Corresponding author. Tel.: +48-58-345-03-53; fax: +48-58-341-03-57.
E-mail address: ww@chemik.chem.univ.gda.pl (W. Wiczk).

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the fluorescence quantum yield of tyrosine is constant and the decay is mono-exponential with lifetime about 3.4 ns, which indicates that the excited-state proton transfer from phenolic hydroxyl to water, though thermodynamically allowed, is too slow to affect the fluorescence decay [5,10,21]. For pH > 8, the fluorescence quantum yield decreases, while the fluorescence lifetime remains constant because of tyrosinate formation which fluorescence is negligible [21,22].

The ground-state pK_a value for tyrosine carboxyl group was determined using spectrophotometric, fluorimetric and potentiometric methods. The published values for tyrosine are as follows: 2.32 ± 0.05 [25], 2.3 [20], 2.8 [21], 2.25 [7], 2.2 [22], 2.25 [26]. The pK_a value of carboxyl group of 3-(4-hydroxy)-propionic acid, an analogue of tyrosine devoid of an amino group, is 4.5 [10] or 4.9 [20], which indicates that the presence of protonated amino group has a strong influence on acid–base properties of carboxyl group. A direct interaction between the carbonyl group and the phenolic ring, resulting in quenching of the excited state and lowering the pK_a of carboxyl group of tyrosine in the excited state was suggested by Pal et al. [7] and Fayet and Whal [22]. Basing on differences between pK_a values in the ground and excited state for tyrosine isomers (*o*-, *m*- and *p*-tyrosine), Pal et al. [7] suggested that the interaction of carboxyl group with the chromophore via hydroxyl of phenolic ring exists in the excited state.

In order to check how the form of an amino group (protonated or acetylated) and/or hydroxyl group of phenolic ring (free or methylated) and position of carboxyl and amino group with respect to the phenol chromophore influence on acidity of carboxyl group, we measured the fluorescence quantum yield of tyrosine and its analogues and derivatives as a function of pH (from 0 to neutral) using steady-state fluorescence method.

2. Material and methods

Tyrosine (99+%) (Tyr), *p*-hydroxy-phenylalanine (Phg (OH)), *O*-methyl-tyrosine (Tyr(Me)), (3-hydroxy)tyrosine (Dopa) were purchased from Aldrich, 3-(4-hydroxyphenyl)-propionic acid (PPA) from Lancaster, *N*-acetyl-tyrosine (AcTyr) and α-methyl-Dopa (α-MeDopa) from Sigma, whereas hydrochloric acid "Suprapur" was received from Merck. Tyr, Dopa, Tyr(Me), α-MeDopa and PPA were used without further purification. The synthesis of an appropriate amino acid and its derivatives were performed according to the published procedures: 7-hydroxy-tetrahydrisoquinoline-3-carboxylic acid (Tic(OH)) [27], Phg(OH) [28], *O*-methyl-β tyrosine (β-Tyr(Me)) [29], β-homo-tyrosine (β-Hty) [30], Dopa and Tyr(Me) derivatives [14]. All prepared derivatives were purified by reversed-phase liquid chromatography (RP-HPLC) and the purity of all compounds studied was assessed by analytical RP-HPLC, ^{1}H NMR and mass spectrometry (FD or FAB) were used to identify them.

The structure of studied compounds are shown in Fig. 1.

Absorption spectra were recorded using a Perkin-Elmer Lambda 40P spectrophotometer. Fluorescence spectra were recorded using a Perkin-Elmer LS-5OB spectrofluorimeter with 2.5 nm band-width for excitation and emission. The excitation wavelength was 275. The optical density of the sample at this excitation wavelength did not exceed 0.1. The fluorescence quantum yields were obtained by comparing the integral intensity of the steady-state emission spectra (corrected for absorbance) with that of tyrosine in water, using a value of 0.14 for the latter [6]. The sample concentration was about 5×10^{-4} M. All measurements were performed in double deionized water (Milipore) and pH was adjusted using HCl solution using a Hanna Instruments pH-meter model HI9327 and HI1330B electrode. The pH was measured directly in a cuvette. All measurements were performed at 20 °C. No buffer was used. The pK_a values calculation for all compounds studied were performed using a nonlinear fitting method according to the equation:

$$\mathrm{QY} = \frac{[\mathrm{QY}_1 + \mathrm{QY}_{\mathrm{int}} 10^{(\mathrm{pH}-\mathrm{p}K_1)}]}{[10^{(\mathrm{pH}-\mathrm{p}K_1)} + 1]} + \frac{[\mathrm{QY}_{\mathrm{int}} + \mathrm{QY}_2 10^{(\mathrm{pH}-\mathrm{p}K_2)}]}{[10^{(\mathrm{pH}-\mathrm{p}K_2)} + 1]} \quad (1)$$

where QY is the fluorescence quantum yield at lowest (QY_1), medium ($\mathrm{QY}_{\mathrm{int}}$) and highest ($\mathrm{QY}_2$) pH, respectively, using Origin version 6.1 software (OriginLab, Northampton, MA).

3. Results and discussion

The absorption and fluorescence spectra of tyrosine its analogues and derivatives in water are characteristic for tyrosine and do not change after the addition of hydrochloric acid. However, the fluorescence intensity decreases considerably, particularly in the pH range from 3 to 0. The dependence of the fluorescence quantum yield on pH for Tyr, its derivatives and PPA are shown in Fig. 2, whereas for β-Hty and its derivatives with blocked amino or hydroxyl group in Fig. 3. The fluorescence quantum yields and pK_a values of carboxyl group (pK_a) and protonated *N*-acetyl group (pK_a^{Ac}) of tyrosine and its analogues and their derivatives in water at room temperature are presented in Table 1. The fluorescence quantum yields presented in Table 1 for the pH = 0, 7 and for intermediate pH (from the plateau for *N*-acetylated compounds) are obtained from the nonlinear fit of the Eq. (1) to the experimental data, with the fluorescence quantum yield and pK_a as floating parameters. For all compounds studied except Tic(OH), the fluorescence quantum yields at pH = 0 are small and do not exceed several percents. For neutral pH, the fluorescence quantum yield of amino acids studied is more diversified and generally much higher than that at pH = 0, which indicates that the protonated carboxyl group is a strong quencher, which is compatible with the literature data concerning tyrosine [4,5,7,8,10,17,18,20,23], PPA

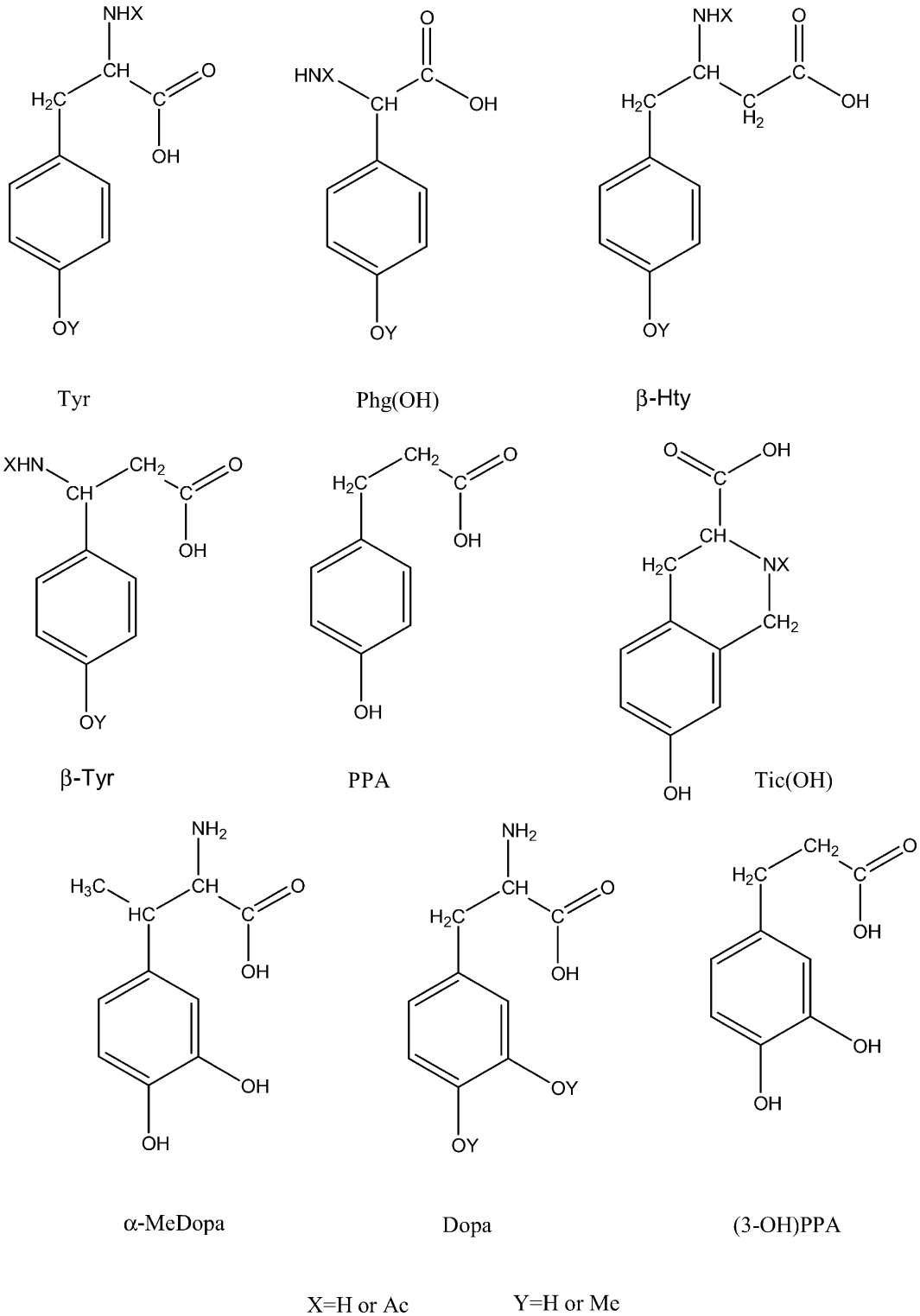

Fig. 1. Line drawing structure of compounds studied.

[5,10], and tyrosine isomers [7]. However, for compounds with the methylated hydroxyl group of phenolic ring the fluorescence quantum yield increases in comparison to that of parent molecules, which indicates that the hydroxyl group fulfills the essential role in the fluorescence quenching of phenolic fluorophore as a result of hydrogen bond formation with the solvent molecule(s) [5]. For *N*-acetylated derivatives, except β-Hty, the fluorescence quantum yields are lower than that for the parent molecule and the efficiency of fluorescence quenching by the acetyl group depends on

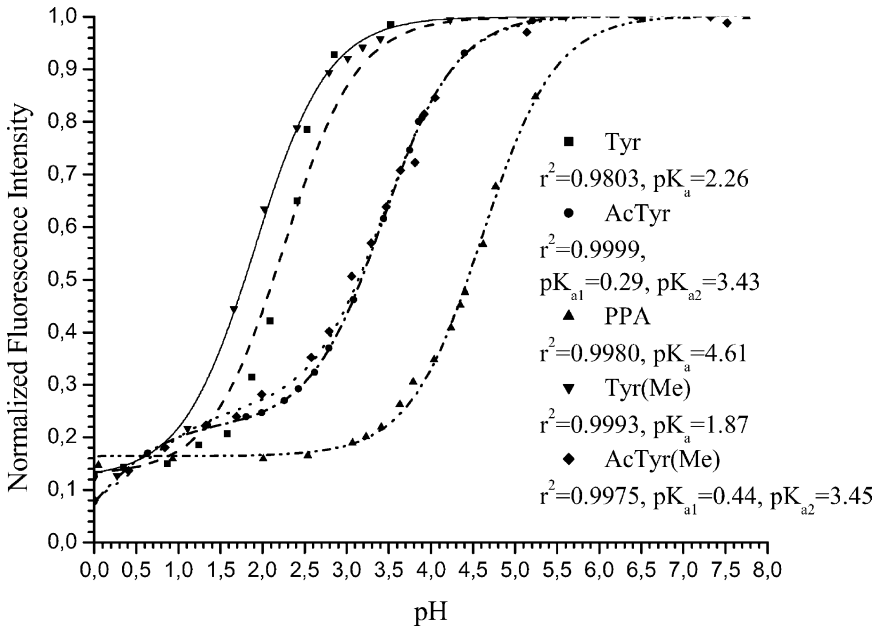

Fig. 2. Fluorimetric titration curves of Tyr and its derivatives and PPA. Lines represent the best fit of Eq. (1) to the experimental points.

both the form of the hydroxyl group and the position of amino group in relation to the phenolic ring.

The pK_a value of PPA, an analogue of tyrosine devoid of the amino group, obtained from the fluorimetric titration or potentiometric titration are 4.612 ± 0.001 and 4.65 ± 0.02, respectively [8], and is comparable to that of alkyl carboxylic acid. For all amino acids studied, possessing protonated amino group, the pK_a values are lower than that of PPA, but higher than for *N*-acetylated derivatives which indicates that there is a strong inductive effect of the protonated amino group compared to *N*-acetyl group or hydrogen atom connected to the α-carbon atom. The position of both, the protonated amino group and the phenolic ring in relation to the carboxyl group as well as the conformational freedom have an influence on the acidity of the carboxyl group. For the compounds with lower conformational freedom, Phg(OH) [28] in which all phenolic ring, amino and carboxyl group are connected to the same carbon atom and Tic(OH) [27] where the amino group is connected with the phenolic ring by methylene group, the pK_a value is lower

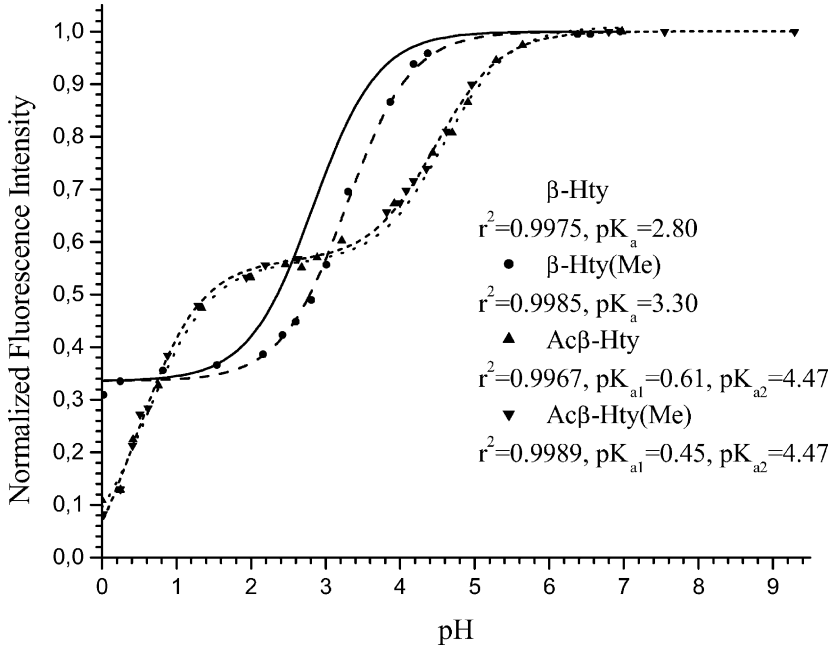

Fig. 3. Fluorimetric titration curves of β-Hty and its derivatives. Lines represent the best fit of Eq. (1) to the experimental points.

than that observed for tyrosine. The displacement of the amino group from the α-carbon to the β-carbon atom causes the lowering of pK_a of carboxyl group (pK_a Tyr(Me) < pK_a β-Tyr(Me) < pK_a β-Hty(Me)), but simultaneously it depends on the position of the phenolic ring in relation to the amino group (pK_a β-Tyr(Me) < pK_a β-Hty(Me)).

The values of pK_a depend, in a small degree, on the kind of substituent of phenyl ring (hydroxy or methoxy group), but in this case it is difficult to find a straight relation between pK_a and distance between the carboxyl and/or amino group and phenolic ring (pK_a for Tyr decreases, for Phg(OH) is unchanged, whereas for β-Hty increases for about 0.5 unit).

As was previously stated, the conversion of the amino group into *N*-acetyl one caused a substantial decrease of the acidity of carboxyl group for all amino acids studied, but also changed the character of the titration curve. The exceptions are AcTic(OH) and AcPhg(OH) for which two-state Henderson–Hasselblach relation observed for parent molecule is preserved. For other compounds studied, an additional inflection point at low pH is observed, which could be connected with a protonation of the *N*-acetyl group. The lack of the fluorescence quenching of parent molecule in the low pH range allows to exclude the protonation of phenolic ring as a process responsible for the fluorescence quenching of *N*-acetyl derivatives at low pH range. The pK_a values of the protonated *N*-acetyl group for *O*-methylated tyrosine derivatives (Tyr(Me), β-Tyr(Me) and β-Hty(Me)), are the same p$K_a^{Ac} = 0.45$, and are higher than expected for model amide group (−0.5) or first protonation constant estimated for simple peptides or proteins (from −3 to −4) [30]. More diversified are pK_a^{Ac} for amino acids with free hydroxyl group (Tyr (0.29 ± 0.08) and Hty (0.61 ± 0.08)) which indicate that there are some interaction between the hydroxyl group of phenolic ring and protonated acetyl group including the hydrogen bond network formation with the solvent molecule(s). The strength of those interactions depends on the spatial proximity of the interacting group thereby on the conformational freedom of the amino acid.

Pal et al. [7] revealed that the position of hydroxyl group in the phenolic ring of the *o*-, *m*- and *p*-tyrosine has an effect on the pK_a value, both in ground and excited states, indicating on the interaction between the hydroxyl of phenolic ring and carboxyl group in the ground and excited state. In order to check the influence of the additional hydroxyl group introduced into the phenolic ring on the acidity of the carboxyl group, we measured the fluorescence intensity of 3,4-dihydroxy phenylalanine (Dopa), its di-*O*-methyl derivative (Dopa(Me)$_2$) and 3-(3,4-dihydroxyphenyl)-propionic acid ((3-(OH)PPA) as a function of pH. The pK_a values of aforementioned compounds are presented in Table 1. For (3-OH)PPA, the pK_a value is a little higher (4.77 ± 0.09) than for PPA, whereas for Dopa it is lower than that for Tyr which indicate on some interaction between hydroxyl and the carboxyl group. The conversion of the hydroxyl group of Dopa to the methoxy group caused the increase of the

Table 1
The fluorescence quantum yields and pK_a values of carboxyl group (pK_a) and protonated *N*-acetyl group pK_a^{Ac} of tyrosine and its analogues and their derivatives in water at room temperature

Compound	QY (calculated from fitted curve)[a]				pK_a	pK_a^{Ac}
	pH = 0	QY (intermediate)	pH (intermediate)	pH = 7		
Tyr	0.018	–	–	0.14	2.26 ± 0.05[b], 2.37 ± 0.04[c]	–
AcTyr	0.010	0.03	2.04	0.131	3.43 ± 0.01, 3.52 ± 0.02[c]	0.29 ± 0.08, 0.45 ± 0.10[c]
Tyr(Me)	0.019	–	–	0.187	1.87 ± 0.02	–
AcTyr(Me)	0.003	0.042	1.7	0.167	3.34 ± 0.01	0.44 ± 0.09
Phg(OH)	0.001	–	–	0.098	2.12 ± 0.02	–
AcPhg(OH)	0.003	–	–	0.08	3.26 ± 0.01	–
Phg(OMe)	0.005	–	–	0.141	2.13 ± 0.01	–
AcPhg(OMe)	0.006	–	–	0.104	3.17 ± 0.02	–
β-Tyr(Me)	0.090	–	–	0.207	3.12 ± 0.04	–
Acβ-Tyr(Me)	0.000	0.043	2.12	0.16	4.24 ± 0.03	0.45 ± 0.06
β-Hty	0.052	–	–	0.148	2.80 ± 0.03	–
Acβ-Hty	0.000	0.087	2.48	0.152	4.47 ± 0.07	0.61 ± 0.08
β-Hty(Me)	0.067	–	–	0.195	3.30 ± 0.02	–
Acβ-Hty(Me)	0.000	0.069	2.69	0.169	4.47 ± 0.03	0.45 ± 0.05
Tic(OH)	0.108	–	–	0.202	2.16 ± 0.05	–
AcTic(OH)	0.006	–	–	0.13	3.44 ± 0.02	–
PPA[b]	0.030	–	–	0.164	4.612 ± 0.001[b], 4.65 ± 0.02[c]	–
Dopa	0.001	–	–	0.094	1.97 ± 0.01	–
Dopa(Me)$_2$	0.009	–	–	0.165	2.36 ± 0.07	–
(3-OH)PPA[c]	0.013	–	–	0.028	4.77 ± 0.09	–
α-MeDopa	0.003	–	–	0.082	2.27 ± 0.03	–

[a] Error in the fluorescence quantum yield estimation was no higher than 0.001.
[b] Data from [8].
[c] From potentiometric titration [31].

pK_a value. The same effect was observed for all studied amino acids, except for tyrosine. The replacement of hydrogen atom at α-carbon atom of Dopa by a methyl group caused increase of pK_a value, probably because of either the decrease of the conformational freedom which prevents from the interaction between the hydroxyl and the carboxyl group or the inductive effect of the α-methyl group.

As was previously observed from time-resolved and steady-state fluorescence measurements [8], the fit of the fluorescence quantum yield of tyrosine to the Henderson–Hasselblach equation is not as good as the fit of the fluorescence quantum yield of all other compounds studied. For tyrosine a substantial deviation of the experimental points from the fitting curve in the middle pH range is observed, whereas for all other compound studied, all experimental points lie (in the experimental range of error) on the fitting curve. Thus, the deviation from the Henderson–Hasselblach equation observed only for tyrosine as well as the differences between pK_a values determined from fluorimetric and potentiometric titrations indicates that for this particular amino acid with unblocked amino and phenolic hydroxyl group the spatial location of functional groups and conformational freedom on the C^{α}–C^{β} bond favor the interaction between them in the excited state causing the increase of acidity of the carboxyl group. The presence of an additional hydroxyl group (Dopa) increases this effect.

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References

[1] A.P. Demchenko, Ultraviolet Spectroscopy of Proteins, Springer-Verlag, New York, 1981.
[2] E.A. Permyakov, Luminescent Spectroscopy of Proteins, CRC Press, Boca Raton, Florida, 1993.
[3] I. Weinryb, R.F. Steiner, The luminescence of aromatic amino acid, in: R.F. Steiner, I. Weinryb (Eds.), Excited States of Proteins and Nucleic Acid, Plenum Press, New York, 1971, pp. 277–318.
[4] J.R. Lakowicz, Principles of Fluorescence Spectroscopy, 2nd Edition, Kluwer Academic Publishers, Dordrecht, 1999, pp. 448–514.
[5] J.B.A. Ross, W.R. Laws, K.W. Roussland, H.R. Wyssbrod, Tyrosine fluorescence and phosphorescence from proteins and polypeptides, in: J.R. Lakowicz (Ed.), Topics in Fluorescence Spectroscopy, Vol. 3, Biochemical Applications, Plenum Press, New York, 1992, pp. 1–63.
[6] C. Seidel, A. Orth, K.-O. Greulich, Photochem. Photobiol. 58 (1993) 178.
[7] H. Pal, D.K. Palit, T. Mukherejee, P. Mittal, J. Photochem. Photobiol. A: Chem. 52 (1990) 391.
[8] K. Guzow, M. Szabelski, A. Rzeska, J. Karolczak, H. Sulowska, W. Wiczk, Chem. Phys. Lett., submitted for publication.
[9] R.F. Chen, Anal. Lett. 1 (1967) 35.
[10] W.R. Laws, J.B.A. Ross, H.R. Wyssbrod, J.M. Beechem, L. Brand, J.C. Sutherland, Biochemistry 25 (1986) 599.
[11] P. Gauduchon, P. Whal, Biophys. Chem. 8 (1978) 87.
[12] P.B. Contino, W.R. Laws, J. Fluorescence 1 (1991) 5.
[13] A.R. Rehms, P.R. Callis, Chem. Phys. Lett. 208 (1993) 276.
[14] W. Wiczk, A. Rzeska, J. Łukomska, K. Stachowiak, J. Karolczak, J. Malicka, L. Łankiewicz, Chem. Phys. Lett. 341 (2001) 99.
[15] C. Seidel, A. Orth, K.-O. Greulich, Photochem. Photobiol. 58 (1993) 178.
[16] J. Łukomska, A. Rzeska, J. Malicka, W. Wiczk, J. Photochem. Photobiol. A: Chem. 142 (2001) 135.
[17] R.W. Cowgill, Biochim. Biophys. Acta 133 (1967) 6.
[18] R.W. Cowgill, Arch. Biochem. Biophys. 100 (1963) 36.
[19] J.E. Tournon, E. Kuntz, M.A. El Bayoumi, Photochem. Photobiol. 16 (1972) 425.
[20] J. Feitelson, J. Phys. Chem. 68 (1964) 391.
[21] D.M. Rayner, D.T. Kraycarski, A.G. Szabo, Can. J. Chem. 56 (1978) 1238.
[22] M. Fayet, P. Wahl, Biochim. Biophys. Acta 221 (1971) 102.
[23] J.R. Lakowicz, G. Laczko, I. Gryczynski, Biochemistry 26 (1987) 82.
[24] K.J. Wilis, A.G. Szabo, J. Phys. Chem. 95 (1991) 1585.
[25] R.B. Martin, J.T. Edsall, D.B. Wetlaufer, B.R. Hollingworth, J. Biol. Chem. 233 (1958) 1429.
[26] T. Kiss, B. Thot, Talanta 29 (1982) 539.
[27] W. Wiczk, K. Stachowiak, P. Skurski, L. Łankiewicz, A. Michniewicz, A. Rój, J. Am. Chem. Soc. 118 (1996) 8300.
[28] W. Wiczk, K. Stachowiak, C. Czaplewski, L. Łankiewicz, A. Michniewicz, J. Photochem. Photobiol. A: Chem. 102 (1997) 189.
[29] W. Wiczk, L. Łankiewicz, C. Czaplewski, S. Ołdziej, K. Stachowiak, A. Michniewicz, B. Micewicz, A. Liwo, J. Fluorescence 7 (1997) 257.
[30] D.B. Weltaufer, Ultraviolet spectra of proteins and amino acids, in: C.D. Anfinsen Jr., M.L. Anson, K. Bailey, J.T. Edsall (Eds.), Advance of Proteins Chemistry, Vol. 17, Academic Press, New York, 1962.
[31] H. Sulowska, T. Ossowski, unpublished results.