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Photophysical properties of β-homo-tyrosine derivatives

Short Communication

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Summary. The observed monoexponential fluorescence decay of N-acetyl-β-homo-tyrosine methylamide (Ac-βHty-NHMe) (I) and N-acetyl-(O-methyl)-β-homo-tyrosine methylamide (Ac-βHty(OMe)-NHMe) (II) is supporting the rotamer population theory, according to which rotamers are responsible for heterogeneity of the fluorescence decay of N-acetyl-tyrosine amide or tyrosine incorporated within a peptide chain, in general.

Keywords: Amino acids – β-Homo-tyrosine derivatives – Fluorescence

Tyrosine zwitterion and analogues with an ionized α-carboxyl group exhibit monoexponential decay kinetics. Conversion of the α-carboxyl group to the corresponding amide results in a fluorescence intensity decay that requires at least a double exponential to fit the data. Gauduchon and Wahl (1978) suggested that the complex kinetics could be explained in terms of rotamer populations resulting from rotation about the C^{α}—C^{β} bond. They proposed, that the shorter time constant, observed for analogues with amide group, was caused by the fluorescence quenching based on a contact of the phenol ring with the carbonyl group and the longer time constant was the average of the decays of the remaining two rotamers. The rotamer population theory has been supported by the fluorescence and ^{1}H NMR studies of tyrosine and tyrosine analogues (Laws et al., 1986), and the measurements of tyrosine fluorescence in oxytocin and desaminodicarbaoxytocin (Ross et al., 1986). The rotamer model has been also used to explain the tyrosineamide fluorescence quenching by acrylamide (Contino and Laws, 1991).

The carboxylate in β-homo-tyrosine (βHty) is further from the phenol ring than in tyrosine because of the incorporation of the one additional methylene moiety (—CH_2—) into the main chain of the molecule. This structural change also resulted in an additional degree of a conformational freedom (apart from the rotation about the C^{α}—C^{β} bond), the possibility of the rotation about the

C^{β}—C^{γ} bond. As can be seen on Figure 1, the conformational map of Ac-βHty-NHMe (I) for rotation about C^{α}—C^{β} vs. C^{β}—C^{γ} bonds shows 9 energy minima (the same tyrosine derivative displays only 3 minima connected with the rotation about C^{α}—C^{β} bond). A relatively low energy barrier of the rotation allows a fast interconversion of rotamers during the lifetime of excited state of electrons of the molecule at room temperature.

The rotation about the C^{α}—C^{β} and the C^{β}—C^{γ} bonds results in an averaging of interactions of the phenol chromophore with the oxygen from the carboxyamide group, what finally leads to an averaging of the fluorescence decay times over all possible rotamers existing in a solution. As a consequence, we have observed the monoexponential fluorescence-decay kinetics for both: Ac-βHty-NHMe (I) and Ac-βHty-(OMe)-NHMe (II).

The calculated monoexponential-decay curves with fluorescence decay times: 2.89 ns for Ac-βHty-NHMe (I) and 4.06ns for Ac-βHty(OMe)-NHMe (II) well fit to the experimental data (X^2_R 1.07 for I and X^2_R 0.99 for II). The quantum yields for both derivatives are: 0.108 (I) and 0.137 (II). The higher quantum yields and the longer decay time for the O-methylated β-homotyrosine derivative (II) than for (I), as the effect of O-methylation, was also observed in tyrosine derivatives (Ross et al., 1992).

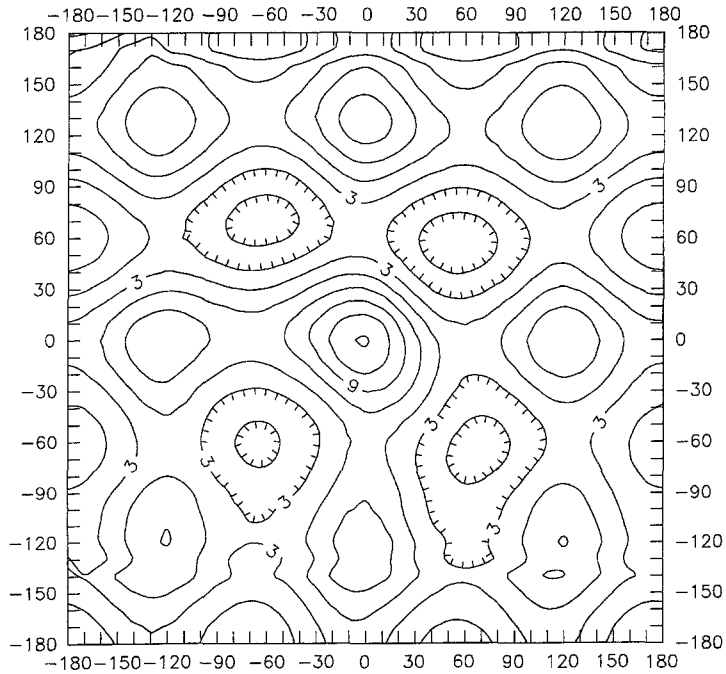

Fig. 1. Conformational map of Ac-βHty-NHMe ordinate: CO—C^{α}—C^{β}—C^{γ} torsional angle, abscissa: C^{α}—C^{β}—C^{γ}—C^{δ} torsional angle. Energy value (in kcal/mol) are shown on respective countours

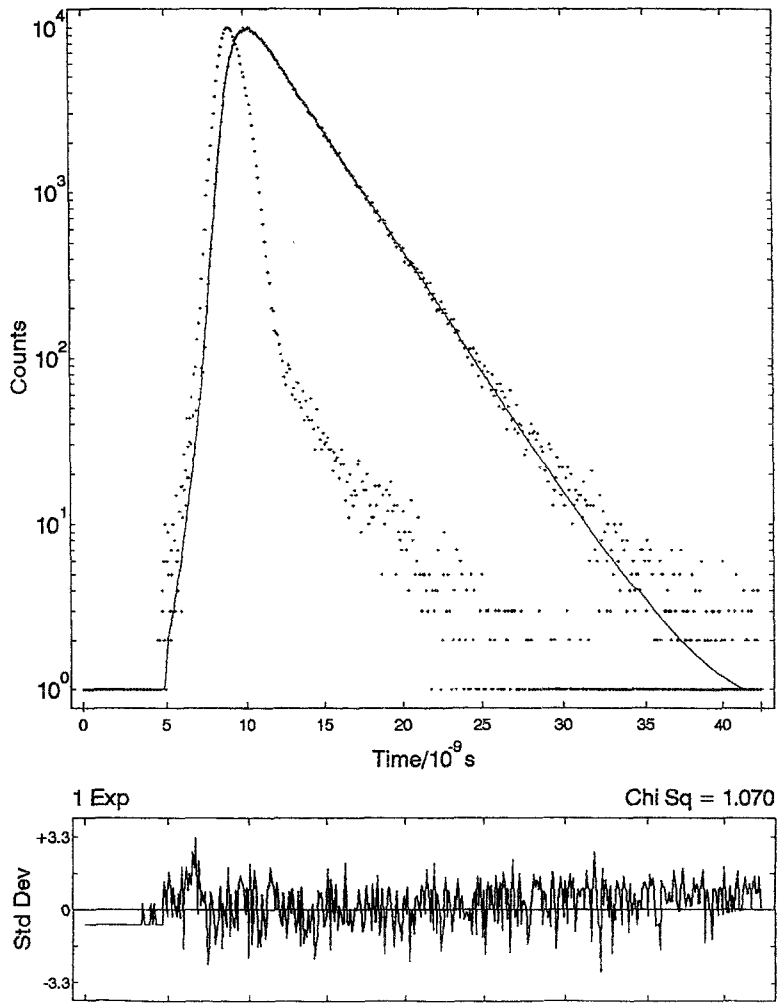

Fig. 2. Measured (doted line) and fitted decay curve (solid line) vs. (left set of points lamp profile) time for Ac-βHty-NHMe (Ac-βHty(OMe)-NHMe give very similar results). The weighted residual are ploted at the lower part of the figure

Materials and methods

Synthesis

We obtained two derivatives of β-homo-tyrosine (βHty) suitable for fluorescence measurements: Ac-βHty-NHMe (I) and Ac-βHty(OMe)-NHMe (II). To prepare these derivatives we first synthesized Boc-βHty(OMe)-OH (A) and Boc-βHty(OBzl)-OH (B) utilizing the sequence of Arndt-Eistert reactions (Plucińska and Liberek, 1987). Compounds A and B were transformed into N-methyl amides by a reaction with appropiate amine using 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoborate (TBTU) as a coupling reagent. The t-butyloxycarbonyl group was removed by action of 4N HCl in dioxane and thus obtained hydrochlorides were acetylated with acetic anhydride in THF in presence of triethylamine and catalytic amount of DMAP. Additionally, in the case of (I) to remove benzyl protection we performed catalytic hydrogenation. After final purification by means of crystalization or reversed phase liquid chromathography, the chemical homogeneity was assessed by mass spectrometry (field desorption) and analytical reversed phase HPLC (a linear gradient of 0–80% of CH_3CN

in 0.1% TFA in H_2O over 60 min at flow rate of 1.0 ml/min; column: Vydac C-18, 4.6 × 250 mm, 5 μm).

Theoretical calculations

The conformational map for Ac-βHty-NHMe was calculated by PCMODEL v.4.0 program (Gajewski and Gilbert, 1991) using the MMX force-field (Gajewski et al., 1990).

Fluorescence measurements

Steady-state fluorescence spectra and quantum yields were obtained on a Perkin Elmer LS-50 spectrofluorimeter using typrosine as reference compound (quantum yield 0.14). Time-resolved measurements were performed on a Edinburgh Analitical Instruments model CD-900 apparatus. The hydrogen lamp was used for excitation at 275 nm. The emission was observed through a monochromator at 315 nm (10 nm bandwidth). All measurements were performed in water at pH = 7.0 at room temperature.

Acknowledgements

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