Journal of Molecular Structure 1118 (2016) 56–67

Contents lists available at ScienceDirect

Journal of Molecular Structure

journal homepage: http://www.elsevier.com/locate/molstruc

Excited state electron distribution and role of the terminal amine in acidic and basic tryptophan dipeptide fluorescence

Azaria S. Eisenberg [a], Moshe Nathan [a, 1], Laura J. Juszczak [a, b, *]

[a] Chemistry Dept., Brooklyn College, The City University of New York, 2900 Bedford Ave., New York, NY, 11210, USA
[b] Depts. of Chemistry and Biochemistry, The Graduate Center, The City University of New York, 365 Fifth Ave., New York, NY, 10016, USA

ARTICLE INFO

Article history:
Received 11 January 2016
Received in revised form
28 March 2016
Accepted 29 March 2016
Available online 31 March 2016

Keywords:
Tryptophan fluorescence
Fluorescence lifetime
Quantum yield
Excited state
Molecular dynamics simulation
Quantum mechanics calculations

ABSTRACT

The results of quantum yield (QY) study of tryptophanyl glutamate (Trp–Glu), tryptophanyl lysine (Trp–Lys) and lysinyl tryptophan (Lys–Trp) dipeptides over the pH range, 1.5–13, show that the charge state of the N-terminal amine, and not the nominal molecular charge determines the QY. When the terminal amine is protonated, QY is low (10^{-2}) for all three dipeptides. As the terminal amine cation is found proximal to the indole ring in Trp–Glu and Trp–Lys conformers but not in those for Lys–Trp, its effect may lie only in the partitioning of energy between nonradiative processes, not on QY reduction. QY is also low when both the N-terminal amine and indole amine are deprotonated. These two low QY states can be distinguished by fluorescence lifetime measurement. Molecular dynamics simulation shows that the Chi 1 conformers persist for tens of nanoseconds such that 10^{0}–10^{1} ns lifetimes may be associated with individual Chi 1 conformers. The ground state electron density or isosurface of high QY (0.30) 3-methyindole has a uniform electron density over the indole ring as do the higher QY Trp dipeptide conformers. This validates the association of ground state isosurfaces with QY. Excited state orbitals from calculated high intensity, low energy absorption transitions are typically centered over the indole ring for higher QY dipeptide species and off the ring in lower QY species. Thus excited state orbitals substantiate the earlier finding that the ground state isosurface charge density pattern on the indole ring can be predictive of QY.

1. Introduction

The fluorescent indole residue of tryptophan is an intrinsic probe of protein structure, responding to its local environment [1–3]. Several spectral parameters are readily obtainable via steady state and time resolved methods [4–6] but their meaning has long been stymied by the complexities of tryptophan (Trp) photophysics [7–9]. Within a protein, several nonradiative processes compete with fluorescence, and several residues—as well as the peptide bond [10–12]—are potential acceptors of an electron transferred from the indole residue of Trp [13,14]. Intramolecular proton transfer quenching is also possible [15,16]. A 1991 study of several Trp dipeptides [17] showed little variation of quantum yield with the identity or position of the second amino acid, but this study was limited to midrange pH values, and avoided amino acids such as histidine, arginine and cysteine, which interact with the indole ring. Additionally, no molecular dynamics or quantum mechanics calculations, which reveal specifics of intramolecular interaction, were carried out.

Through the application of computational methods [18–27] the contribution of solvent, protein, and the indole residue can be separated out. In our previous molecular dynamics study of the Lys–Trp dipeptide in different charge states [28], optimized molecular dynamics conformations did not show the juxtaposition of the terminal amine cation next to the indole ring. Changes in ground state isosurface charge density on the indole ring, resulting from pH-induced changes in off-ring charge state, were shown to correlate with reduced quantum yield and weighted average lifetime, suggesting a static quenching mechanism as an explanation. In this work, Trp–Lys and Trp–Glu dipeptides are similarly examined. Here, the charge state of the terminal amine is found to govern QY up until the point of indole amine deprotonation. To reinforce our findings, we also consider excited state orbitals from

* Corresponding author. Chemistry Dept., Brooklyn College, The City University of New York, 2900 Bedford Ave., New York, NY, 11210, USA.
E-mail address: LJUZAK@brooklyn.cuny.edu (L.J. Juszczak).
[1] Current address: Technion-The Ruth and Bruce Rappaport Faculty of Medicine, Technion City, Haifa, Israel.

http://dx.doi.org/10.1016/j.molstruc.2016.03.098
0022-2860/

calculated absorption spectra, and highest occupied molecular orbital-lowest unoccupied molecular orbital (HOMO–LUMO) orbital overlap. Since fluorescence is a vertical transition and we are focusing on fluorescence rather than radiationless decay processes, topology of the excited state potential energy surface, where the energy tracks with motion of a single molecular group, is not considered here. Indeed, energetic overlap (conical intersection) between excited and charge transfer states along a potential energy surface does not guarantee spatial overlap, i.e., that the charge transfer state will be populated. Others have made extensive and valuable study of indole charge transfer states, and convincingly demonstrated that both electron and proton transfer operate as part of the indole deexcitation process [10–16]. Our work yields insight into the electron distribution responsible for the partition of excitation energy between the light (fluorescence) and dark (radiationless) mechanisms of energy dissipation but does not distinguish between the myriad radiationless mechanisms.

In an earlier paper, we examined the fluorescence lifetimes and Chi 1/Chi 2 dihedral angle preferences of Trp–Glu [29]. We discussed the significance of Chi 1 dihedral angle to conformation, the digital nature of conformational transitions, and noted the juxtaposition of the terminal amine cation to the indole ring in several conformers [29]. In that Trp–Glu paper, we also first noted the unusually high charge density on the beta methylene carbon of the Trp–Glu dipeptide from ring planar charge distribution images. Here, we extend our examination of Trp–Glu dipeptides to include isosurfaces, orbital analysis and experimental QYs. We also examine a dipeptide containing a basic amino acid, the Trp–Lys dipeptide, finding a similar role for the terminal amine cation in controlling quantum yield. In the absence of excited state isosurfaces, molecular orbital calculations for Trp–Lys and Trp–Glu species absorption transition are also carried out. We are especially interested in the HOMO and LUMO for the fluorescent ^1L_a transition because similarities between the isosurfaces of higher quantum yield Lys–Trp species and the HOMO have been observed [28]. We examine the LUMOs from the most intense absorption transitions to distinguish between fluorescence, a vertical transition between excited and ground state, and dark state processes, where the excited state electron density can be found off-ring.

2. Experimental

Trp–Glu and Trp–Lys dipeptides were purchased from Research Plus, Inc. (Barnegat, NJ) and used without further purification. Aqueous solutions of dipeptide were adjusted to the required pH by additions of 1 mM HCl or 1 mM NaOH. Quantum yield standards, L-tryptophan and 2-aminopyridine were purchased from Acros Organics/Thermo Scientific (Waltham, MA) and Alfa Aesar (Ward Hill, MA), respectively, and used without further purification.

2.1. Absorption measurements

Background corrected absorption measurements were recorded on a Perkin–Elmer Lambda 650 UV–vis spectrometer (Waltham, MA) with a 2 nm slit width, 1 cm path length cuvette and 1 nm interval.

2.2. Fluorescence emission measurements

Fluorescence emission spectra were recorded on a Fluorolog 3 model FL-1000 (Horiba Jobin Yvon, Edison, NJ) fluorimeter with 280 nm excitation, 2 nm slits, 1 nm interval and 0.1 s integration time. Sample concentrations were all <0.01 mM to avoid inner filter effects.

2.3. Quantum yield determination

Quantum yield measurement and analysis were carried out using the method of Williams et al. [30]. Details of the method can also be found in Eisenberg and Juszczak [28].

2.4. Time-resolved fluorescence lifetime decay measurements

Fluorescence lifetime measurements (20 K counts, 2 nm slits) of the Trp–Lys and Trp–Glu dipeptide species were carried out using a laser diode excitation source with a broad (1.47 ns full-width half maximum, FWHM) instrument response function (Horiba Jobin Yvon, Edison, NJ). In addition, the Trp–Lys dipeptide species (pH 1.5–11) lifetimes were measured on a Ti:sapphire-pumped, lab-built laser system at the NIH-sponsored Ultrafast Optical Processes Laboratory, (University of Pennsylvania, Philadelphia, PA). Instrument response function width for the Ti:sapphire system is 45 ps FWHM.

Additional details about the Ti-Sapphire system can be found in Eisenberg and Juszczak [28]. Excitation was achieved at 280 nm while emission was monitored at 340 nm on both instruments.

2.5. Fluorescence lifetime decay analysis

The lifetimes for decays collected on the diode instrument were determined by iterative convolution using the vendor-provided Decay Analysis software, DAS6. Details of this method can be found in Eisenberg and Juszczak [29]. The lifetime decay data acquired on the Ti-sapphire-pumped system were analyzed with the fitting program, FluoFit (Picoquant, Photonics North America, West Springfield, MA).

3. Computational details

3.1. Molecular dynamics simulation

All dipeptide species conformations were simulated via the program, GROMACS 4.5.3 [31]. Roughly 1000 water molecules in a cubic box 1 nm greater than the dipeptide boundary were used with periodic boundary conditions in three dimensions. To be sure that all Chi 1 dihedral space was sampled, a 20–30 ns simulation with 0.5 fs time steps was executed. Other details are given in Eisenberg and Juszczak [29].

3.2. Density functional theory calculations

Ab initio quantum mechanical calculations were carried out via the Gaussian 09 software program [32]. Details of our method are given in Eisenberg and Juszczak [28,29] with the following addition and change. Time-dependent density functional theory (TD-DFT) excited state calculations were carried out with the B3LYP method and the 6-31++g(df,p) basis set to produce excited state orbitals as well as oscillator strengths for the electronic transitions; oscillator strengths are included in the Supporting information. Range-separated basis sets, such as LC-BLYP and LC-PBE [33–35], correct for errors in delocalization, HOMO-LUMO gaps and fluorescence energies, generated by the B3LYP basis set. Fluorescence energy errors are not of concern here because we do not calculate fluorescence energies. However, the results generated by using the B3LYP basis set and by the long-range corrected CAM-B3LYP basis set were compared (Data not shown). Orbitals and isosurfaces were almost identical. In fact, the lowest energy transition for the standard B3LYP basis set was found to be a closer match to the experimental absorption band than for the long-range corrected basis set. GaussView 5.0 software [36] was used for visualization of the