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# A FLUORESCENCE, $^1$H NMR SPECTROSCOPY AND MOLECULAR DYNAMICS STUDY OF THE INFLUENCE OF ROTAMER POPULATION ON FLUORESCENCE DECAY OF TYROSINE, PHENYLALANINE AND THEIR DERIVATIVES

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**Abstract:** Molecular dynamics simulations were carried out on tyrosine and phenylalanine and their derivatives with various terminal groups to determine the populations of side-chain rotamers. The obtained populations were compared with those calculated from fluorescence-decay lifetime distributions and NMR studies. It was found that theoretically calculated populations do not match the experimental ones, which suggests that the static rotamer model is inadequate to explain the dynamics of tyrosine and phenylalanine side chain in fluorescence and NMR experiments.

**Keywords:** tyrosine, phenylalanine, rotamers, fluorescence, molecular dynamics, NMR spectroscopy

## 1. Introduction

The tyrosine zwitterion exhibits monoexponential fluorescence decay kinetics. Conversion of the carboxyl group into the amide group or protonation of the carboxyl group results in a fluorescence intensity decay that requires at least a double exponential to fit the data [1]. Gauduchon and Whal [2] suggest that this complex kinetics could be explained in terms of rotamer populations resulting from the rotation about the $C^{\alpha}$–$C^{\beta}$ bond (Figure 1).

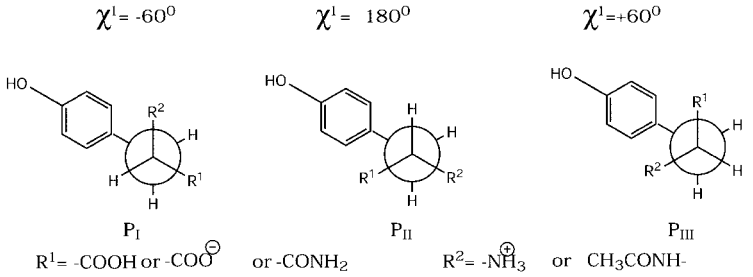


**Figure 1.** Newman projections about the $C^{\alpha}$–$C^{\beta}$ bond, showing the possible positions of the phenol ring in relation to the $C^{\alpha}$ substituent

They suggested that the short, subnanosecond time constant, observed for analogues with an amide group, was due to quenching phenol ring fluorescence in rotamer III by contact with the carbonyl group and that the longer time constant was due to the decays of rotamers I and II (see Figure 1). Cowgil [3] and Tournon *et al.* [4] suggested that the more efficient fluorescence quenching in rotamer III, compared to that in rotamer I and II was caused by short distance between the tyrosine and C-terminal amide moiety or the protonated carboxyl group in rotamer III. Laws *et al.* [5–7] have elaborated on this rotamer model of fluorescence decay of tyrosine and tyrosine analogues and tyrosine incorporated in peptide chain, and tyrosine amide quenching by acrylamide. This rotamer model of the fluorescence decay of an aromatic amino acid also predicts that the amplitudes of the kinetic components should correspond to the ground-state populations, provided that interconversion between rotamers is slow compared to the lifetime of the excited state. It should be noted that some tyrosine derivatives exhibit monoexponential fluorescence decay [1] which could indicate that rotamer interconversion is faster than the excited-state decay; therefore the rotamer model would not be valid in all cases. However, monoexponential fluorescence decay, can also be explained in terms of similar, unresolvable fluorescence lifetimes of the rotamers [5–7]. For phenylalanine derivatives with a protonated or an amidated carboxylic group fluorescence quenching is observed (which does not occur for the parent molecule); however the fluorescence intensity decay remains monoexponential.

## 2. Methods

Molecular dynamic calculations were performed using the AMBER 4.1 software package [8]. The molecule was immersed in a water box, with a 7 Å solvent layer in each direction. After the system had been set up, energy minimization using 10000 conjugate-gradient steps was carried out prior to the actual MD simulation. Simulations were carried out in the NVT scheme, at a temperature of 298 K. The minimum-image convention was applied and a spherical cut-off of 9.0 Å was used on non-bonded and electrostatic interactions. The total duration of a simulation was about 7.0 ns and the integration step was 0.5 fs. The structures were saved every 0.25 ps.

One- and two-dimensional histograms of the populations of the dihedral angles under consideration were constructed by binning the angles calculated from the structures saved during MD simulations. The structures from the first 50 ps, which corresponded to the equilibration period were removed from this analysis. The bin size was 10 degrees.

## 3. Results and discussion

The trajectories of the dihedral angles ($\chi^1$, $\chi^2$ $\phi$, $\psi$ and $\chi^3$; see Figure 2 for abbreviations) for Ac-Tyr-$NH_2$ in water are shown in Figure 3.

During the simulation time (about 5 ns), which is longer than the fluorescence decay time for this compound (see Table 1), frequent changes of the values of dihedral angles are observed, especially for $\chi^1$, $\phi$ and $\chi^3$. For the other compounds studied: Tyr, Phe and Ac-Phe-$NH_2$ similar changes of the dihedral angles during simulation time were observed too (data not shown). The same was observed by Kungl for Tyr and Gly-Tyr-Gly *in vaco* and in solution [9]. Based on the calculated trajectories, the populations of appropriate rotamers for the four compounds studied were computed and the results are presented in Table 1 and as contour plots in Figure 4 (for Ac-Tyr-$NH_2$) and Figure 5 (for Ac-Phe-$NH_2$).

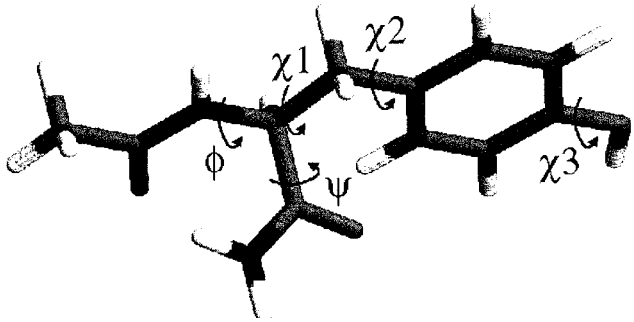


**Figure 2.** Definition of the dihedral angles ($\phi$, $\psi$, $\chi^1$, $\chi^2$ and $\chi^3$)

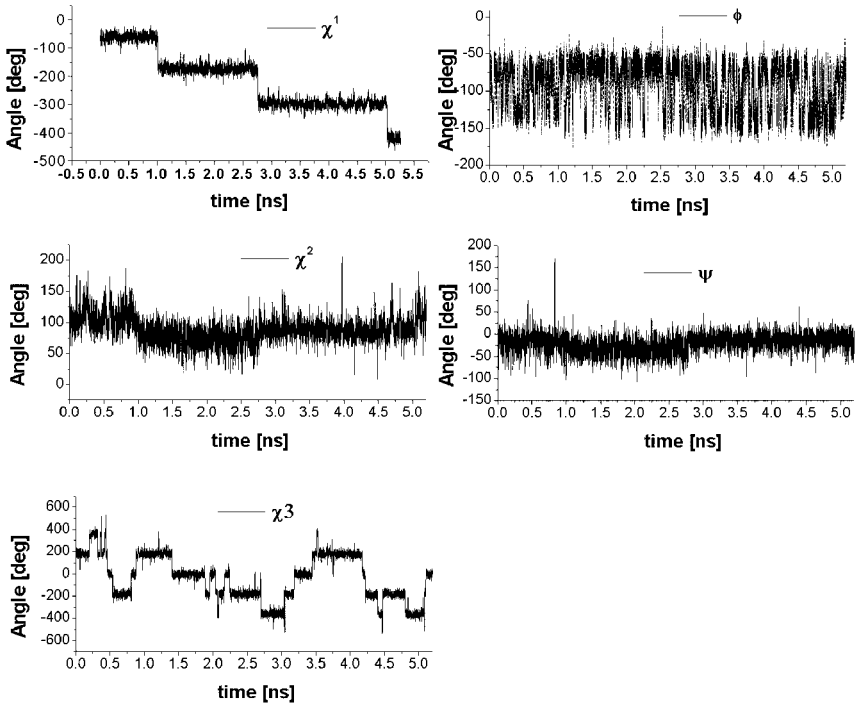


**Figure 3.** The trajectories of the dihedral angles for Ac-Tyr-$NH_2$ in water obtained from MD simulations

For the most populated rotamer of Tyr, $\chi^1$ is centered around $\chi^1 \pm 180°$, whereas for Phe two rotamers occur with $\chi^1$ centered at $-60°$ and $\pm 180°$, respectively; both of them have approximately the same populations. There is also difference in rotamer population for Ac-Tyr-$NH_2$ and Ac-Phe-$NH_2$ (Table 1). The difference in rotamer populations between Tyr and Phe, as well as between their N-acetylated amides indicates that the hydroxyl group of the phenol chromophore plays an important role in determining rotamer population.

The $\chi^1$ rotamer populations obtained from molecular dynamics calculations do not conform with those obtained from NMR and fluorescence spectroscopy (Table 1). The discrepancy between the ground state rotamer population obtained from NMR, and fluorescence spectroscopy and with respect to those obtained from MD calculations might indicate that the rotamer theory is not appropriate to explain the heterogeneity of

**Table 1.** Rotamer populations calculated from NMR data, molecular dynamics (MD), and fluorescence lifetime and pre-exponential factor for tyrosine and their derivatives in water. The values of the angles corresponding to maxima of the distribution are in parentheses

| Compound | $\chi^1$ rotamer populations | | | | Fluorescence lifetime $\tau_i$ [ns] | Preexp. Factor ($\alpha$) |
|---|---|---|---|---|---|---|
| | Method | −60° | ±180° | 60° | | |
| Tyr | MD | 0.22 (−65°) | 0.73 (187°) | 0.1 (54°) | 3.38[2]<br>3.35[10] | 1.00<br>1.00 |
| | NMR[11] | 0.49 | 0.26 | 0.25 | | |
| Phe | MD | 0.48 (−61°) | 0.47 (171°) | 0.05 (58°) | 7.12[12]<br>6.80[13] | 1.00<br>1.00 |
| | NMR[14] | 0.48 | 0.24 | 0.28 | | |
| Ac-Phe-$NH_2$ | MD | 0.46 (−60°) | 0.38 (173°) | 0.16 (57°) | 4.91[13] | 1.00 |
| | NMR | – | – | – | | |
| Ac-Tyr-$NH_2$ | MD | 0.18 (−65°) | 0.34 (187°) | 0.49 (61°) | 2.22; 0.93[5]<br>1.66; 0.11[15] | 0.86; 0.14<br>0.65; 0.35 |
| | NMR[11] | 0.58 | 0.32 | 0.1 | | |

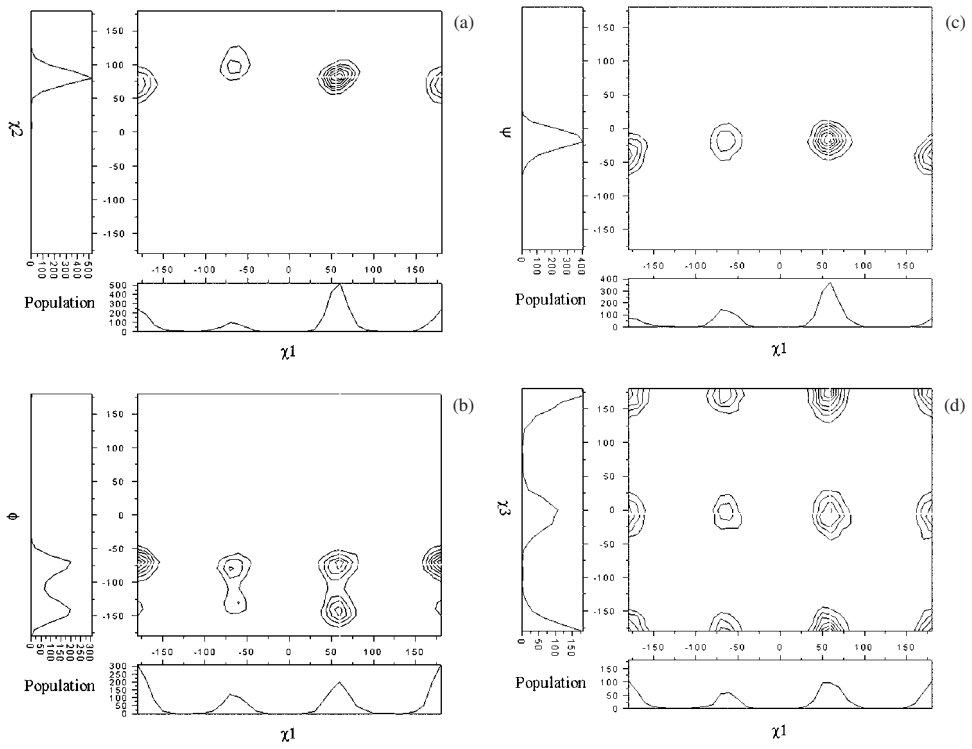


**Figure 4.** The contour plots of the two-dimensional distribution of $\chi^1$ and $\chi^2$ angles (a), $\chi^1$ and $\phi$ angles (b), $\chi^1$ and $\psi$ angles (c), and $\chi^1$ and $\chi^3$ angles (d) for Ac-Tyr-$NH_2$ in water

fluorescence intensity decays of tyrosine derivatives in water solution. Probably, the rate of the rotation about the $C^\alpha$–$C^\beta$ bond is similar to that of deactivation of the excited state. Consequently, the amplitudes derived from kinetic measurements need not correspond to the ground-state rotamer population. However, the discrepancy between the rotamer populations calculated from MD runs and those determined by NMR, which clearly correspond to the

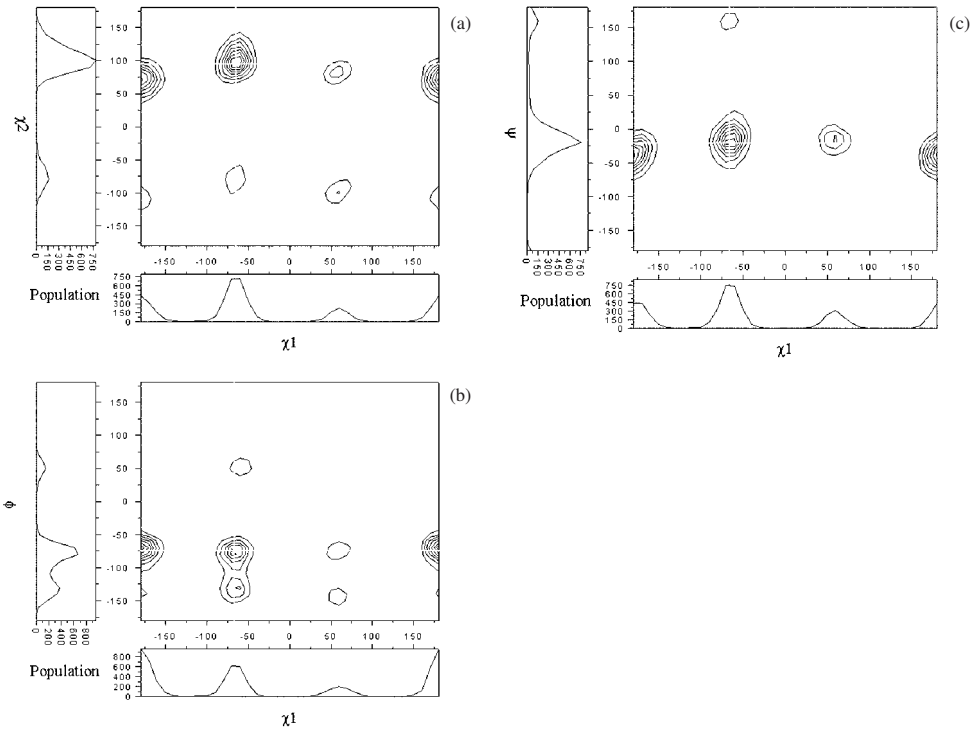


**Figure 5.** The contour plots of the two-dimensional distribution of $\chi^1$ and $\chi^2$ angles (a), $\chi^1$ and $\phi$ angles (b), $\chi^1$ and $\psi$ angles (c) for Ac-Phe-$NH_2$ in water

ground state of the molecule, suggests that the force field or the MD procedure applied might not be fully adequate to describe the dynamics of the systems studied at quantitative level. It also follows from this study that the solvent and the specific interactions between the phenol ring and the amino acid functional group have influence on the fluorescence intensity decay of tyrosine and its derivatives [3].

## *Acknowledgements*

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