

Nonlinear optical properties of aromatic amino acids in the femtosecond regime

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We report experimental and theoretical investigations of the third-order optical nonlinearities of aromatic amino acids (Phenylalanine, Histidine, Tryptophan, and Tyrosine) in aqueous solutions. The Z-scan technique with femtosecond laser pulses at 800 nm was explored for the determination of the nonlinear refractive index, nonlinear absorption coefficient, and the second-order hyperpolarizability of each amino acid. Experimental results were compared with theoretical analysis based on post-Hartree Fock MP2/6-311+G^{**}. © 2010 Optical Society of America

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1. INTRODUCTION

The nonlinear optical properties of organic materials have been subject to extensive studies aiming at the understanding of their intrinsic origin as well as the possible use of these systems in photonic applications. For instance, the nonlinear refractive index, n_2 , of several amino acid (Alanine, Proline, Arginine, Threonine, and Serine) solutions employed on crystal growth for ultrashort-pulse second-harmonic generation has been measured [1].

Amino acids are important molecules for life, and have different roles and functions in the body metabolism. Amino acids that include an aromatic ring (Phenylalanine, Tryptophan, and Tyrosine with a benzene ring and Histidine with an imidazole ring) are known as aromatic amino acids. In particular, Histidine, Phenylalanine, and Tryptophan are essential amino acids (building blocks of proteins) that cannot be synthesized by the human body and therefore must be part of its diet.

Except for Histidine, the aromatic amino acids fluoresce when excited with ultraviolet light. Most of the intrinsic fluorescence emission of a folded protein is due to excitation of Tryptophan residues, with additional contributions by Tyrosine and Phenylalanine. The optical properties of the aromatic amino acids such as fluorescence and second-harmonic generation are influenced by the local environment. Therefore, they are useful spectroscopy tools to track conformational states and chemical behavior of proteins [2,3]. In particular, Tryptophan fluorescence has been the esubject of a recent detailed study aiming at providing a new insight into the interpretation of the fluorescence origin [4].

The intrinsic fluorescence of aromatic amino acids has been explored in nonlinear cellular optical microscopy of cells, where three-photon ($\lambda=800$ nm) absorption is used to excite levels corresponding to wavelengths deep into

the UV [5]. Recently it was demonstrated that metallic nanoparticles can be used to increase the Tryptophan autofluorescence [6,7]. Although the intrinsic multiphotonic and second-harmonic microscopy (using near infrared light sources in the femtosecond regime) of aromatic amino acids present in different protein structures are commonly used [5,8], some of their nonlinear optical property values are not well known. In this work, we have measured and analyzed the third-order optical nonlinearities of water solutions of Phenylalanine (Phen, C₉H₁₁NO₂), Tyrosine (Tyr, C₉H₁₁NO₃), Tryptophan (Tryp, C₁₁H₁₂N₂O₂), and Histidine (Hist, C₆H₉N₃O₂) using the Z-scan technique [9]. The main interest here is to characterize the samples' macroscopic nonlinear refractive index (n_2), nonlinear absorption coefficients (a_2), and the second-order hyperpolarizabilities (γ) of the aromatic amino acids to advance the understanding of the light-molecule interactions and guide further works with organic materials. The experimental nonlinear optical values were compared with theoretical results analysis based on post-Hartree Fock MP2/6-311+G^{**}.

2. METHOD

The amino acids used in our experiments were obtained from Ajinomoto, Inc. The measurements were performed using water solutions of the amino acids with concentrations below the solubility limit in water for 25 °C. All the amino acids studied were in a zwitterionic form such that their carboxylate (COO⁻) and protonated amino (NH⁺³) groups are charged [10]. The amino acid concentrations and solution pH used are summarized in Table 1.

To characterize the optical nonlinearities, the well known Z-scan technique was used [9] exploiting the wavefront distortion (self phase changes) of the beam that propagates inside a nonlinear medium. The laser beam

Table 1. Experimental and Theoretical Hyperpolarizability Values for the Studied Amino Acid Solutions

Amino acid	Abb.	Concent. (μM)	pH	n_2 ($\times 10^{-20}$ m ² /W)	a_2 ($\times 10^{-14}$ m/W)	γ_{exp} ($\times 10^{-35}$ esu)	γ_{the} ($\times 10^{-35}$ esu)
Tryptophan	Tryp	55.3	6.0 \pm 0.5	9.9 \pm 0.2	7.5 \pm 0.1	28 \pm 2	23.0
Phenylalanine	Phen	179.2	6.0 \pm 0.5	6.7 \pm 0.2	6.1 \pm 0.1	17 \pm 2	16.7
Tyrosine	Tyr	2.5	6.0 \pm 0.5	7.6 \pm 0.2	6.0 \pm 0.1	21 \pm 2	20.9
Histidine	Hist	270.2	7.0 \pm 0.5	1.5 \pm 0.2	0.5 \pm 0.1	3.8 \pm 1	4.2
Proline	Prol	363.1	6.0 \pm 0.5	4.4 \pm 0.2	1.1 \pm 0.1	0.15 \pm 0.1	0.21

was focused by a 15 cm focal distance lens, and the studied material was placed inside a 2 mm thick quartz cell such that the thin-lens condition is satisfied [sample length (L) smaller than Rayleigh range ($z_0=3.5$ mm)] [9]. The sample was mounted in a translation stage that moved along the beam propagation direction z , where $z < 0$ corresponds to locations of the sample between the focusing lens and its focal plane. By measuring the variation of the transmitted beam intensity through a circular aperture placed in front of a detector in the far-field region, one can determine the sign and magnitude of the nonlinear refractive index and the nonlinear absorption coefficient of the analyzed medium. The aperture size r_a is related to the linear aperture transmittance (S) by $S = [1 - \exp(-2r_a^2/\omega_a^2)]$, with ω_a denoting the beam radius at the aperture. A small-aperture Z-scan experiment corresponding to $S \ll 1$ was employed on the measurement of the real components of the nonlinear optical susceptibilities. A wide or absent aperture, $S=1$, is necessary for the determination of the nonlinear absorption coefficients. The experiment was performed using an amplified femto-second laser system (Coherent Libra) delivering 80 fs optical pulses at 800 nm and 1 kHz repetition rate.

The values of n_2 and a_2 can be determined by measuring the difference between the normalized peak and the valley transmittance (ΔT) in the Z-scan curve. The ΔT value was obtained using the normalized Z-scan transmittance given by [9]

$$T(z, \Delta\varphi_0) \approx 1 - \frac{4\Delta\varphi_0 x}{(x^2 + 9)(x^2 + 1)}, \quad (1)$$

where $x=z/z_0$. For small phase distortion and small aperture ($S \ll 1$), the on-axis phase shift at the focus $\Delta\varphi_0$ is defined as $|\Delta\varphi_0| \approx \Delta T/0.406$. The n_2 value is related to the phase distortion given by [9]

$$n_2 \approx \left(\frac{\lambda}{2\pi} \right) \frac{\Delta\varphi_0}{I_0 L_{\text{eff}}}, \quad (2)$$

where λ is the laser wavelength, I_0 is the on-axis irradiance at focus, $L_{\text{eff}} = [1 - \exp(-a_0 L)]/a_0$, L is the sample length, and a_0 is the linear absorption coefficient of the sample. Simultaneously the a_2 value can be measured by the transmission change for the open aperture configuration ($S=1$) as [9]

$$a_2 \approx \frac{\Delta T}{2^{(-3/2)} I_0 L_{\text{eff}}}. \quad (3)$$

3. RESULTS AND DISCUSSION

Figure 1 shows the Z-scan signature in a small aperture setup ($S \ll 1$) for the amino acid solutions studied and for pure water. The solid curves are the theoretical fitting for the far-field condition described by Eq. (1). The focus intensity used for this case was $I_0 = 18$ GW/cm².

To measure the solutions' nonlinear absorption coefficients, an intensity of one order of magnitude higher than previously, $I_0 = 180$ GW/cm², was used. The experimental results of the open aperture Z-scan setup are presented in Fig. 2. The real and the imaginary part of the effective third-order optical susceptibility can be determined through $\chi_R^{(3)} = 2n_o^2 \epsilon_o c n_2$ and $\chi_I^{(3)} = (\lambda/2\pi) n_o^2 \epsilon_o c a_2$.

For an isotropic liquid the macroscopic third-order optical susceptibility and the microscopic (γ) second-order hyperpolarizability can be related as $\chi^{(3)} = NF^4 \langle \gamma \rangle$, where N is the concentration in number of molecules per cm³ and F is the local field or Lorentz correction given by $F(\lambda) = (n^2(\lambda) + 2)/3$ [11]. Hence, the γ values are independent of the solution concentration.

The amino acids' second-order hyperpolarizability values are summarized in Table 1. Note that the nonlinear refraction of the amino acid solutions has a significant contribution from the water nonlinearity. Here we subtracted the water contribution to deduce the amino acids' second-order hyperpolarizabilities. The obtained amino acid γ values are in accordance with the values reported by Rustagi and Ducuing, where the molecular hyperpolarizability for a molecule with a low number of double bonds is expected to be in the 10^{-35} esu to 10^{-33} esu range [12]. The experimental methodology was also applied on a water solution of Proline (Prol, C₅H₉NO₂). Proline is an amino acid whose γ value is already known in the litera-

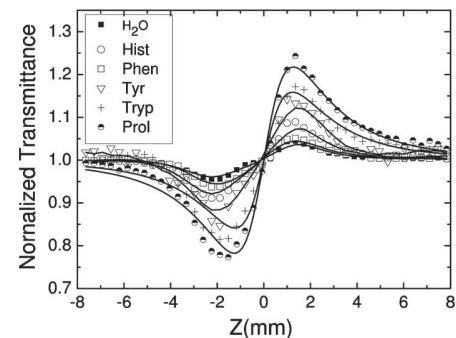


Fig. 1. Closed aperture Z-scan signature obtained from the amino acid solutions. Symbols are the experimental data and the curves the theoretical fit by using Eq. (1). Label abbreviations are explained in Table 1.

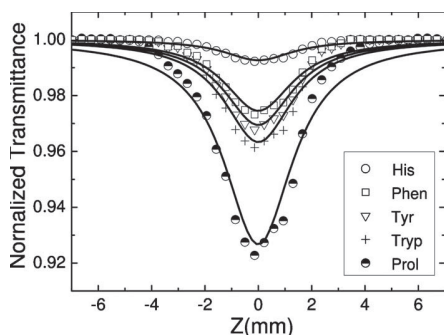


Fig. 2. Open aperture Z-scan signature obtained from the amino acid solutions. Label abbreviations are explained in Table 1.

ture [1]. The obtained hyperpolarizability value of Proline was $\gamma = 0.15 \times 10^{-35}$ esu, in agreement with [1].

The experimental γ values may be compared with theoretical results guiding insight on the relation between the second-order hyperpolarizability and the molecular structure. The γ values have been calculated utilizing the *ab initio* post-Hartree–Fock derivative methodology by the GAUSSIAN package utilizing the preceding implementation of numerical differentiation with respect to the applied field taking into account the fourth derivative of the energy with respect to the field [13,14]. In essence, the theoretical method consists of a numerical differentiation with respect to the electric field for each tensorial component given by $\gamma(zzzz) = d\beta(zzz)/dFz$; $\gamma(zzxx) = d\beta(zzx)/dFz$; allowing obtain the γ values by using the μ , α , and β tensors obtained previously by a GAUSSIAN package (μ is the dipole moment, α is the polarizability, and β is the first hyperpolarizability). The ground-state geometries of all molecules present were fully optimized using the MP2/6-311+G^{**}. Table 1 also shows the γ theoretical values obtained by the applied method.

Although an order of magnitude difference between the theoretical and experimental second-order hyperpolarizabilities values can be observed, it is known that a difference between experimental and theoretical results could be linearly shifted when solvent effects are explicitly considered in the theoretical method [15,16]. However, the correct description of the general trends of the change of γ as a function of the molecular structure is as important as the agreement between the absolute values of the theoretical and experimental hyperpolarizabilities. One can observe that the measured and calculated γ values increase with the increase of the double bonds (π bond) of the molecules. This is in agreement with the π electrons model of a conjugated chain-like polymer [17]. In Fig. 3 the obtained Proline γ value (γ_{Proline}) was used in a comparison of the experimental data to the corresponding estimated results. Figure 3 shows a very good agreement between the relative values obtained using both methods.

4. CONCLUSIONS

We have experimentally investigated the third-order optical nonlinearities of aromatic amino acids in water solution using the Z-scan technique with femtosecond laser pulses at 800 nm. The nonlinear refraction index and the

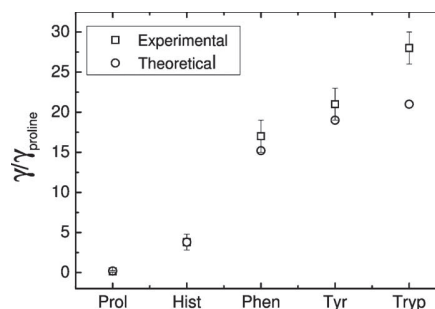


Fig. 3. Theoretical and experimental values of the aromatic amino acids' hyperpolarizability.

nonlinear absorption coefficient were measured and the second-order hyperpolarizabilities determined, ranging from 10^{-36} to 10^{-34} esu. The experimental results are supported by theoretical calculations using the post-Hartree–Fock technique, showing good agreement between the methods. The presented results can guide further basic work and applications with aromatic amino acids.

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REFERENCES

1. J. J. Rodrigues, C. H. T. P. Silva, S. C. Zilio, L. Misoguti, and C. R. Mendonça, "Femtosecond Z-scan measurements of nonlinear refraction in amino acid solutions," *Opt. Mater.* **20**, 153–157 (2002).
2. P. R. Callis and J. T. Vivian, "Mechanisms of tryptophan fluorescence shifts in proteins," *J. Biophys.* **80**, 2093–2109 (2006).
3. M. Ikeda, H. Tsuji, S. Nakamura, A. Ichiyama, Y. Nishizuka, and O. Hayaishi, "Studies on the biosynthesis of nicotinamide adenine dinucleotide: a role of picolinic carboxylase in the biosynthesis of nicotinamide adenine dinucleotide from tryptophan in mammals," *J. Biol. Chem.* **240**, 1395–1401 (1965).
4. J. R. Albani, "New insights in the interpretation of tryptophan fluorescence: origin of the fluorescence lifetime and characterization of a new fluorescence parameter in proteins: the emission to excitation ratio," *J. Fluoresc.* **17**, 406–417 (2007).
5. C. A. Mirkin, R. L. Letsinger, R. C. Mucic, and J. J. Storhoff, "A DNA-based method for rationally assembling nanoparticles in macroscopic materials," *Nature* **382**, 607–609 (1996).
6. J. Lakowicz, "Radiative decay engineering: Biophysical and biomedical applications," *Anal. Biochem.* **298**, 1–24 (2001).
7. D. Rativa, A. S. L. Gomes, S. Wachsmann-Hogiu, D. L. Farkas, and R. E. de Araujo, "Nonlinear excitation of tryptophan emission enhanced by silver nanoparticles," *J. Fluoresc.* **18**, 1151–1155 (2008).
8. W. R. Zipfel, R. M. Williams, R. Christie, A. Yu. Nikitin, B. T. Hyman, and W. Webb, "Live tissue intrinsic emission microscopy using multiphoton-excited native fluorescence and second harmonic generation," *Proc. Natl. Acad. Sci. U.S.A.* **3100**, 7075–7080 (2003).
9. M. Sheik-Bahae, A. A. Said, T. H. Wei, D. J. Hagan, and E. W. Van Stryland, "Measurement of optical nonlinearities using a single beam," *IEEE J. Quantum Electron.* **26**, 760–769 (1990).
10. D. Voet and J. G. Voet, *Biochemistry* (Wiley, 1995).

11. R. Boyd, *Nonlinear Optics*, 3rd ed., Elsevier Science & Technology Books (Elsevier, 2008).
12. K. C. Rustagi and J. Ducuing, "Third order optical polarizability of conjugated organic molecules," *Opt. Commun.* **10**, 258–261 (1974).
13. M. Ferrero, M. Rérat, B. Kirtman, and R. Dovesi, "Calculation of the static electronic second hyperpolarizability or $\chi^{(3)}$ tensor of three-dimensional periodic compounds with a local basis set," *J. Chem. Phys.* **129**, 244110 (2008).
14. I. B. Bersuker and I. Ya. Ogurtsov, "The Jahn-Teller effect in dipole (multipole) moments and polarizabilities of molecules," *Adv. Quantum Chem.* **18**, 1 (1986).
15. T. Andrade-Filho, T. C. S. Ribeiro, and J. Del Nero, "The UV-vis absorption spectrum of the flavonol quercetin in methanolic solution: A theoretical investigation," *EPJ. E* **29**, 253–259 (2009).
16. T. Andrade-Filho, H. S. Martins, and J. Del Nero, "Theoretical investigation of the electronic absorption spectrum of Piceatannol in methanolic solution," *Theor. Chem. Acc.* **121**, 147–153 (2008).
17. J. Hermann and J. Ducuing, "Third-order polarizabilities of long-chain molecules," *J. Appl. Phys.* **45**, 5100–5102 (1974).