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Analysis of size-dependent optical properties of lysine carbon dots produced by femtosecond laser synthesis

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Abstract. Pulsed laser synthesis of fluorescent nanoparticles from amino acids is promising technique for fluorescent imaging. Understanding relation between nanoparticle size and absorption and fluorescence characteristics sheds light on mechanism of fluorescence and enables control of optical properties of products. To study this relation, we separated products obtained by laser synthesis from L-lysine into fractions of different size and analyzed optical properties and chemical composition of these fractions.

Keywords: luminescent carbon dots, femtosecond laser pulses, nanomaterials, photostability

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Материалы конференции

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Анализ оптических характеристик фракций углеродных точек, полученных из лизина методом фемтосекундного лазерного синтеза

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Аннотация. Лазерный синтез углеродных точек с флуоресцентными свойствами на основе аминокислот – многообещающая методика для флуоресцентного имаджинга. Исследование соотношения размера точки с ее флуоресцентными параметрами является необходимым для понимания механизма флуоресценции и точного контроля характеристик продуктов лазерного синтеза. В данной работе мы разделили углеродные точки, полученные в ходе лазерного синтеза из лизина, на фракции в соответствии с их молекулярной массой (<2 kDa и >2 kDa), проанализировали ключевые оптические характеристики и химический состав соответствующих фракций.

Ключевые слова: люминесцентные углеродные точки, фемтосекундные лазерные импульсы, наноматериалы, фотостабильность

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Introduction

Production of fluorescent species from biomolecules in living cells and tissues *in situ* by femto-second laser irradiation holds promise for targeted, localized, rapid and non-invasive fluorescent labelling of intracellular material [1–2]. Previously, we demonstrated that femtosecond laser synthesis of fluorescent carbon dots-like nanoparticles from essential amino acids, in particular, L-lysine provides a potential route of fluorescent species formation in living cells [3].

Hence, there is a practical interest in understanding and tailoring fluorescent properties of amino acid-derived carbon dots. Nanoparticle size is one of parameters through which optical properties of carbon dots can be controlled and manipulated. Also, relation between size and fluorescence characteristics is indicative of the fluorescence mechanism. When energy levels are controlled by quantum confinement effects, absorption and emission bands of carbon dots strongly depend on their diameter [4–5].

On the other hand, fluorescence of carbon dots can originate from specific organic fluorophore groups in their structure, in this case its spectra are independent of the nanoparticle diameter [6]. Fluorescence of these groups can be bright, when they exist as separate molecules in solution, but becomes strongly quenched when they combine into large nanoparticles [7].

In order to reveal the relation between nanoparticle size and optical properties and gain insight into fluorescence mechanisms, we employed dialysis to separate fluorescent products, obtained from L-lysine aqueous solution, into several fractions of different sizes. Absorption and emission spectra, fluorescence quantum yields and lifetimes and other optical characteristics were then registered for each fraction and effect of nanoparticle size on these properties was revealed.

Materials and Methods

Synthesis and separation of products. Aqueous solution of L-lysine (2 mL, 0.1 g/mL) in a quartz cuvette was irradiated with trains of femtosecond laser pulses focused by a spherical lens ($f = 8$ mm, 0.5NA). Central wavelength of laser pulses was 800 nm, repetition rate 1 kHz, duration 50 fs, pulse energy 1.4 mJ. Irradiation resulted in color change of solution and fluorescent carbon dots formation (Lys-CD). This solution was dialyzed for 72 hours in a 2,000 MWCO dialysis unit and separated into dialysate with molecular weight < ca. 2 kDa (Lys-CD-1) and retentate (Lys-CD-2, molecular weight > ca. 2 kDa) (Fig. 1).

Samples characterization. Absorption of photoluminescence spectra of samples in water were recorded with Shimadzu spectrophotometer (UV-3600) and spectrofluorometer (RF-5031 PC). Fluorescence quantum yield (350 nm excitation) was estimated with the slope method using ethanol solution of anthracene (quantum yield 0.27) as a standard.

Fluorescence decay and fluorescence anisotropy decay kinetics in aqueous solution were registered with femtosecond pulsed laser excitation at 360 nm at 450 nm emission wavelength using time-correlated single-photon counting module (SPC-150N, Becker&Hickl GmbH). Fluorescence lifetime and anisotropy decay time were calculated as amplitude-weighted averages from three-exponential fit of decay kinetics.

Photobleaching kinetics were recorded by exposure of carbon dots aqueous solutions of the same concentration to continuous irradiation with excitation light of the spectrofluorometer

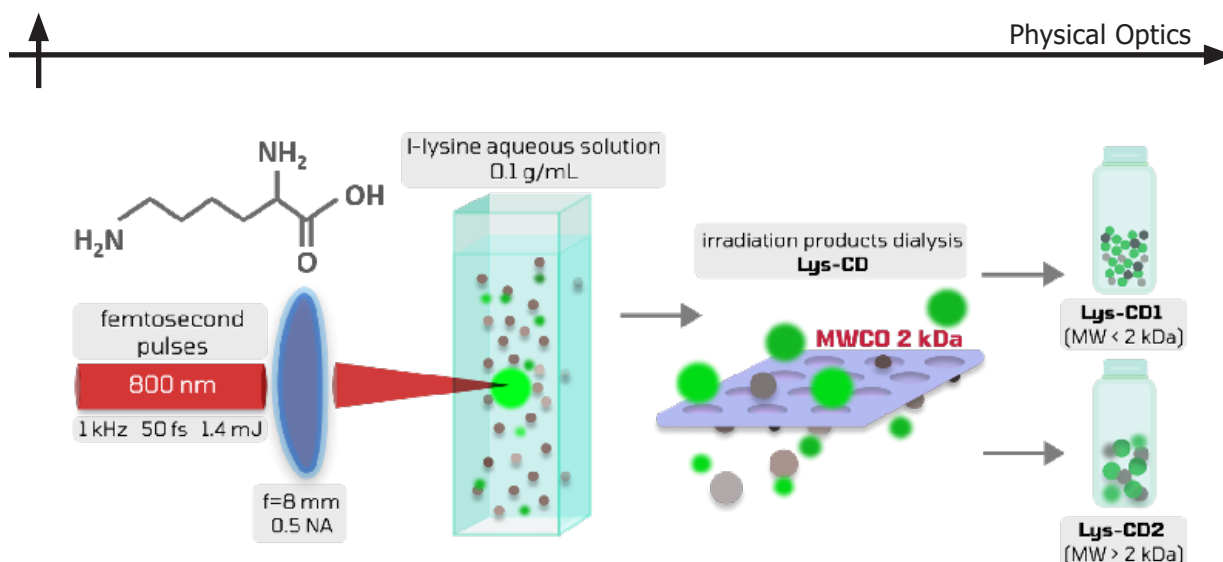


Fig. 1. Synthesis and separation of lysine carbon dots

(central wavelength is 350 nm, spectral width is 20 nm); fluorescence intensity was recorded at 430 nm every 2 minutes for 80 minutes.

Infrared absorption spectra of dried samples were collected using Bruker Lumos II FTIR microscope-spectrometer in an attenuated total reflection mode.

Results and Discussion

As described previously, laser irradiation of L-lysine solution resulted in formation of water-soluble colored and fluorescent nanometer-sized particles (carbon dots). This solution was separated using dialysis into lower and higher weight fractions (Lys-CD-1 and Lys-CD-2 respectively). Effective separation of the sample into fractions was confirmed using measurements of fluorescence anisotropy decay time, which is proportional to the average hydrodynamic volume of carbon dots (Fig. 2, *a*, Table 1). Emission anisotropy of Lys-CD-1 decayed much faster and Lys-CD-2 – much slower than of Lys-CD which confirms their different volumes. Characteristically, Lys-CD-1 exhibited relatively long anisotropy decay corresponding to the hydrodynamic volume of approximately 3.1 nm³. Thus, even in the lower-weight fraction, fluorescence is mostly attributed to nanoparticles (carbon dots) and few, if any, fluorescent moieties exist as separate small molecules in solution.

Infrared spectra of irradiated solution, separated from unreacted L-lysine, demonstrated characteristic amide peaks (Fig. 2, *b*), indicating that carbon dots were formed by polymerization of

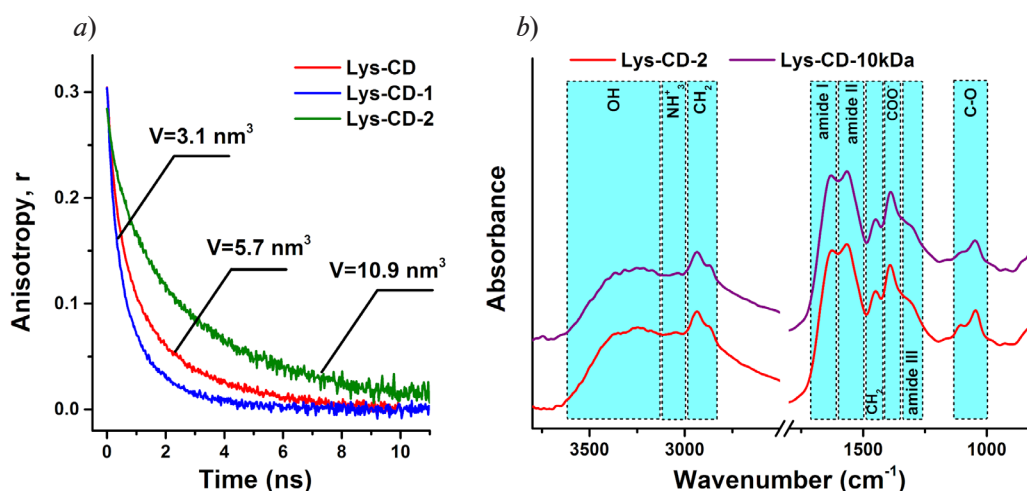


Fig. 2. Fluorescence anisotropy decay kinetics of Lys-CD, Lys-CD-1, and Lys-CD-2 (excitation at 360 nm, registration at 430 nm) (*a*). Comparison of infrared spectra of Lys-CD dialyzed using 2 and 10 kDa membranes (*b*)

amino acid monomers through formation of amide bonds. Additionally, there were also bands of hydroxyl, alkoxy-, methylene and carboxylate groups. We compared infrared spectra of carbon dots of different weights, dialyzed through 2 kDa and 10 kDa membranes, which were essentially similar (Fig. 2,*b*). Thus, chemical composition of carbon dots demonstrates little dependence on their size.

Absorption and emission spectra demonstrated, that, unlike original L-lysine, the Lys-CD exhibited a broad absorption spectrum in the near ultraviolet and visible range and excitation-dependent visible fluorescence with the maximal intensity at ca. 430 nm and excitation maximum at ca. 350 nm. Absorption and fluorescence characteristics of Lys-CD-1 and Lys-CD-2 fractions were generally the same as of Lys-CD. The small difference between them was in slightly larger visible absorption and broader emission spectrum of Lys-CD-2 (Fig. 3, *a*, *b*) and small shifts in the excitation and emission maxima (Table 1). Lack of obvious dependence of the absorption and emission bands position on the carbon dot size proves that quantum size effects do not make appreciable contribution to their optical characteristics. Rather absorption and fluorescence are attributed to organic chromophore groups, which are the same in carbon dots of different sizes.

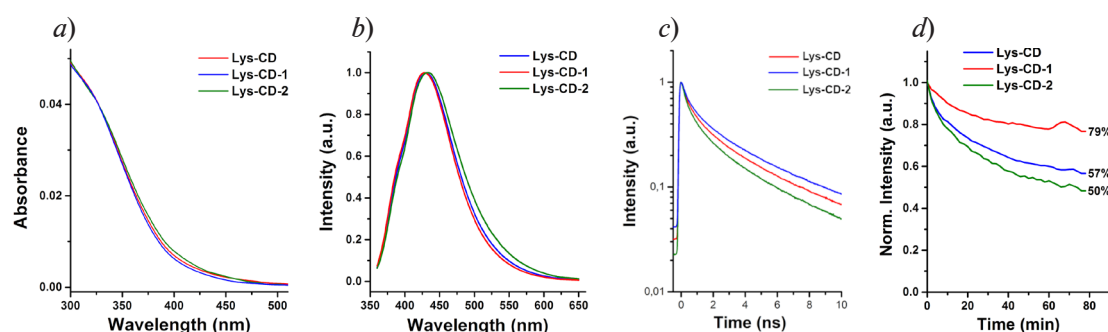


Fig. 3. Comparison of UV-Vis absorption (*a*) and fluorescence emission (excitation at 350 nm) (*b*) spectra, fluorescence decay kinetics (*c*) and fluorescence photobleaching kinetics (*d*) of Lys-CD, Lys-CD-1 and Lys-CD-2

Unlike emission spectra, fluorescence lifetimes and quantum yields demonstrated a clear dependence on the nanoparticle size and declined with increase of the carbon dot size (Fig. 3,*c*, Table 1). The natural radiative lifetime, equal to the ratio of fluorescence lifetime to quantum yield, was approximately constant at 35-40 ns for all the three samples. Thus, the difference in yields and lifetimes originates from higher nonradiative relaxation rate in carbon dots of larger diameter. This fluorescence quenching effect in Lys-CD-2 is accompanied by broadening of the emission spectrum to the higher wavelengths area (Fig. 3,*b*).

These observations indicate that the quenching mechanism is likely related to resonance energy transfer between donor and acceptor chromophores within the same carbon dot [8]. Larger nanoparticles are more likely to include several chromophore groups and thus are exhibit stronger energy transfer effects which agrees with stronger fluorescence quenching in Lys-CD-2.

Table 1

Fluorescence characteristics of Lys-CD and its fractions Lys-CD-1 and Lys-CD-2

Sample	Em. minimum, nm	Ex. maximum, nm	Φ , %	Lifetime, ns	Anisotropy decay time, ns	I_{450}/I_{350}
Lys-CD	429	351	6.5	2.29	1.39	0.109
Lys-CD-1	428	348	7.9	2.72	0.77	0.091
Lys-CD-2	433	352	4.6	1.85	2.68	0.149

Notations. Φ is the fluorescence quantum yield, I_{450}/I_{350} is the ratio of integral fluorescence intensities under 450 and 350 nm excitation



Finally, we analyzed photostability of lysine carbon dots. Under continuous wave irradiation at 350 nm fluorescence intensity of all the three samples decreased, the fastest photobleaching rate and the largest magnitude of decrease was observed for larger dots (Lys-CD-2) (Fig. 3,d). Thus, the smallest dots possessed the largest photostability.

Conclusion

Our results indicate that the same type of chromophores is responsible for absorption and emission of lysine carbon dots regardless of their size and quantum size effects do not contribute to their absorption and fluorescence. These chromophores are only formed within carbon dots and don't exist as separate molecules in solution. Quenching of blue fluorescence in carbon dots of large size indicates resonance energy transfer to acceptors within the same nanoparticle. Due to higher quantum yield and larger photostability smaller-sized lysine carbon dots are more attractive for practical applications.

REFERENCES

1. Sun Q., Qin Z., Wu W., Lin Y., Chen C., He S., Li X., Wu Z., Luo Y., Qu J., In vivo imaging-guided microsurgery based on femtosecond laser produced new fluorescent compounds in biological tissues, *Biomedical Optics Express*. 9 (2018) 581–590.
2. Astafiev A.A., Shakhov A.M., Osychenko A.A., Syrchina M. S., Karmenyan A.V., Tochilo U.A., Nadtochenko V.A., Probing intracellular dynamics using fluorescent carbon dots produced by femtosecond laser in situ, *ACS Omega*. 5 (21) (2020) 12527–12538.
3. Astafiev A.A., Shakhov A.M., Gulin A.A., Vasin A.A., Gubina M.V., Syrchina M.S., Nadtochenko V.A., Femtosecond laser synthesis and comparative analysis of fluorescent carbon dots from L-lysine aqueous solution, *Journal of Physics: Conference Series*. 2086 (2021) 012121
4. Zhu S., Song Y., Zhao X., Shao J., Zhang J., Yang B., The photoluminescence mechanism in carbon dots (graphene quantum dots, carbon nanodots, and polymer dots): current state and future perspective, *Nano Research*. 8 (2015) 355–381.
5. Yuan F., Yuan T., Sui L., Wang Z., Xi Z., Li Y., Li X., Fan L., Tan Z., Chen A., Jun M., Yang S., Engineering triangular carbon quantum dots with unprecedented narrow bandwidth emission for multicolored LEDs, *Nature Communications*. 9 (2018) 2249.
6. Schneider J, Reckmeier C.J., Xiong Y., von Seckendorff M., Susa A.S., Kasák P., Rogach A.L., Molecular Fluorescence in Citric Acid-Based Carbon Dots, *The Journal of Physical Chemistry C* 121 (3) (2017) 2014–2022.
7. Essner J.B., Kist J.A., Polo-Parada L., Baker G.A., Artifacts and Errors Associated with the Ubiquitous Presence of Fluorescent Impurities in Carbon Nanodots, *Chemistry of Materials*. 30 (6) (2018) 1878–1887.
8. Astafiev A.A., Shakhov A.M., Nadtochenko V.A., Resonance Energy Transfer Reveals the Presence of Multiple Luminescence Emission Centers within a Carbon Nanodot, *JETP Letters*. 116 (2022) 505–513.

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