Adsorption 11: 595–602, 2005

Synthesis and Characterization of Silica Gel Particles Functionalized with Bioactive Materials*

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Abstract. Bioactive materials (having an amino acid, Ac-Tryptophan, A or a peptide, Ac-Trp-Ala-Ala, B) were anchored onto silica particles. A photoactive linker (*N,N'*-bis(2-phosphonoethyl)-1,4,5,8-naphthalenediimide (DPN)) was initially attached to the particles and next the amino acids were bound by using both Zr/P chemistry and 1,3-diisopropylcarbodiimide/1-hydroxybenzotriazole coupling. In A derivative extensive complexation of Trp with DPN was observed. Photolysis studies showed the presence of excited state reactions on the silica particles, moreover the radical species (DPN$^{\cdot-}$, TrpH$^{\cdot+}$, Trp$^{\cdot}$) remained alive ~100 times longer on the particle surface than in solution. These studies show that the formation rate of these radicals is a function of the supramolecular structures of the peptide and of the amino acid derivative on the silica particle.

Introduction

The mechanisms involved in photoinduced reactions between biological systems and photoactive molecules are important points to be elucidated to the technological application of hybrid materials composed of inorganic matrices with bioactive materials. Relevant properties of these materials start with the molecular recognition where proteins and sugars are the typical components. In sequence, a selected property of the biological material needs to be demonstrated (Sisido et al., 1998) for its usage. Among the properties of interest, the photoinduced redox activity is a typical choice due to its possibility of destroying tumor cells, parasites and so far (Schuitmaker et al., 1996). Photoinduced phenomena involve two main routes: either by direct interaction of the photoactived species with the target or via singlet oxygen production (Foote, 1968; Kim et al., 1993). The rate of these two processes is dependent on the specific location of the sensitizer, oxygen diffusion rate in the microenvironment and proximity of redox targets in the biological systems. Besides its very low cost silica gel particles offers, a roughness to the interactions between sensitizer and target which is appropriate to mimic biological media. In parallel, it is also important to several applied areas such as chromatography, combinatorial synthesis and photocalysis (Chen and Liu, 2001; Balzani and Scandola, 1983). Furthermore, the control of the chemical and photochemical properties of peptide and photoactive

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*This paper was presented in the 5th Brazilian Meeting on Adsorption, held at Natal, Brazil, 18–21 July, 2004.

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drugs bound to a solid support is a challenging issue in chemistry and supramolecular photochemistry.

Tryptophan (Trp) is one of the most important targets involved in photoinduced damages because of its property to participate in redox reactions either in ground or in excited states and of course, because it is a quite common amino acid present in practically all proteins (Morozova et al., 2002).

The photoactive chromophore group is a naphthalenediimide (NDI) derivative due to its straightforward synthetic chemistry and to its favorable photochemical properties (Rodrigues et al., 1999; Aveline et al., 1997) Excitation of NDI produces an extensive amount of triplet state due to a high yield intersystem crossing (Rogers and Kelly, 1999), as well as radical species in the presence of appropriate electron donors.

In this work a derivative of NDI having two phosphonates at its ends, the (*N,N′*-bis(2-phososphonoethyl)-1, 4, 5, 8-naphthalenodiimide-DPN), was anchored to silica gel by the strong interaction of phosphonate groups and zirconium. A tripeptide (Ac-Trp-Ala-Ala-OH) and an amino acid (Ac-Trp-OH) derivatives were following attached to the silica surface by using Fmoc methodology (Chan and White, 2000). Mechanistic studies show the photoactivity of these particles by the formation of DPN and Trp radicals.

Experimental Section

Materials

Silica-gel 60 was obtained from Aldrich. *N,N′*-bis (2-phosphnooethyl)-1,4,5,8-naphthalenediimide (DPN) and *N,N′*-bis(dibutyl)-1, 4, 5, 8-naphthalenediimide (DBN) were prepared as previously described (Rodrigues et al., 1999). Fmoc-Ala-OH, Fmoc-Trp-OH1, 3-diisopropylcarbodiimide (DIC), 1-hydroxybenzotriazole (HOBt) and piperidine were Aldrich products. All other materials for the synthesis were from commercially available sources. $POCl_3$, DMF and acetonitrile were previously treated by distillation and the other solvents were used as obtained from the suppliers. The derivatization of silica particles follows the route depicted in the Scheme 1 and it is described in details in reference (Rodrigues et al., 2005).

Analytical Methods

Silica particles were suspended in ethylene glycol : water (12.5:1, v:v) for the spectroscopic measurements to decrease the rate of particles settling. The synthetic peptide and amino acid derivatives were analyzed by LC-MS after the treatment of silica particles with NaF which cleaves the Zr/phosphonate bond and releases the phosphonate derivative. Product separation was performed in a Supelco ODS column (25 cm × 4.6 mm, 5 μm) by using a linear gradient of H_2O:TFA (100:0.1); over, H_2O:ACN:TFA (90:10:0.08); 1 mL min^{-1}. The mass spectra were obtained with a Quatro 11 spectrometer (electron spray ionization) from Micromass. The extent of substitution of DPN, Ac-Trp and the Tripeptide is presented in Table 1. The particles assemble was based in Zr/phosphate interaction (Kaschak and Mallouk, 1996) and Fmoc solid phase peptide syntheses strategy (Chan and White, 2000).

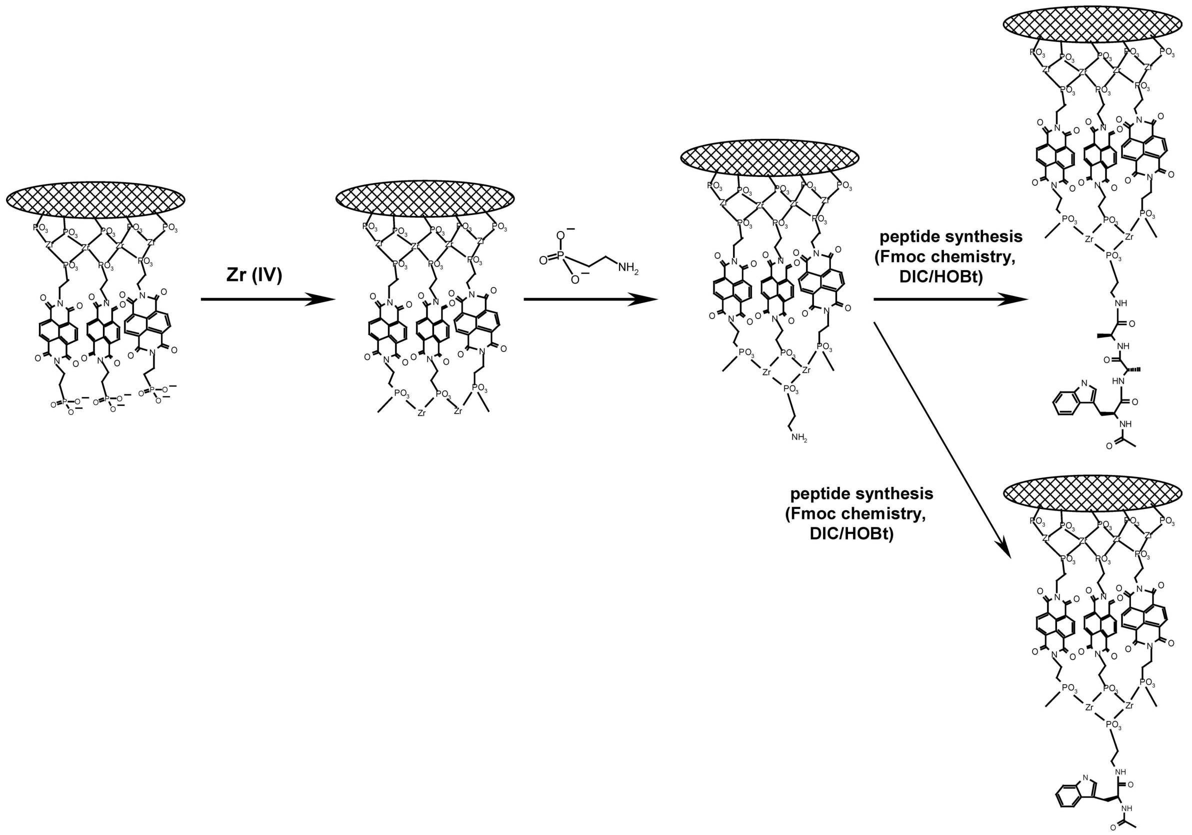
Instruments

UV-Vis absorbance spectra were obtained in a Hitachi-2000 or a Shimadzu UV-2400-PC spectrophotometers. Fluorescence spectra were recorded in a SPEX DM 3000-F fluorometer. Spectral data were obtained with a 1 cm optical path length quartz cuvettes. Laser flash photolysis experiments were realized with an "Applied Photophysics" system composed of a Nd:YAG laser (Spectron Laser System, England) operating at 355 nm, delivering pulses with ∼20 mJ/20 ns full width at of half maximum, a pulsed 150 W Xe lamp, control electronics and a digitizing oscilloscope (Hewlett-Packard 54510 B) for data capture. Data were analyzed and stored in a PC compatible microcomputer. The experiments were conducted at room temperature (∼23°C).

Results and Discussion

Before studying the interaction of DPN and Trp on silica particles, it was necessary to understand the process in solution. It was shown previously that DNI and Trp have favorably a charge transfer mechanism which is probably via π complex (Rogers and Kelly, 1999). This interaction was observed in the present work, as the decrease in the UV-Vis absorbance spectra of DPN as a function of added Trp (Fig. 1). In parallel a decrease in the fluorescence emission yield was also observed (Fig. 2). Data treatment using a 1:1 stoichiometry resulted in a good linear relationship (Connors, 1987). A complexation constant of ∼75 M^{-1} was obtained.

The DPN triplet state in solution was monitored by laser flash photolysis studies as depicted in the Fig. 3 (circles in the lower panel). Transient signals due to



Scheme 1.

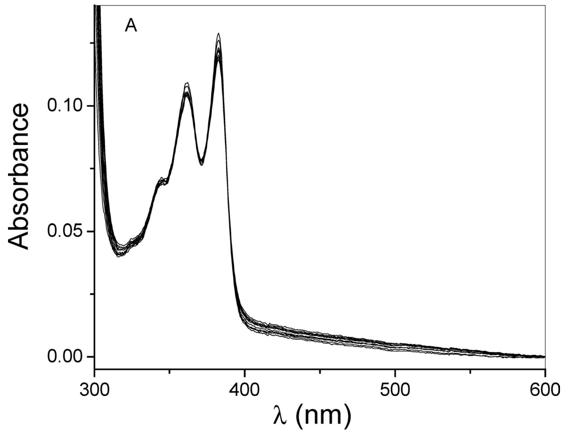


Figure 1. DPN absorbance spectra ([DPN] = $1 \times 10^{-5}\,M^{-1}$) *versus* [Trp] (from top to bottom Trp varied from 1×10^{-3} to $1 \times 10^{-2}\,M^{-1}$).

^{3}DPN and to $DPN^{\cdot -}$ are observed at 450 and 475 nm, respectively (Rodrigues et al., 1999; Aveline et al., 1997). For clarity purpose the transient signals of a longer lived NDI derivative (a *N*-dibutyl-naphthalenediimide DBN, up right panel) dissolved in a rigid matrix (sucrose octaacetate) and of DPN in water using a good electron donor (1,4-diaza-biciclo [2.2.2] octano, DABCO) are included. In the case of DBN, the peaks due to the triplet (450 nm) and to the radical (475 nm) are easily detected. In the case of DPN in the presence of DABCO, only the signal due to $DPN^{\cdot -}$ (475 nm) is observed. In the absence of electron donors the proposed mechanism for the formation of $DPN^{\cdot -}$ occurs by the reaction between ^{3}DPN with DPN ground state species(Aveline et al., 1997).

The behavior of ^{3}DPN in the presence of Trp is presented in Fig. 3 (squares in the bottom panel). In this condition the amount of Trp was sufficient to provide DPN and Trp free species, once it was observed the lack of photoactivity in the complex. In other words, the complex DPN-Trp simply decays vibronically from the singlet excited state. The transient spectra of NDI in the presence of Trp showed a high yield of $DPN^{\cdot -}$ and as well as of the oxidized species of Trp ($Trp^{\cdot}$ and $TrpH^{\cdot +}$ at 550 and 575 nm, respectively) Rogers et al. (2000).

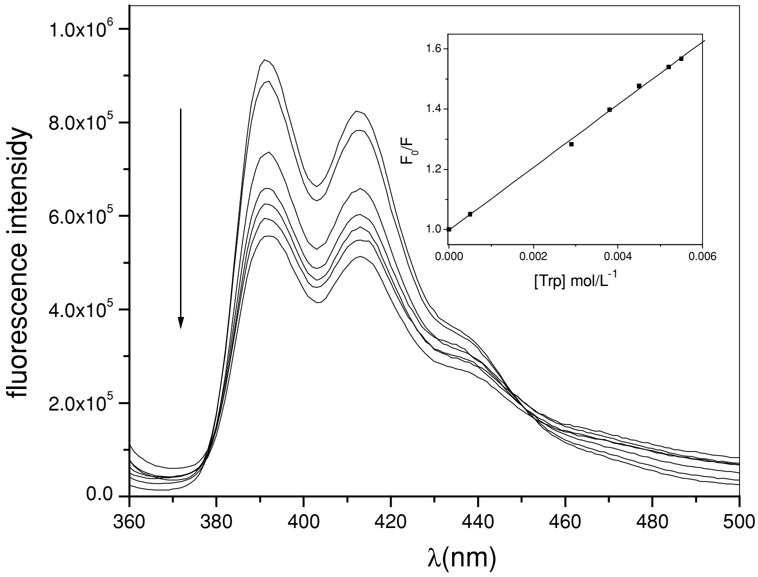


Figure 2. Emission spectra of DPN ([DPN] = 1×10^{-5} mol L^{-1}) *versus* Trp concentration (from top to bottom at 400 nm Trp concentration varied from 1×10^{-3} to 6×10^{-6} M^{-1}). Inset: Fluorescence intensity ratio of DPN without (F_0) and with (F) Trp as function of unbound Trp.

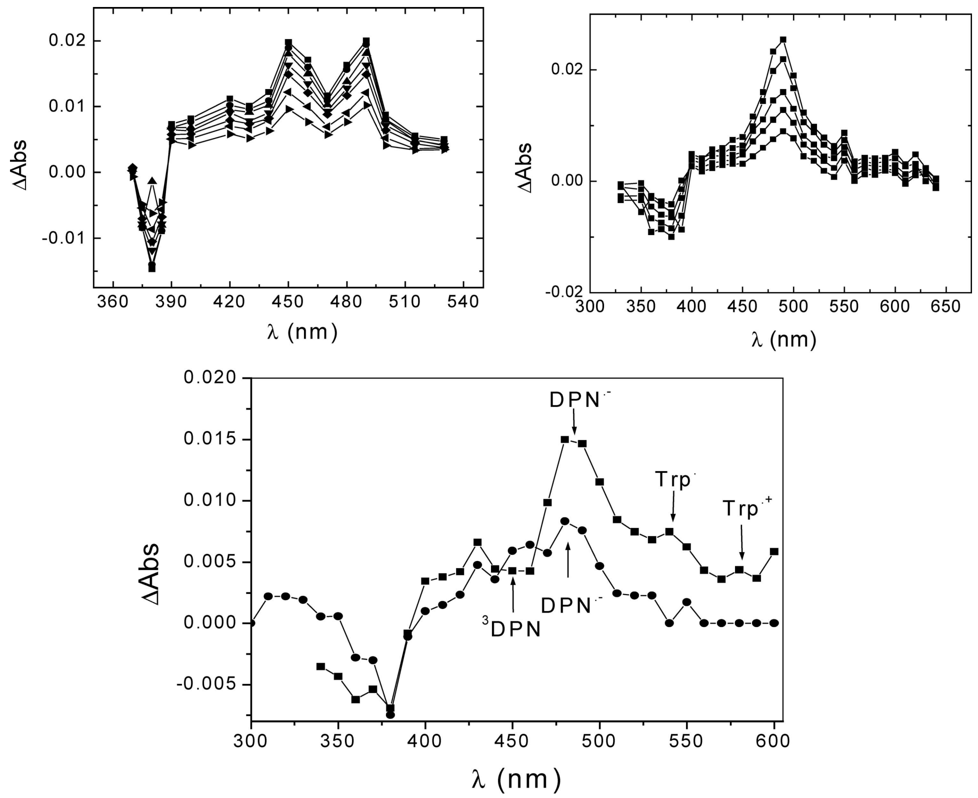


Figure 3. Transient spectra of di-butyl naphthalene diimide in sucrose octaacetate glass at 2, 5, 10, 20, 30, 50, 80 μs after the laser pulse (*up right panel*). Transient spectra of DPN (1×10^{-5} M) in the presence of DABCO ([DABCO] = 1 mM) at 0.8, 5, 20, 40, 80 μs after the laser pulse (*up left panel*)**.** Transient spectra of DPN ([DPN] = 1×10^{-5} M) in aqueous solution in the absence (●) and in presence (■) of Trp ([Trp] = 5×10^{-4} M) 1 μs after the laser pulse. $\lambda_{exc} = 355$ nm.

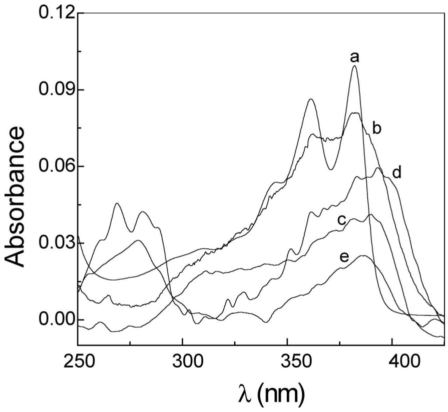


Figure 4. UV-Vis spectra of DPN in: (a) water, (b) DPN-silica, (c) APA-Zr-DPN-silica, (d) Ac-Trp-Ala-Ala-DPN-silica and (e) Ac-Trp-DPN-silica.

The UV-Vis spectra of DPN free in solution and anchored in silica particles are presented in the Fig. 4. The spectrum of DPN free in aqueous media (trace a) shows its usual vibronic aspect with maxima at ~380, 360, 335, and 325 nm (Rodrigues et al., 1999). The spectrum of DPN with one extremity bound to silica particles and another free (Fig. 4, trace b) resembles that of DPN in solution and shows that the chromophore experiences a certain degree of mobility. The addition of a new Zr layer leads to DPN immobilization resulting in the broadening of the spectrum observed with aggregated DPN (Fig. 4, trace c; Rodrigues et al., 1999). The spectra of silica gel with Trp or tripeptide (traces e and d respectively) are similar to that of immobilized DPN and shows an extra transition at ~280 nm due to Trp.

The fluorescence emission properties of DPN in the same conditions (Fig. 4) are presented in the Fig. 5. A spectral mirror image was found for DPN free in solution (trace a in Fig. 5 top panel), whereas DPN bound to silica particles showed a featureless red shifted emission. These emissions are assigned to a like excimer transition. It is interesting to observe in the Fig. 5, in which the maximum emission wavelength is the highest for DPN anchored on the silica having its end free (trace

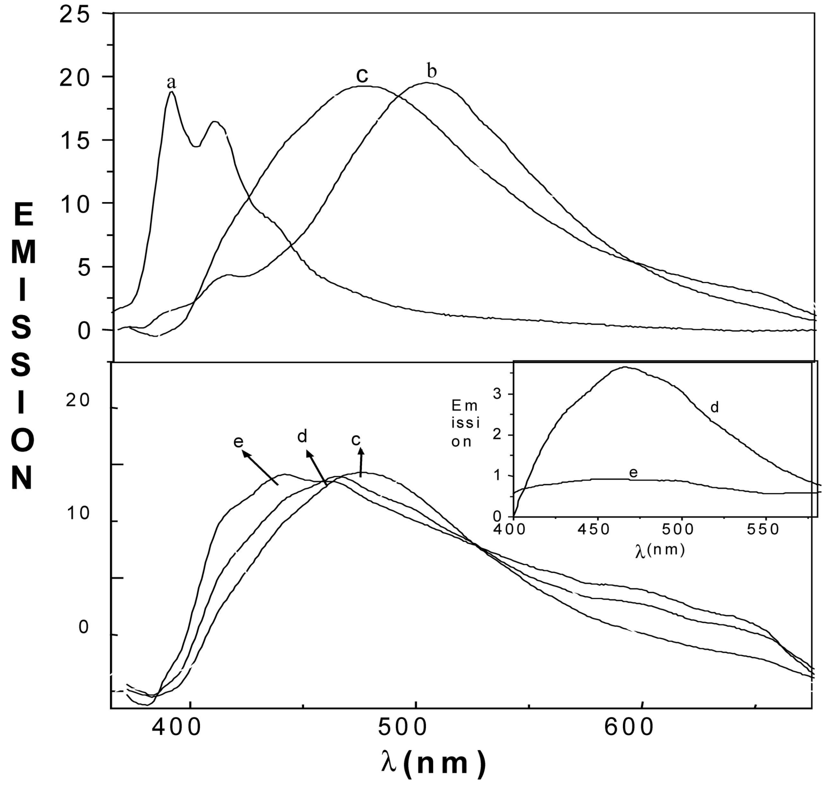
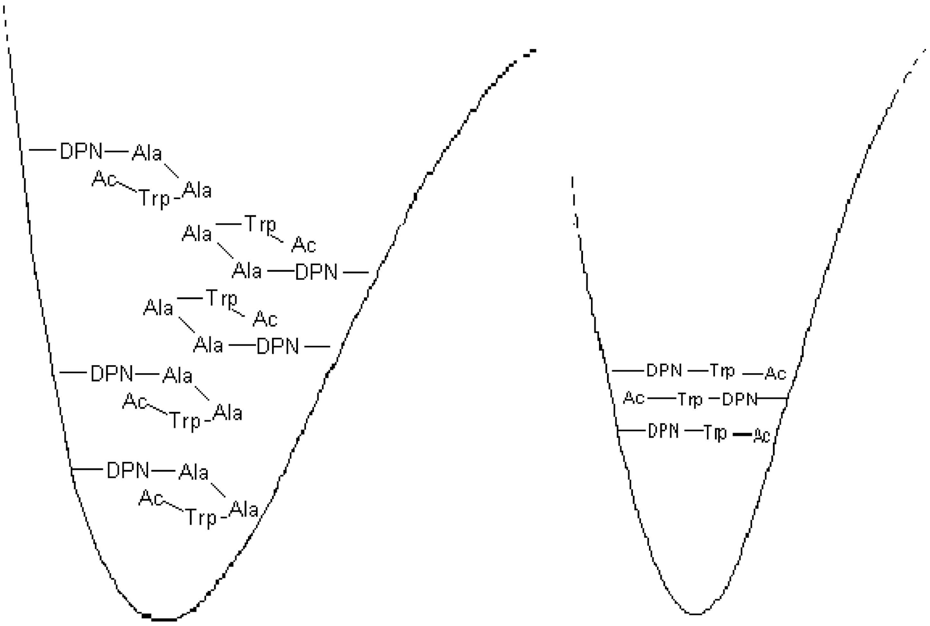
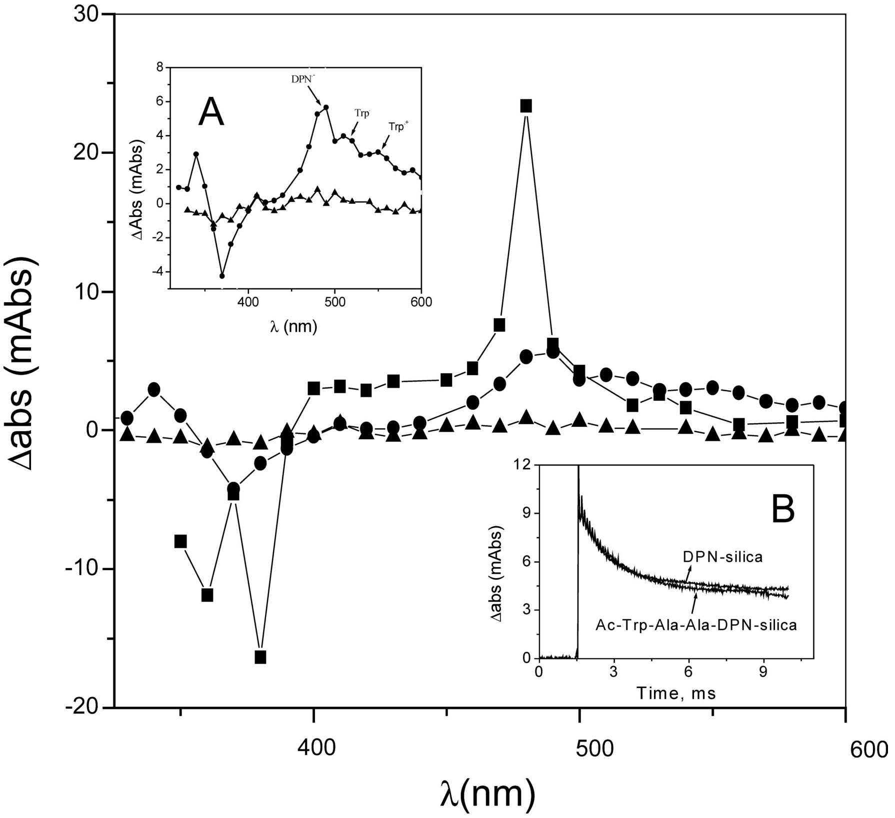


Figure 5. Fluorescence emission spectra of DPN in water (a), (b) DPN-silica, (c) APA-Zr-DPN-silica (top panel), and in (c) APA-Zr-DPN-silica, (d) Ac-Trp-Ala-Ala-DPN-silica, and (e) Ac-Trp-DPN-silica, $\lambda_{exc} = 350$ nm. The spectra are normalized. In the figure inset the relative emissions for the tripeptide (d) and for the Ac-Trp (e) are presented.



Scheme 2. Artist version of DPN silica particles in the silica gel crevices.



Scheme 6. Transient spectra of (■) DPN-silica, (●) Ac-Trp-Ala-Ala-DPN-silica and (▲)Ac-Trp-DPN-silica. Insets. (**A**) Scale expansion of the transient spectra of Ac-Trp-Ala-Ala-DPN-silica (●) and Ac-Trp-DPN-silica (▲) from the main figure. (B) ΔAbs transients decay curves for Ac-Trp-Ala-Ala-DPN-silica and for Ac-Trp-DPN-silica obtained under the same conditions. $\lambda_{exc} = 355$ nm.

b, top panel), followed by DPN immobilized with a Zr layer (traces c in both panel) and after by the tripeptide (trace d in the bottom panel) and Ac-Trp (trace e in the bottom panel). It is also noticiable the very low emission of Ac-Trp compared with that of the tripeptide (figure inset in the lower panel). It worth to notice that Trp is transparent in the excitation wavelength used and thus does not contribute to the observed emissions. These set of evidences show that the chromophore is sensitive to distinct freedom degrees within the particles. For the particles modified with Ac-Trp and for the tripeptide the DPN environment is depicted in the Scheme 2.

Finally, the transient spectra of DPN in the silica particles are presented in Fig. 6. It is observed for DPN-silica a strong contribution of $DPN^{\cdot -}$ at ~480 nm (squares), for the tripeptide formation of $DPN^{\cdot -}$ and Trp oxidized species ($Trp^{\cdot}$ and $TrpH^{\cdot +}$, circles and top inset), whereas for Ac-Trp-DPN-silica (triangles) no transient could be detected. Another observed feature is the relatively long-lived transients compared to DPN in solution (lower inset). These observations are in good agreement with the picture presented in Scheme 2. When DPN is immobilized very close to a Trp moiety in the particle (Scheme 2, right figure) very fast processes should occur below the resolution of our system. On the other hand, the electron transfer process for tripeptide is slower and the signals can be easily detected. (Scheme 2, figure on the left).

Conclusion

In this study the derivatization of silica gel particles with a photoinduced diimide and with amino acid residues is presented. Anchoring of the diimide was perfomed via Zr/Phosphonate linkage followed by Fmoc synthesis of Ac-Trp and (Ac-Trp-ALA-ALA).

The phoactivity of the tripeptide derivative showed to occur by the appearance of the radical species $DPN^{\cdot -}$, $Trp^{\cdot}$, and $TrpH^{\cdot +}$ from an electron exchange process. These species presented lifetimes relatively long. These results pointed to a further usage of such materials for biotechnological applications.

Nomenclature

Ac	Acetyl
Trp	Tryptophan
Ala	Alanine
DPN	*N,N′*-bis(2-phosphonoethyl) -1,4,5,8-naphthalenediimide
$DPN^{\cdot -}$	DPN radical anion
$TrpH^{\cdot +}$	Trp radical cation
$Trp^{\cdot}$	Trp radical neutra
NDI	naphthalenediimide
DBN	*N,N′*-bis(dibutyl)-1,4,5,8 -naphthalenediimide
HOBt	1-hydroxybenzotriazole
DABCO	1,4-diaza-biciclo [2.2.2] octano
^{3}DPN	DPN triplet state

Acknowledgments

We wish to express our deep gratitude to the brazilian granting agencies FAPESP, CNPQ and CAPES.

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