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BEYOND DINITROPHENOL INTERACTION WITH TRYPTOPHAN-BASED COMPOUNDS

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Abstract: The effects of 2,4-dinitrophenol (2,4-DNP) on the spectroscopic parameters (UV-Vis or FT-IR absorbance) or fluorescence emission of tryptophan and glycyl-tryptophan were studied. A quenching phenomenon of fluorescence was observed, attributed to interactions between the indole ring of the fluorophore and the aromatic ring of the quencher. The analysis of fluorescence spectra confirms that the quenching is dictated by 2,4-DNP concentration and pH. A combined mechanism of static and dynamic quenching was detected. The quenching phenomenon observed in this work could be employed to explain the mechanism of action of such compounds on large fluorescent peptides or proteins.

Keywords**:** Spectroscopy; fluorescence; tryptophan; dipeptide, 2,4-dinitrophenol.

Introduction

Dinitrophenols are commonly used in agriculture, dye industry or as dangerous slimming agents.[1,2] However, more than 60 deaths have been related to 2,4-DNP consumption.[3] Nevertheless, 2,4-DNP could act as a neuroprotective agent, but its adverse effects on the blood-brain barrier during ischemia were reported.[4] Furthermore, 2,4-DNP was still able to enhance caspase-activation via an uncoupled oxidative phosphorylation.[5] Besides, 2,4-DNP increases the expression of hypoxia-inducible factor and increases the rate of hepatic prostaglandin production.[6,7]

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It is well known that the fluorescence quenching studies and theoretical calculations are valuable tools for studying the interaction of tryptophan with various nitroderivatives.[8] Therefore, in the present study, the interaction of tryptophan or glycyl-tryptophan with 2,4-dinitrophenol was investigated using spectroscopic and fluorimetric measurements.

Results and Discussion

Initially, the behavior of tryptophan in the presence of dinitrophenol was investigated by UV-Vis spectroscopy. Thus, the UV spectrum of an aqueous 24 μM tryptophan solution (in 20 mM Tris buffer, pH 7) reveals two absorption peaks (219 and 279 nm) (Figure 1). Furthermore, 2,4-DNP solution displays three maxima and a shoulder in both UV and Vis regions (229, 259, 360 and 394 nm). A stoichiometric mixture of these components (24 μM each) displays a different UV-Vis spectrum when compared with the sum of the spectra of individual compounds. For clarity, the individual spectral contributions were subtracted from the last spectrum. A 13 nm blue shift (in the UV region) of the maximum was observed from these data.

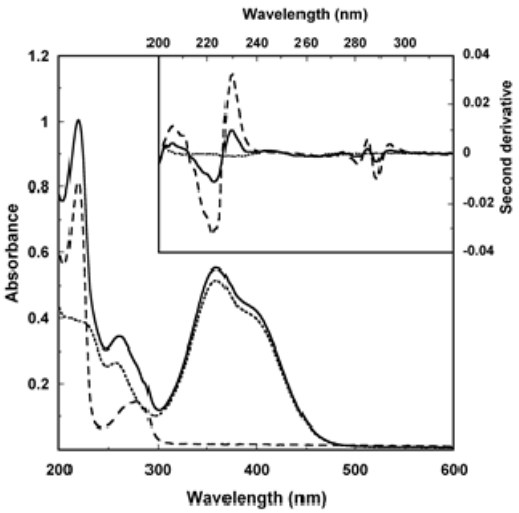

Figure 1. UV-Vis spectra of tryptophan (Trp 24 μM, dashed line), 2,4-dinitrophenol (2,4-DNP 24 μM dotted line) and their 1:1 stoichiometric mixture (line).

The second derivative absorption spectrum for this binary system (Figure 1, inset), reveals a negative band at 220 nm and two positive bands at 204 and 215 nm. However, these bands have lower intensities compared to the second derivate spectrum of tryptophan alone. This fact suggests that dinitrophenol may uptake a part of energy devoted to the π-π* transition of indole when tryptophan is also present.

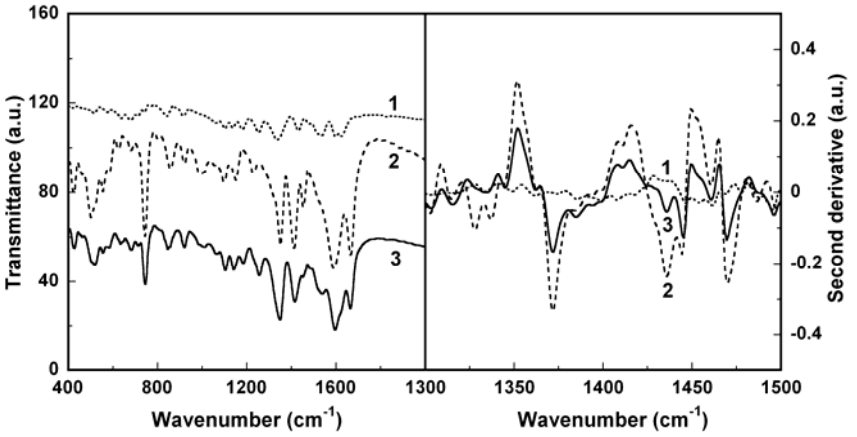

Figure 2. FT-IR spectra (left) and their second order derivatives (right) corresponding to tryptophan (Trp, dashed line), 2,4-dinitrophenol (2,4-DNP, dotted line) and their stoichiometric mixture (line).

The FT-IR spectra of the Trp and 2,4-DNP alone or their stoichiometric complex are presented in Figure 2. Two spectral shifts were observed in the case of this binary system when a thorough analysis was conducted. More specifically, the bands from 906 cm^{-1} and 1416 cm^{-1} were found to have shifted to 904 cm^{-1} and 1414 cm^{-1}, respectively. Thus, this interaction could be mainly attributed to 2,4-DNP interaction with the indole aromatic system. The aromatic ring also underwent a small disturbance. More exactly, the 2,4-DNP's band from 1060 cm^{-1} was shifted down with 2 cm^{-1} in the presence of Trp.

The fluorescence quenching method is a sensitive and cost effective tool for nitroaromatic compound detection. Dinitrophenols and their

derivatives are able to quench the fluorescence of various biological compounds. Therefore, this study is focussed on their interaction with the tryptophan molecule, in its excited state. This approach enables us to gain more details about both electron and energy transfer from the excited molecule to dinitrophenol.

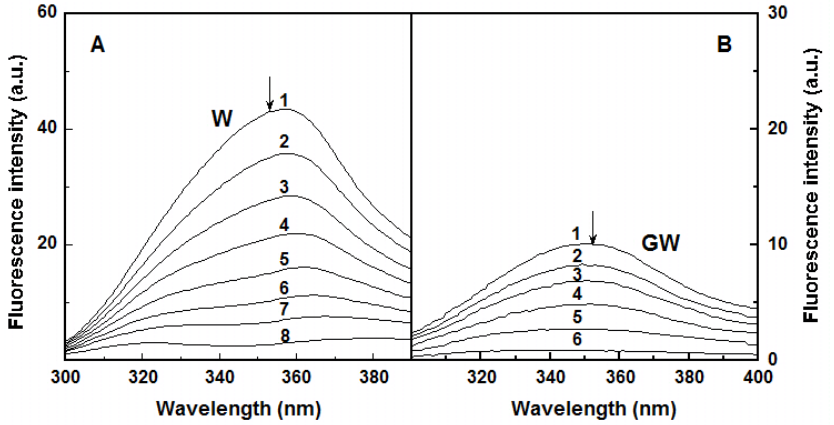

Figure 3. Fluorimetric titration of tryptophan (W) and glycyl-tryptophan (GW) with 2,4-dinitrophenol (2,4-DNP) at pH 7.0: a) emission spectra of W in the absence (curve 1) or presence of 1.4; 2.04, 2.72; 3.4; 4.08; 4.76 and 6.12 equivalents of 2,4-DNP (spectra 2-8); b) emission spectra of GW in the absence (curve 1) or presence of 2; 4, 8; 16; 32 equivalents of 2,4-DNP (spectra 2-6).

Tryptophan alone in Tris buffer exhibits a strong emission band at 357 nm. In this respect, the first set of experiments envisaged changes in the Trp fluorescence intensity at various concentrations of 2,4-dinitrophenol. A gradual quenching of tryptophan fluorescence is illustrated by emission spectra (Figure 3, Panel A). Contrary, a splitting of the fluorescence signal for 2,4-dinitrophenol was noticed. Thus, a red shift (about 23 nm) of the emission band was observed in the presence of 2,4-DNP. Supplementary, a shoulder at 315-325 nm appeared at higher concentration of 2,4-DNP. These emission wavelength shifts support the evidence of 2,4-DNP interaction with tryptophan. Glycyl-tryptophan, a fluorescent dipeptide, exhibits a

moderate emission band at 350 nm.[9] A blue shift (about 8 nm) of the emission band was denoted in the presence of 2,4-DNP (Figure 3, Panel B). When a Stern–Volmer equation (modified according to Lehrer) was used, the fluorescence quenching constant of W by 2,4-DNP was calculated to be 9.40 mM^{-1}, at neutral pH. A smaller value (7.22 mM^{-1}) was found in a similar manner for GW at pH 6.7 (Figure 4). Tryptophan is more easily quenched by 2,4-DNP than the dipeptide GW even at higher pH values. All experiments were performed at pH values that exceeded the isoelectric points of the investigated fluorophores. The calculated value of fractional maximum fluorescence intensity, f_a, was within the range 1.14 to 1.37.

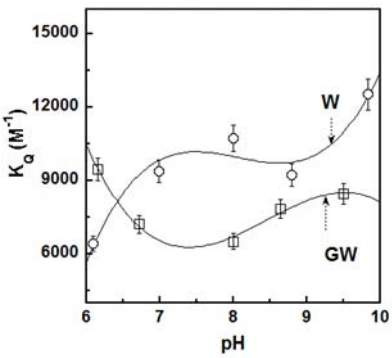

Figure 4. The pH dependence of quenching constants (K_Q, M^{-1}) for both tryptophan (W) glycyl-tryptophan (GW) using 2,4-dinitrophenol (2,4-DNP) as a quencher.

Experimental

All chemicals were of analytical reagent grade and all solutions prepared with milliQ grade water with R = 18.2 MΩ·cm. Dinitrophenol was purchased from Merck. L-tryptophan (W) and glycyl-tryptophan (GW) were supplied from Sigma. The spectral measurements were performed with a Libra S35 PC UV/VIS spectrophotometer (Cambridge, UK) with 1 cm matched cells of quartz.

The pH values were measured with a HANNA PH 211 microprocessor pH meter. All fluorescence spectra were recorded on a

SFM-25 Spectrofluorimeter (*Kontron* Instruments S.P.A., Milan, Italy) equipped with 1.0 cm quartz cell (volume 1.7 mL) and a thermostated bath (37 ºC). An excitation wavelength of 275 nm was chosen for all experiments. All experiments were carried out in a 50 mM Tris buffer.

The quenching constants of fluorophore (K_Q) were determined from the Stern–Volmer equation modified by Lehrer [34]:

$$F_0/\Delta F = [Q]^{-1} f_a^{-1} K_Q^{-1} + f_a^{-1}$$

where: f_a is the fractional maximum fluorescence intensity of W or GW, and [Q] is the 2,4-DNP concentration.

Conclusions

This is the first report describing interactions of 2,4-DNP, a well-known metabolic inhibitor and a slimming agent, with two fluorophores. These binary systems were investigated spectroscopically. The binding of 2,4-DNP to the investigated fluorescent compounds influences the intensity of both UV-Vis and FT-IR spectra. The fluorescence quenching experiments are more sensitive and useful to study such interactions. Tryptophan is easier quenched by 2,4-DNP than glycyl-tryptophan. Most probably, the dipeptide GW requires some conformational rearrangements in order to promote the interaction with 2,4-DNP. Nevertheless, the quenching constants did not differ substantially in comparison with their parent amino acid fluorophore. Further research is needed to clarify the whole picture of such interactions.

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