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Short Communication

Molecular level investigation of the role of peptide interactions in the glyphosate analytics

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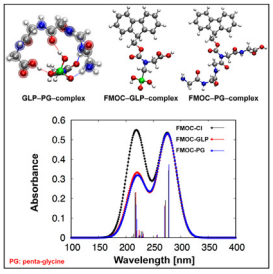
HIGHLIGHTS

- This study included calibration experiments and quantum chemical modeling.
- Strong interaction between GLP and peptides in solution has been explored.
- FMOC-Cl showed similar reactions with GLP and peptides.
- Peptides inhibit the GLP derivatization reaction causing the GLP underestimation.
- The presence of peptides may lead to an overestimation of the GLP concentration.

GRAPHICAL ABSTRACT



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ABSTRACT

The detection of the herbicide glyphosate (GLP) in environmental samples is most often conducted after derivatizing the target molecule with the chromophore 9-fluorenylmethyloxycarbonyl chloride (FMOC-Cl). However, this method is sensitive to all primary and secondary amines, which can occur in the sample matrix as well. In order to quantify the interference of primary and secondary amines on GLP detection, we have used well-defined peptides such as pentaglycine (PG) and albumin as well as mixtures of peptides such as peptone. These peptides have been added to the derivatization solution of GLP at different constant concentration levels and UV extinction coefficients have been determined. Data analysis supported by quantum chemical modeling of the GLP–peptide, FMOC–GLP, and FMOC–peptide complexation reactions facilitated the identification of two interfering impacts of peptide on GLP derivatization: (i) increase of the signal due to reaction with FMOC-Cl leading to an overestimation of GLP concentration and (ii) decrease of GLP recovery due to complex formation and therefore inhibition of GLP derivatization, which leads to an underestimation. Specifically, our results indicated that the GLP-peptide- and peptide-FMOC-interactions are mainly affected by type of interfering peptides as well as concentration of each peptide and GLP in the environmental samples.

1. Introduction

Glyphosate (GLP) is the most commonly used herbicide worldwide. Although it is assumed that GLP is nearly immobile in soil

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(Borggaard and Gimsing, 2008) it was found in ground and surface waters (Aparicio et al., 2013) and recently in the Baltic Sea (Skeff et al., 2015). This emphasizes the need for reliable analytical methods to support the ongoing discussion of potential risks of unlimited GLP usage. The most frequently employed detection method for GLP is a chromatographic separation of the reversed phase and UV- or fluorescent-spectrometric detection (Stalikas and Konidari, 2001), often combined with mass-spectrometry (Ramirez et al., 2014). Recently, Koskinen et al. (2016) reported that the GLP direct analytical detection methods, i.e. without GLP derivatization, are almost not suitable for the GLP determination in samples of complex and rich matrix constituents such as plant and soil materials. To this end, GLP has to be converted into a non-polar and UV-detectable form *via* a derivatization step such as reaction with 9-fluorenylmethyloxycarbonyl chloride (FMOC-Cl) as described by Hanke et al. (2008).

The composition of environmental samples is generally complex, containing numerous diverse organic and inorganic constituents. These matrix components potentially interact with GLP in solution, e.g., by formation of stable complexes between GLP and multivalent cations (Freuze et al., 2007). Also, polar organic compounds containing phenolic, hydroxylic, carboxylic, and amino functional groups can form stable complexes with GLP *via* H-bond formation with the GLP phosphonic and carboxylic moieties (Gros et al., 2017). Primary and secondary amines such as peptides have the ability to interact with GLP (Castellino et al., 1989) as well as with the derivatization agent FMOC-Cl (Carpino and Han, 1972; Moye and Boning, 1979). This indicates that the GLP detection or quantification may be affected by peptides in the samples. The impact of possibly produced FMOC–peptide complexes can be avoided by chromatographic separation combined with mass-spectrometry (environmental samples with unknown concentration, Vreeken et al., 1998) or blank correction in the data evaluation (samples with known GLP concentrations such as those for sorption isotherms). However, possible interactions between peptides and GLP preventing the derivatization with FMOC-Cl cannot be corrected using the established analytical procedures. This is due to the lack of knowledge about concentration of GLP and peptides, and their interaction strength in environmental samples. Here, we hypothesize that the interaction of peptides with GLP can suppress the GLP reaction with FMOC-Cl, and this inhibiting effect on GLP derivatization may cause underestimations of GLP concentrations. Surprisingly, this possible disturbance of the GLP quantification has not been examined and described in the literature before.

The objective of the present study was to scrutinize possible inhibition effects at a molecular level by investigating the GLP derivatization with FMOC-Cl in the presence of peptides of different compositions. Well-defined peptides such as pentaglycine (PG) and albumin as well as mixtures of peptides such as peptone will be used in the current contribution. Specifically, the interactions between GLP and peptides, FMOC-Cl and GLP, and FMOC-Cl and peptides will be explored experimentally by calibration studies and theoretically by quantum chemical modeling.

2. Materials and methods

2.1. Chemicals and calibration solutions

GLP (CAS: 1071-83-6), FMOC-Cl (CAS: 28920-43-6), dichloromethane (CAS: 75-09-2), sodium tetraborate decahydrate (CAS: 1330-43-4), albumin (pig, CAS: 9048-46-8), and peptone (from casein, CAS: 91079-40-2) were purchased from Sigma Aldrich. PG (CAS: 7093-67-6) was purchased from Fluorochem Ldt. Hydrochloric acid and sodium hydroxide were used for pH adjustment of GLP and PG solutions. All the used chemicals were of analytical grade. Concentration levels of single components for GLP and PG were prepared in the range of 2.96–94.67 $\mu mol\,L^{-1}$ and range of 0.5–32.0 $mg\,L^{-1}$ for albumin and peptone. The GLP calibration solution was also prepared in the presence of three distinct levels of PG, albumin and peptone (0.5, 16.0, and 32.0 $mg\,L^{-1}$). Four replicates per concentration level were prepared.

2.2. Derivatization and UV-detection

The method proposed by Waiman et al. (2012) for complex environmental samples like soil was used for GLP, PG, albumin, and peptone derivatization. Briefly, 0.5 mL borate buffer solution (pH 9) was added to 4 mL of sample solution. Next, an excess concentration of FMOC-Cl (0.5 mL, $c = 1\,g\,L^{-1}$; dissolved in acetonitrile) was added. After vigorously shaking the derivatization solution was allowed to react for 2 h with occasionally shaking. Subsequently, by-products of FMOC-Cl (FMOC-OH) were removed by extracting with 4 mL dichloromethane. The mixture was centrifuged (10 min, 1558x*g*) to separate the two phases. The supernatant aqueous phases of each derivatization solution were used for UV/Vis spectroscopy at $\lambda = 264$ nm (Specord200, Analytik Jena AG, 07745 Jena, Germany). The averaged signals of the calibration series of the respective analyte were corrected by subtracting the signal intensity of the blank level containing no analyte. In this way effects on signal intensity resulting from matrix constituents were eliminated.

2.3. Quantum chemical modeling

Here, we are mainly focusing on the interaction between GLP and PG that was simulated through 1:1 complex formation between them, i.e. 1GLP + 1PG → GLP–PG complex. Different initial geometries for this complex were constructed by selecting the expected preferential binding situations between GLP and PG. The aqueous solution around the complex was simulated by introducing an implicit treatment through the conductor-like polarizable continuum model (CPCM, Cossi et al., 2003). Full geometry optimization, using CPCM, was performed for the complexes as well as for the individual species (GLP and PG). The calculations have been performed using density functional theory (DFT) implemented in the Gaussian09 program package (Frisch et al., 2013). Specifically, the B3LYP hybrid functional (Becke, 1988; Lee et al., 1988) combined with the 6–311++G (d,p) basis set (Hehre et al., 1972) and Grimme's D3 dispersion correction (Grimme et al., 2011) have been applied. The basis set superposition error (BSSE) has been corrected using the counterpoise scheme (Jansen and Ros, 1969). For more details about the different methods of computational chemistry and their application to soil science, see also Kubicki (2016).

For the complexation reaction, 1GLP + 1PG → GLP–PG complex, the reaction energy (ΔE) is calculated as follows:

$$\Delta E = E_{GLP-PG\ complex} - (E_{GLP} + E_{PG}) \quad (1)$$

where, $E_{GLP-PG\ complex}$, E_{GLP}, and E_{PG}, are the electronic energies of the GLP–PG complex, GLP, and PG, respectively. Similarly, the corresponding reaction free energy (ΔG) is calculated by including the zero-point energy and thermal correction to the Gibbs free energy.

The derivatization reactions of FMOC-Cl with GLP as well as with PG have been simulated at the same level of theory. For PG, we have considered five possibilities for the 1:1 FMOC–PG derivatization reaction according to the five amino groups (for details see Figs. S1 and S2 in the Supplemental Material (SM)). For the derivatization reaction, 1FMOC-Cl + 1GLP(PG) → FMOC–GLP(PG) complex + HCl, the reaction energy (ΔE) is calculated as follows: