Quantitative evaluation of noncovalent interactions between polyphosphate and dissolved humic acids in aqueous conditions

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ABSTRACT

As one kind of phosphorus species, polyphosphate (poly-P) is ubiquitous in natural environments, and the potential interactions between poly-P and humic substances in the sediments or natural waters would influence the fate of poly-P in the environments. However, the mechanism of the interactions has not yet been understood clearly. In this work, the characteristics and mechanisms of the interactions between humic acids (HA) and two model poly-P compounds with various chain lengths have been investigated. Results show that a stable polyphosphate-HA complex would be formed through the noncovalent interactions, and hydrogen bond might be the main driving force for the binding process, which might be formed between the proton-accepting groups of poly-P (e.g., P$=$O and P$-$O$^-$) and the oxygen containing functional groups in HA. Our findings implied that the presence of humic substances in natural waters, soils and sediments would influence the potential transport and/or mobility of environmental poly-P.

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1. Introduction

Polyphosphate (poly-P), condensed forms of inorganic phosphorus (P) compounds, is a linear polymer of orthophosphate residues linked via phosphoanhydride bonds. They were widely used as commercially synthetic detergent products and in the water works industry (Davis and Wilcomb, 1967). Their residues in the natural water body contribute directly to the aquatic environmental pollution. As a energy and nutrient storage molecule, poly-P can be accumulated intracellularly by microorganisms (Brown and Kornberg, 2008). Under sufficient external P supply, high amounts of poly-P can be found in many heterotrophic bacteria and phytoplanktonic organisms, especially cyanobacteria (Eixler et al., 2005). A high proportion of poly-P was detected within particulate samples collected from a eutrophic lake (Read et al., 2014). Phytoplankton and bacteria containing significant quantities of poly-P may act as efficient carriers for poly-P deposition to the sediment surface.

Poly-P have been found in the sediments from variety of water bodies with different trophic states, especially in eutrophic lakes (Carman et al., 2000; Hupfer et al., 2007, 2008; Shinohara et al., 2012). It is estimated that about 6.9 g poly-P P/m^2/yr would deposited to the aquatic sediments, and poly-P account for a substantial fraction of total P in the surface sediments (Sannigrahi and Ingall, 2005). The releasing phosphorus-containing compounds from poly-P in the sediment are considered as a potential source of internal P loading to explain the enhanced benthic P fluxes commonly observed under anoxic conditions (Hupfer et al., 2004; Ingall et al., 2005). This phenomenon may be of high ecological importance, since they increase the P loading of the benthic food chain and affect the permanent P deposition in sediments.

Humic substances are ubiquitous in fresh waters, soils and sediments (Gerke, 2010; McDonald et al., 2004). Because they are enriched in organic functional groups and high chemical reactivity, humic substances can provide a great number of attachment sites for environmental pollutants, and thus influence the biogeochemistry of the pollutants in the environments (Piccolo, 1996). The growing threat from eutrophication in aquatic water systems suggests that more attention should be paid on the transport and/or mobility of the poly-P. Though the presence of poly-P in the humic fractions of natural soils has been found for years (Bedrock et al., 1994; Makarov et al., 1997), potential interactions between poly-P and humic substances have not been well understood. Mechanistic information about the interactions between poly-P and humic substances is needed.

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In this work, the interactions between humic acids (HA) and the two model poly-P compounds with various chain lengths (sodium tripolyphosphate, STPP; sodium hexametaphosphate, SHMP) were investigated in details. The thermodynamic parameters of the binding between HA with poly-P could be calculated by isothermal titration calorimetry (ITC) at different pHs. ^{31}P nuclear magnetic resonance (NMR) technique was employed here to prove the formation of complex structure between HA and poly-P. To further characterize the interaction, spatial configuration changes of HA after binding with STPP or SHMP were evaluated using laser lighting scattering (LLS) analysis. This study allows for a quantitative description of the interactions between HA and poly-P, and would improve the understanding the roles of humic substances on the transport of poly-P in natural environments.

2. Materials and methods

2.1. Characterizations of STPP and SHMP

STPP and SHMP were purchased from Sigma Aldrich Corp., and used without further purification. STPP ($Na_5P_3O_{10}$) gave ^{31}P NMR signals between −7 and −22 ppm (Figure S1a). The doublet peak (P-1) arised from the interaction between the two terminal P atoms with the central P atom, while the triplet peak (P-2) in the spectrum indicated the interaction between the central P atom with the two terminal P atoms. SHMP is a poly-P with long chains. According to the quantitative ^{31}P NMR spectrum (Figure S1b), the chain length of SHMP could be calculated as 56 from the ratio of the peak area between the end-chain groups (P-1) and middle-chain groups (P-2) in the ^{31}P NMR spectrum (Choi et al., 2010). Then the molecular formula of this SHMP could be defined as $Na_{58}P_{56}O_{169}$ with 56 monomer ($-PO_3Na$).

2.2. HA properties analysis

HA (CAS no. 1415936) was purchased from Sigma−Aldrich Corp. Before experiments, the HA used in this study was purified preliminarily. The dissolved HA solution was sonicated for 20 min and centrifuged at 14,000 rpm for 5 min, and then filtered to remove the residues. Elemental analysis was conducted on an Elemental Vario EL III (Elementar Analysensysteme, Hanau, Germany), sample contains 38.01% C, 0.80% N, 26.42% O, and 3.87% H. The solid state ^{13}C NMR spectrum showed in Figure S2 suggests the presence of carboxyl carbon, aromatic and phenolic carbon, substituted aliphatic carbon (including alcohols, amines, carbohydrates, ethers, methoxyl,

(100 mg/L) was adjusted to below 3 with 0.1 M HCl. After 20-min stirring, the HA solution was automatically titrated using 0.05 M NaOH solution under N_2 atmosphere programmed in incremental monotonic mode with a dosing unit of 10 μL. The acquired titration data were analyzed with the PROTOFIT 2.1 software by using the Donnan Shell Model. The Donnan shell model with two discrete sites was applied to simulate our results (Turner and Fein, 2006). The pK_a values of −COOH and phenolic −OH groups were estimated to be 3.06 and 8.01, and the site densities were calculated to be 4.9 and 2.8 mmol/g, respectively (Figure S3). Compared to the pK_a values of other HA used in previous study (Wang et al., 2015), results indicated that HA from various sources had different pK_a values, implying the structures of these HA might be slightly different. The total contents of Fe and Al in HA were determined by inductively coupled plasma-mass spectrometry (ICP-MS, X Series 2, Thermo Scientific, USA). Results showed that the percentages by weight of Fe and Al in HA were 0.77% and 1.32%, respectively.

2.3. Isothermal titration calorimetry

The thermodynamic characteristics of the binding of HA with these two poly-P compounds were investigated using an ITC-200 calorimeter (MicroCal Co., USA), the interactions between HA and poly-P under different pHs (6−9) were investigated. All solutions were prepared in phosphate buffer solution (PBS), which was prepared by mixing 0.05 M Na_2HPO_4 and 0.05 M NaH_2PO_4 solutions to the desired pH (Rex et al., 1986). All solutions were sonicated for 20 min and centrifuged at 14,000 rpm for 5 min, and the supernatants were collected. The actual concentrations of HA solutions were measured using a total organic carbon (TOC) analyzer (muli N/C 2100, Analytic Jena, Germany). The titration conditions were given in Table S1. Experiments were carried out with a working volume of 199.3 μL at 20 °C at a stir rate of 1000 rpm. For each experiment, there was an initial thermal equilibrium time of 60 s, and subsequently 19 injections. Titrations of poly-P into HA and buffer solution were performed in 2 μL aliquots injected over 4 s. Analysis of the data was performed using Origin 8.0.

The background calorific effect in the dilution of HA and poly-P solutions had been subtracted. Thus, the released heat was attributed mainly to the binding reaction between HA and poly-P. The total heat content Q is related with the total volume of poly-P solution (ΔV) injected during the experiment (Perry et al., 2005; Xu et al., 2013). The change in heat content δQ from the completion of the i-1 injection to completion of the i injection is only related to ΔV, which can be acquired by the first derivative of Eq. (1).

$$\begin{aligned}
Q = \frac{\Delta H V_0}{2} &\left[NM_0 \left(\frac{1 - \frac{\Delta V}{2V_0}}{1 + \frac{\Delta V}{2V_0}} \right) + S_0 \frac{\Delta V}{V_0} \left(1 - \frac{\Delta V}{2V_0} \right) + \frac{1}{K_a} \right. \\
&\left. - \sqrt{\left[NM_0 \left(\frac{1 - \frac{\Delta V}{2V_0}}{1 + \frac{\Delta V}{2V_0}} \right) + S_0 \frac{\Delta V}{V_0} \left(1 - \frac{\Delta V}{2V_0} \right) + \frac{1}{K_a} \right]^2 - 4NM_0 \left(\frac{1 - \frac{\Delta V}{2V_0}}{1 + \frac{\Delta V}{2V_0}} \right) S_0 \frac{\Delta V}{V_0} \left(1 - \frac{\Delta V}{2V_0} \right)} \right] \\
= f(\Delta V) & \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad (1)
\end{aligned}$$

acetal carbon) and alkyl carbon in the HA molecules. The contents of the main functional groups in HA (e.g., −COOH and phenolic −OH) could be acquired by the acid-base titration using an automated titrator (DL50 Mettler-Toledo Inc., Switzerland) at 25 °C (Dewit et al., 1993; Ritchie and Perdue, 2003). Firstly, the pH of HA solution

where ΔH is the molar heat of poly-P binding to HA; N is the maximum binding capability of HA; K_a is the binding constant related to the affinity of the binding sites; M_0 is the initial concentration of HA (mg/L); S_0 is the initial phosphate units concentration (mmol/L); ΔV is the total volume of phosphate units injected