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Biomolecule–nanoparticle interactions: Elucidation of the thermodynamics by isothermal titration calorimetry☆

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ABSTRACT

Background: Nanomaterials (NMs) are often exposed to a broad range of biomolecules of different abundances. Biomolecule sorption driven by various interfacial forces determines the surface structure and composition of NMs, subsequently governs their functionality and the reactivity of the adsorbed biomolecules. Isothermal titration calorimetry (ITC) is a nondestructive technique that quantifies thermodynamic parameters through in-situ measurement of the heat absorption or release associated with an interaction.
Scope of review: This review highlights the recent applications of ITC in understanding the thermodynamics of interactions between various nanoparticles (NPs) and biomolecules. Different aspects of a typical ITC experiment that are crucial for obtaining accurate and meaningful data, as well as the strengths, weaknesses, and challenges of ITC applications to NP research were discussed.
Major conclusions: ITC reveals the driving forces behind biomolecule–NP interactions and the effects of the physicochemical properties of both NPs and biomolecules by quantifying the crucial thermodynamics parameters (e.g., binding stoichiometry, ΔH, ΔS, and ΔG). Complimentary techniques would strengthen the interpretation of ITC results for a more holistic understanding of biomolecule–NP interactions.
General significance: The thermodynamic information revealed by ITC and its complimentary characterizations is important for understanding biomolecule–NP interactions that are fundamental to the biomedical and environmental applications of NMs and their toxicological effects. This article is part of a Special Issue entitled Microcalorimetry in the BioSciences — Principles and Applications, edited by Fadi Bou-Abdallah.

1. Introduction

With the advance of nanotechnology, a vast diversity of nanomaterials (NMs) with novel properties has been developed for a wide range of applications. In the context of biomedical applications and NM toxicity, these NMs are exposed to a broad range of biomolecules of different abundances. The interaction between these biomolecules and NMs is fundamental to the application of NMs and the understanding of their toxicological mechanisms [1]. One of the main goals in these research fields is to identify the biomolecules to be coated around nanoparticles (NPs) of different properties [2]. Therefore, it is necessary to measure and compare the affinity of different biomolecules to the same NP or the same biomolecules to different NPs under relevant physiological conditions [3,4]. Another goal is to understand the stability and functionality of the NPs following coating of biomolecules, which are largely determined by the interaction mechanisms (e.g., interfacial forces that are involved and interaction affinity). In the context of environmental research, the production and applications of NMs ultimately lead to the release of NPs into the environment [5,6]. Natural nanosized particles such as mineral NPs and proteins are also ubiquitous in the environment [7,8]. These natural and man-made NPs commonly interact with natural organic matters (NOM), and the interaction greatly determines their environmental behaviors and influences the biogeochemical cycling of elements and contaminants [9].

Understanding the thermodynamics of biomolecule–nanoparticle interactions is challenging and of paramount importance, as the molecular interaction at this interface largely controls subsequent processes and effects. For example, the binding strength and binding moiety of the adsorbed protein may govern its structural integrity and activity. Few analytical techniques can compete with isothermal titration calorimetry (ITC) in tackling this challenge. ITC derives quantitative thermodynamic parameters (binding stoichiometry, binding constant, enthalpy, free energy, and entropy change) by monitoring the interaction and its associated heat change in real time. The results can help explore the interaction mechanisms for biomolecule–NP interaction (in terms of driving forces, affinity and stoichiometry) and allow

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comparative analysis of the effects of NP properties (e.g., size, shape, and surface chemistry). Due to the in-situ and nondestructive nature of ITC measurement, and its quantitative and informative results, it can even be a standalone technique in some cases.

This review summarized the applications of ITC in studying biomolecule–NP interactions. Standard methodology, experimental protocols, and the results from representative studies were highlighted to illustrate the application of ITC. Relevant articles and their references were identified using Web of Knowledge and Google Scholar (using "isothermal titration calorimetry" in combination with other keywords such as "nanoparticle", "protein" or "nucleic acid"). Instead of sorting by types of NMs, these articles were generally sorted into two main categories — proteins and other biomolecules. This kind of classification was preferred for two reasons: 1) there is a great variation in the types and properties of NPs studied, in terms of their size, shape, core materials and surface coatings; 2) the research context, objective and interaction mechanism differ significantly between proteins and other biomolecules. We revolved around the key information provided by ITC, while complementary results provided by other techniques to better understand biomolecule–NP interaction were also included. This review illustrates that the rich thermodynamic information provided by ITC could help identify the main driving forces for NP-biomolecule interactions and to evaluate the relative effects of different NP properties.

2. Methodology

Since biomolecule–NP interaction is a complex process and the measurement of its associated heat signal is rather delicate, great cares have to be taken during the experimental design, execution, and data analysis to obtain accurate and meaningful information. A few excellent reviews on the fundamentals, experimental design and data analysis of ITC can be found in the literature [10–13]. The theory and methodology described in these earlier studies are applicable to biomolecule–NP interactions after taking a few additional factors into account.

2.1. Experimental design

Two critical questions should be answered before choosing ITC to study a system: what is the appropriate concentration range of NPs and biomolecules? Are these concentrations enabling interactions that will generate measurable heat signals? The ability to quantify biomolecule–NP interactions using ITC is constrained by the nature of the interaction and the capability of the ITC instrument.

Similar to any calorimetric binding experiment, an appropriate concentration range of NPs and biomolecules has to be determined in order to obtain a satisfactory titration heat profile that can be accurately fitted by specific models. The Wiseman "*C*" parameter [Eq. (1)] as determined by binding stoichiometry, binding constant and NP concentration can be used to evaluate the appropriateness of the NP concentration [10].

$$C = nK_b[NP]_T \quad (1)$$

where K_b is the binding constant, $[NP]_T$ the total NP number (molar) concentration in the cell, and n is the stoichiometry of interaction (number of biomolecules per NP). Regarding the number concentration of NP, assuming a gold NP suspension has an Au concentration of 5 g/L and the AuNPs are spherical with an average diameter of 4.5 nm, the mass of individual NP will be 9.21×10^{-19} g (ρ = 19.3 g/cm^3), and the number concentration of NP will be about 9.02 μM (see more below on NP counting).

C value between 1 and 1000 is typically considered to be the optimal range, thus a rough estimation of K_b and n is needed in order to determine the optimal NP concentration required. For example, for an interaction with a binding constant in the order of 10^6 M^{-1}and $n \sim 5$, since the minimum *C* value is 1, the number concentration of NPs should be at least 0.2 μM. As shown in a study of lectin PA-IL adsorption to three types of NPs (with increasing terminal galactose/glucose ratio), with similar n and NP concentration, larger K_b yielded larger *C* value, and a "S″ shape titration curve began to emerge (Fig. 1) [14]. Therefore, to obtain an optimal titration curve like Fig. 1C, a higher or lower NP concentration is needed for interaction with smaller nK_b and larger nK_b, respectively. Since the binding constants and stoichiometry can still vary for an order of magnitude even when the NP sizes are similar (Table 1), it is important to conduct preliminary experiments (after a rough estimation based on published values from similar system) when optimizing the concentration range.

Once the number (molar) concentration of NPs is selected, the actual concentration of NPs used has to be accurately determined. Unfortunately, despite the critical importance of the molar concentration of NPs, details of its experimental determination were usually missing in most of the studies. A recent review summarized the methodologies available for the determination of NP number concentration [15]. Essentially, the selection of NP counting techniques depends on the characteristics of the studied NPs, and it would be wise to use at least two methods to validate the counting. For metal-based NPs, it can be 1) determined by measuring the total mass concentration then divided by average mass per NP or 2) counted by using TEM from a predetermined solution. For carbon-based and organic NPs, light scattering techniques and TEM counting might be more suitable.

Small ligands are usually prepared in the titration syringe because 1) the solute concentration in the syringe has to be at least $10 \times n$ times of that of the solute in the cells (in order to have sufficient ligands to fully titrate the NPs in the cell), and 2) there are more constraints in preparing NP solution with high concentration.

Accurate thermodynamic information from ITC relies on heat signals generated mainly from the interaction between the biomolecules and NPs, without interferences such as impurities in the solutions. The synthesis of NPs usually involves a variety of organics and inorganics as size stabilizer or surface coating, these unbound ligands may still present in the stock despite the purification by manufacturers [16,17]. The types and quantity of impurities in a biomolecule may depend on its source and purification methods. It is crucial to purify the NPs and biomolecules before use, to minimize potential interferences. The most common way to purify biomolecules and NPs is dialysis, as most of the impurities are salts and organics that are much smaller than the biomolecules. Membranes with specific size cut-off can be used to retain the NPs or biomolecules while removing the impurities. It is advantageous to use dialysis when preparing NPs and biomolecules of similar solution conditions (to minimize potential interference as a result of the difference). The characteristics of NPs are often useful in guiding the selection of appropriate purification strategies. For example, ligands that are sorbed to NPs through nonspecific adsorption may be desorbed during dialysis. In this case, multiple centrifugations and resuspensions may be a better choice. Size distribution of NPs is another parameter that has to be constrained since interaction is usually size-dependent. A broad size distribution may affect the accuracy of molarity determination. Therefore, it is desirable to use NPs with a relatively uniform size distribution. Depending on the types of NPs, a narrow size distribution can be obtained by filtration or differential centrifugation.

2.2. Experimental execution

Following concentration determination and solution purification, control experiments are needed to measure the heat-of-dilution prior to the actual titration. These control experiments should be properly conducted: 1) to identify potential interferences from background solution alone, and 2) to provide background signals for final data subtraction. The heat-of-dilution measurement includes the titration of ligands into the background solution and the titration of background solution from the syringe into NP solution in the cell. To accurately