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Combination of UV–vis spectroscopy and chemometrics to understand protein–nanomaterial conjugate: A case study on human serum albumin and gold nanoparticles

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ABSTRACT

Study of the interactions between proteins and nanomaterials is of great importance for understanding of protein nanoconjugate. In this work, we choose human serum albumin (HSA) and citrate-capped gold nanoparticles (AuNPs) as a model of protein and nanomaterial, and combine UV–vis spectroscopy with multivariate curve resolution by an alternating least squares (MCR-ALS) algorithm to present a new and efficient method for comparatively comprehensive study of evolution of protein nanoconjugate. UV–vis spectroscopy coupled with MCR-ALS allows qualitative and quantitative extraction of the distribution diagrams, spectra and kinetic profiles of absorbing pure species (AuNPs and AuNPs–HSA conjugate are herein identified) and undetectable species (HSA) from spectral data. The response profiles recovered are converted into the desired thermodynamic, kinetic and structural parameters describing the protein nanoconjugate evolution. Analysis of these parameters for the system gives evidence that HSA molecules are very likely to be attached to AuNPs surface predominantly as a flat monolayer to form a stable AuNPs–HSA conjugate with a core–shell structure, and the binding process takes place mainly through electrostatic and hydrogen-bond interactions between the positively amino acid residues of HSA and the negatively carboxyl group of citrate on AuNPs surface. The results obtained are verified by transmission electron microscopy, zeta potential, circular dichroism spectroscopy and Fourier transform infrared spectroscopy, showing the potential of UV–vis spectroscopy for study of evolution of protein nanoconjugate. In parallel, concentration evolutions of pure species resolved by MCR-ALS are used to construct a sensitive spectroscopic biosensor for HSA with a linear range from 1.8 nM to 28.1 nM and a detection limit of 0.8 nM.

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1. Introduction

Exploration and development of the bio–nano interfaces between biocomponents and nanomaterials have important academic and practical significance in the field of chemistry, materials science and biology [1]. In the last few years, considerable scientific attention has been paid to the integration of proteins with nanomaterials to form protein nanoconjugates [2–4]. The protein nanoconjugate possesses numerous potential applications related to bioanalytical science [5–7], biosensors [8–10], biocatalysis [11–13], biofuel cells [14,15] and bio-based nanodevices [16,17]. The central focus, however implicit in most of these researches, is firmly on understanding how these proteins interact with nanomaterials [18,19].

An important aspect of this problem is to qualitatively and quantitatively know about thermodynamic, kinetic and structural information of proteins that binds to the nanomaterials [18,19]. To achieve the goal, various interactions of proteins and nanomaterials have been probed during the past years using a range of different analytical techniques [20,21], including nuclear magnetic resonance, quartz crystal microbalance, mass spectroscopy, circular dichroism spectroscopy, microscopy, surface plasmon resonance, separation-based, thermal-based, and scattering-based techniques.

However, almost all techniques cannot simultaneously give important information about thermodynamics, kinetics and structural aspects of the binding process of protein–nanomaterial conjugate. In addition, most of these techniques require sophisticated instrumentation, have relatively high analytical costs, involve cumbersome pretreatment procedures prior to the analysis or ex situ complicated operations, and some techniques show poor sensitivity in practical applications. Therefore, the development of a universal means to provide comparatively comprehensive descriptions of protein nanoconjugate formation is always

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attractive, especially if the analytical technique is in situ, less complicated, cost-effective and relatively fast.

UV–vis spectroscopy is one of the most common techniques used for conventional analysis. To date, considerable literature has presented its application for research on protein nanoconjugates [20–25]. However, the technique usually provides only a limited amount of qualitative information on binding of protein to nanomaterials by observation of peak shift, broadening of the absorption spectra and spectral intensity variations [20–25]. And quantitative results on the binding are difficult to achieve likely because of very subtle changes in spectra, severe spectral overlap of optically coexisting species and spectral analysis in a univariate mode. Thus, there is an urgent need to find an effective tool to extract implicit information about thermodynamics, kinetics and structure of protein attachment onto nanomaterials from the collected spectral data quantitatively.

Chemometrics, generally known as multivariate statistics analysis, is an exploratory advanced tool to extract multiplex information of pure chemical species directly from the measured overlapping signals of complex systems without any previous physical or chemical separation [26–28]. Most chemometrics methods belong to the self-modeling or model-free methods, which are mainly factor analysis methods that easily meet the intrinsic property of the measurement and their multivariate data structure [26–28]. Among them, multivariate curve resolution by an alternating least squares (MCR-ALS) algorithm developed by Tauler and co-workers [29] is the most widespread used method, predominantly because the method is versatile, robust and rather flexible in dealing with many kinds of complex data structures and chemical problems, requires no priori knowledge or model about the studied system, and provides much qualitative and quantitative information with physical or chemical meaning to allow a full interpretation of the studied complex systems. The only requirements for MCR-ALS application are that data structure should conform to a given bilinear model, and some general knowledge about chemical features and the internal mathematical structure of the system studied, such as non-negativity, implying that all spectra take positive values, should be given [29]. Spectroscopy follows an additive nature of Beer–Lambert's law and has a typical bilinear data structure, so it fulfills well the requirements for application of MCR-ALS.

A detailed explanation of the MCR-ALS algorithm applied for spectral data resolution has been described in previous works [29–34]. Herein, we give only some relevant aspects of the algorithm for brevity.

The mathematical model established by the algorithm usually takes three steps. The first step is to determine the number of optically pure species that the evolving system possesses. This can be usually achieved by means of singular value decomposition [29] and the theory of error in factor analysis [35]. The second step is the construction of an initial estimate of concentration evolutions or spectra of the pure component present in a system. The estimate can be obtained from chemometrics techniques based on evolving factor analysis (EFA) or the purest variables or techniques based on the selection of representative spectra or evolving curves from chemical insight about the process. Finally, once the initial estimate is generated, an iterative alternating least squares (ALS) optimization step is carried out until convergence is achieved. During this iterative process, the introduction of constraints is of crucial importance to obtain physically or chemically meaningful evolving profiles and spectra and to drive the optimization to the sought solution, decreasing the rotational and intensity ambiguities of the resolved profiles. The main constraints are non-negativity, unimodality (the presence of only a single maximum per profile or a plateau-like maximum), closure (the mass balance for total concentration along the evolving process), selectivity or local rank information (some species are forced to be absent in some ranges of resolving profiles based on known information, such as at the beginning and end of the evolving process), component correspondence (the information related to the species in the different samples; if known, set the absent species to be null) and trilinearity (the resolved profiles of the same component in the different data matrices for a particular dimension) [29].

Another outstanding feature of the MCR-ALS method is that it can be easily extended to the simultaneous analysis of multiexperiments under different conditions and/or together with simpler subsystems, by column-wise data matrix augmentation [29]. Generally, column-wise augmented data matrices can be accomplished by appending matrices, belonging to different processes, one on top of each other. They share common spectra in a particular technique (e.g. UV–vis spectroscopic technique), but lengthen in the process directions. The augmented matrices were demonstrated to be able to minimize the ambiguities and remove the rank deficiency problem associated with the decomposition of a single data matrix, and allow reliable and accurate estimation of the number of pure components and description of the chemical evolving system.

During the past few years, spectroscopy coupled with a MCR-ALS tool has been a common phenomenon, and has been satisfactorily applied to study biocomponents or nanomaterials of complex systems involving equlibria or kinetic chemical process [30–34]. To the best of our knowledge, there are, however, no literature reports on investigating the thermodynamics, kinetics and structure of protein nanoconjugate evolution in detail by spectroscopy combined with MCR-ALS. In this context, we herein make an attempt to establish a universal method for integrated research into the evolutionary processes of the protein nanoconjugate using UV–vis spectroscopy together with the MCR-ALS technique. The powerful resolution advantage of MCR-ALS allows extracting distribution diagrams, spectra and kinetic profiles of pure species of interest involved in the evolutionary process. The distribution diagrams can provide quantitative thermodynamic information about equilibrium constant, stoichiometry, molecule footprint, standard free energy change (ΔG), standard enthalpy change (ΔH) and standard entropy (ΔS) change of the binding of protein to nanomaterials, and about possible arrangement and orientation information (monolayer or multilayer structure and “flat-on” or “end-on” conformation) of protein attached to the surface of nanomaterials. The kinetic profiles can present quantitative information about the formation mechanism of protein nanoconjugate, such as reaction rate constant, reaction order, reaction activation energy and so on. The features of the recovered pure spectra (molar extinction coefficient, peak wavelength, peak width and peak shape) can help in the qualitative analysis of structural characteristics of the species of interest involved.

As a case study, human serum albumin (HSA) and citrate-capped gold nanoparticles (AuNPs) are respectively selected as a model of protein and nanomaterial. HSA is readily available and the most abundant protein in the circulatory system, and its related structural information (for example, HSA is a prism-shaped-structure protein of dimensions 8.4 nm × 8.4 nm × 3.15 nm) [36,37] has been better understood. Citrate-capped AuNPs have well-established synthesis methods, and possess good optical and electronic properties [38]. Concurrent with our study of protein–nanomaterial interaction, citrate-capped AuNPs are exploited as spectrophotometric probes to fabricate a biosensor for sensitive detection of HSA.

2. Materials and methods

2.1. Reagents and chemicals

Chloroauric acid tetrahydrate and trisodium citrate dihydrate were purchased from Sinopharm Chemical Reagent Co. (Shanghai,

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