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Chemometrics: An important tool for monitoring interactions of vitamin B7 with bovine serum albumin with the aim of developing an efficient biosensing system for the analysis of protein

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ABSTRACT

For the first time, interaction of vitamin B7 (VB7) with bovine serum albumin (BSA) was investigated with the aim of developing a method for the analysis of BSA. The interaction of VB7 with BSA was investigated by cyclic voltammetry (CV), linear sweep voltammetry (LSV), and differential pulse voltammetry (DPV) at a multi-walled carbon nanotubes-modified glassy carbon electrode (MWCNTs/GCE). The recorded electrochemical data was combined with UVvis and fluorescence (F) spectroscopic data into a row- and column-wise augmented matrix and resolved by multivariate curve resolution-alternating least squares (MCR-ALS) as an efficient chemometric tool, and this assisted in the further elucidation of the above interaction. Also, with aid of MCR-BANDS method, the absence of rotational ambiguity was verified in the obtained results and we confirmed that the obtained results were unambiguous and reliable. The binding of VB7 to BSA was also modeled by molecular docking methods. Excellent agreement was found between the experimental and computational results. The differences of DPV responses of VB7 in the absence and presence of BSA (ΔI) were found to be linearly related to BSA concentration between 0.5×10^{-9} mol L^{-1} and 35.0×10^{-9} mol L^{-1}, and a limit of detection (LOD, $3S_b/b$) of 0.22×10^{-9} mol L^{-1} was calculated. Finally, the DPV method was further applied to the determination of serum albumin (SA) in serum samples obtained from Holstein cows and the results were in good agreement with those obtained by a medical diagnostic laboratory whose method was based on traditional cellulose acetate electrophoresis. The MWCNTs/GCE showed enhanced electron transfer kinetics, large electroactive surface area, and was highly sensitive, selective, and stable towards SA determination. The satisfactory analytical performance of the proposed method would make it potentially advantageous for a broad range of biosensing and clinical applications.

1. Introduction

In living organisms, there are a variety of small molecules with various biological and pharmaceutical functions. Vitamin B7 (VB7, also called vitamin B8 or H, Fig. 1) is a water-soluble vitamin and an essential co-factor for five biotin-dependent carboxylase enzymes. It is synthesized in a wide variety of bacteria and plants. However, several microorganisms as well as higher animals are notable to synthesize it and their needs in this vitamin are met by dietary intake [1]. Besides the typical clinical features, recent evidence indicates that the pregnant women develop biotin deficiency during normal pregnancy [2,3].

Abbreviations: VB7, vitamin B7; BSA, bovine serum albumin; Trp, tryptophan; SA, serum albumin; CV, cyclic voltammetry; LSV, linear sweep voltammetry; DPV, differential pulse voltammetry; MWCNTs, multi-walled carbon nanotubes; GCE, glassy carbon electrode; F, fluorescence; MCR-ALS, multivariate curve resolution-alternating least squares; MD, molecular dynamic; LOD, limit of detection; TBS, Tris–HCl buffer solution; MVD, molegro virtual docker; EFA, evolving factor analysis; PCA, principal component analysis; SIMPLISMA, simple to use self-modeling mixture analysis; ITTFA, iterative target transformation factor analysis

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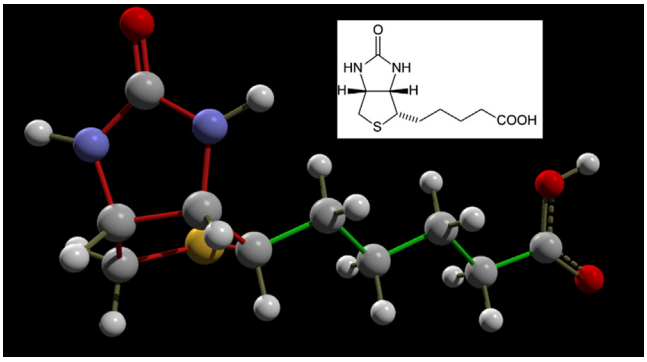

Fig. 1. Molecular structure of VB7.

Among biomacromolecules, serum albumin (SA) is a soluble protein, which is a major constituent of the circulatory system, and it commonly serves as a depository and a transport molecule for many exogenous compounds [4,5]. Consequently, simple, rapid and low cost analysis for SA proteins would be useful [6,7]. Studies on the binding of small molecules with plasma proteins will facilitate the interpretation of the metabolism and transport processes of such substances, and the binding of small molecules with bovine serum albumin (BSA) is a typical example of such interactions. Thus, it is an appropriate protein to use for such investigations, partly because of its structural homology with human serum albumin (HSA) [8].

There are a variety of techniques currently available for obtaining information about protein–ligand interactions such as the measurement of kinetics and binding affinities. Two traditional methods, X-ray diffraction and NMR spectroscopy, employed to obtain structural information of proteins and protein–ligand complexes. Both of these methods have disadvantages: X-ray diffraction requires the preparation of a crystal, which can be time consuming or even impossible; and NMR spectroscopy is not easily applied to larger proteins of more than a few hundred amino acids. Other analytical techniques that can be applied to proteins in solution are spectrophotometry, chemiluminescence, resonance light scattering [9–12]. Compared with spectroscopic methods, electrochemical assay is simple, easily implemented, and has low-cost and fast response. On the other hand, the interpretation of electrochemical data can contribute to elucidation of the interaction of ligand with biomacromolecules.

Chemometrics methods of data analysis, such as multivariate curve resolution-alternating least squares (MCR-ALS), are well known for their ability to resolve different kinds of data and extract profiles of individual chemical components from composite responses. Such results generate valuable information, which other-wise cannot be obtained by conventional methods, and which consequently, enable further analysis of the problem under investigation as is the case with the biosystems in this work.

To the best of our knowledge there is no report on the interactions between VB7 and BSA in the literature, and this work reports a detailed study on the mentioned system for the first time. The main aims of this study were: 1. Investigation of interaction of VB7 with BSA at a MWCNTs/GCE at physiological conditions by CV, LSV, and DPV and also in combination with UV–vis and fluorescence (F) techniques by the use of MCR-ALS. 2. Research and development of an electrochemical method for BSA analysis in conjunction with VB7 which could be suggested for biosensing and clinical applications.

2. Experimental and theoretical details

2.1. Chemicals and solutions

BSA, and VB7 were purchased from Sigma Chemical Company (St. Louise, MO). Multi-walled carbon nanotubes were purchased from io-li-tec, Ionic Liquid Technologies. All other reagents were of analytical grade, and doubly distilled water was used throughout. All solutions used in this work were prepared in Tris–HCl buffer solution (TBS, 0.05 mol L^{-1}, pH 7.4, containing 0.1 mol L^{-1} sodium chloride to maintain the ionic strength of solution). Stock solutions of VB7, and BSA was prepared by dissolving proper amount of their solid powder in the TBS (0.05 mol L^{-1}, pH 7.4) and were kept at dark in a refrigerator for about a week only.

2.2. Instruments and softwares

Electrochemical experiments were performed using a μ-AutolabIII/FRA2 driven by the Nova 1.8 software. A conventional three-electrode cell was used with a saturated calomel electrode (SCE) as reference electrode, a Pt wire as counter electrode and a bare or modified GCE as working electrode. The SEM experiment was made on a KYKY-EM 3200 scanning electron microscope. All fluorescence spectra were measured on a Cary Eclipse fluorescence spectrophotometer equipped with a water bath and a 1.0 cm quartz cell. The UVvis spectra were measured on an Agilent 8453 UVvis Diode-Array spectrophotometer controlled by the Agilent UVvis ChemStation software. A JENWAY-3345 pH-meter equipped with a combined glass electrode was used to pH measurements. The chemical structure of the VB7 was constructed by Hyperchem package (Ver. 8.0), and energy minimization for VB7 was performed by AM1 semi empirical method with Polak-Ribiere algorithm until the root mean square gradient of 0.01 kcal mol^{-1}. The known crystal structure of BSA (PDB Id: 3V03) was obtained from the Brookhaven Protein Data Bank. Water molecules were removed, and hydrogen atoms were added. The molegro virtual docker (MVD) software was employed to generate a docked conformation of VB7 with BSA. LIGPLOT [13], a program for automatically plotting protein–ligand interactions, was used to analyze the interactions between BSA and VB7. MCR-ALS and MCR-BANDS were implemented using the graphical interfaces provided by Prof. Tauler in his web page [14]. The recorded experimental data was smoothed, when necessary, and converted to matrices by means of several homemade MATLAB (Version 7.14) programs. A simple homemade MATLAB program was used for computing the concentrations of VB7 and BSA and their ratio in all voltammetric and spectroscopic experiments. All calculations were run on a DELL XPS laptop (L502X) with Intel Core i7-2630QM 2.0 GHz, 8.0 GB of RAM and Windows 7-64 as its operating system. The MD simulations were performed on a computer with a Linux Fedora 15 as its operating system.

2.3. Fabrication of MWCNTs/GCE and BSA/MWCNTs/GCE

Prior to the electrode modification, the GCE surface was polished with PK-4 polishing kit, BASi MF-2060 successively followed by rinsing thoroughly with redistilled deionized water until a mirror like finish was obtained. It was then dipped in a beaker containing 0.2 mol $L^{-1}H_3PO_4$ solutions to remove the adhered powder, rinsed with distilled water and dried at room temperature for 10.0–15.0 min. The MWCNTs (4.0 mg) was added to 1.0 mL dimethylformamide. A homogeneous and stable suspension of 4.0 mg mL^{-1} MWCNTs was obtained with the aid of ultrasonic agitation for about 30.0 min. A known volume (40.0 μL) of this solution was adsorbed onto the surface of the clean and dried GCE using a micropipette and dried under room temperature. The BSA/MWCNTs/GCE was prepared with the following procedure: an 8.0 mL of the BSA solution (0.40 mg mL^{-1} dissolved in TBS (0.05 mol L^{-1}, pH 7.4)) was dripped on the MWCNTs/GCE surface and dried by passing very slow rate of air for 10.0 min to