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Interaction of brilliant red X-3B with bovine serum albumin and application to protein assay

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Abstract

The interaction of brilliant red X-3B (BRX) with bovine serum albumin (BSA) in three pH media has been characterized by the spectral correction technique. The binding number maximum of BRX was determined to be 102 at pH 2.03, 82 at pH 3.25 and 38 at pH 4.35 and the binding mechanism was analyzed in detail. The effects of ionic strength from 0 to 1 $mol\,L^{-1}$ and temperature from 20 to 70 °C on the binding were investigated. The results showed that the interaction of BRX with BSA responded to the Langmuir adsorption isothermal model and the binding constant was determined. From the correlation between the binding number and the number of basic amino acid residues, the ion-pair attraction induced the union of non-covalent bonds including H-bond, van der Waals force and hydrophobic bond and the binding model was illustrated. The binding of BRX to BSA has resulted in change of the BSA conformation confirmed by means of circular dichroism. Using this interaction at pH 2.03, a sensitive method named the absorbance ratio difference spectrometry was established and applied to the protein assay and the limit of detection of protein was only 6 $\mu g\,L^{-1}$. Two samples were determined and the results were in agreement with those obtained by the classical coomassie brilliant blue colorimetry.
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Keywords: Brilliant red X-3B; Bovine serum albumin; Non-covalent binding; Conformational change; Molecular spectrometry; Protein assay

1. Introduction

Recently, the small molecule–biomacromolecule interactions are focused increasingly for many biochemists and thousands of new findings are reported every year. Understanding the interaction between organic ligand and biomacromolecule is always helpful for us to recognize the structure, function and activity of macromolecule as well as the toxicity of an organic toxicant, *e.g.* protein–protein [1], protein–DNA [2], protein–glycosaminoglycan [3] and protein–ligand [4] interactions. Though people have clarified the structure, conformation, activity and function of many proteins, the protein interaction with organic substance as well as conformational changes of protein is still in the extensive research so as to be realized the structure-activity relationships and particular biological roles of biomacromolecule. The spatial structure of protein is the representation of the foundation of the biological function. Generally speaking, the structure of crude protein is relatively stable. The structural stability keeps individual function and relative stability of species. Organic substance–protein interactions modulate the structure of protein and thus affect its biological functions. So, structural transformation that occurs in the process of protein folding and functioning is of great significance in biological organisms [5]. In fact, the interaction occurring in protein medium is so complicated. In the formation of complex, small organic compound may insert into the protein inner to regulate their structures and functions [6] through non-covalent union, *e.g.* hydrophobic bond, van der Waals force, dipole effect and hydrogen bond. The non-covalent binding is often weak and non-specific [7]. With the weak binding of the non-covalent interaction, this complex is often affected by the urea, dioxane or electrolyte and high temperature [8].

In the present work, we undertook in an attempt to clarify the general principle involved in the protein–organic substance

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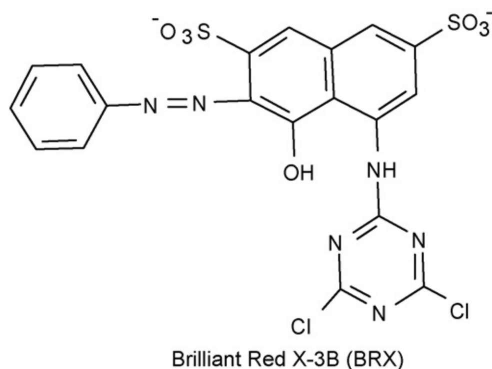

Fig. 1. Chemical structure of BRX.

interaction, characterization of the complex and effect of organic substance on protein conformation. Bovine serum albumin (BSA) obtained easily with well-known properties is often used as a model protein [9]. In an acidic medium, the basic amino acid residues (AARs) of protein are often protonated to form the poly-cationic amino groups. Brilliant red X-3B (BRX) as a kind of azo dye is commonly used in textiles and its chemical structure is given in Fig. 1. Absorption of BRX through the skin is a possible exposure route. We investigated the non-covalent interaction of BRX with BSA by means of molecular spectrometry. The BRX binding product with BSA was characterized by the combination of the spectral correction technique and the Langmuir isothermal adsorption. The binding number is corresponded to the number of basic AARs. A new model is advanced for the ion-pair electrostatic attraction inducement to promise the protein–organic substance binding by the co-action of the non-covalent poly-bonds, *i.e.* the ion-pair electrostatic interaction pulls BRX molecule to insert near the basic AARs and then the other non-covalent bindings form a union to bind BRX between BRX and AARs. By means of circular dichroism (CD) spectrometry [10], the secondary structure of BSA was measured in the presence of BRX and the increase of α-helix transferred from β-sheet was analyzed. Although the target acceptor of BRX may not be BSA, the aim of the research was to study non-covalent bridging of BRX with protein so as to provide a theoretical basis and methodology for estimation of target toxicity of an organic toxicant. In addition, though BRX is only a kind of industrial dyestuffs but not a normal chromogenic agent, it was found to be very sensitive to bind proteins. As a result, the determination of trace amounts of protein was advanced using the interaction of BRX with protein.

2. Experimental

2.1. Apparatus and instruments

The absorption spectra of BRX and its BSA solutions were recorded with a Model Lambda-25 spectrometer (Perkin-Elmer, USA) equipped with a thermostatic cell holder attachment to link with a Model TS-030 water-circulated thermostatic oven (Yiheng Sci. Technol. Shanghai, China). The spectrometer was computer controlled using UV WinLab software (Version 2.85.04). The pH of solutions was measured with a Model pHS-25 acidity meter (Shanghai Precise Sci. Instrum., China). The temperature of solutions was warmed with a Model HHS-11-2 thermostat water bath (Shanghai Precise Sci. Instrum., China). A Model BCD-196 refrigerator freezer (Meiling Production, Hefei, China) was used to store the protein solutions. A Model J-715 Circular Dichroism Spectropolarimeter (JASCO, Japan) was used to measure the conformation of proteins. The spectropolarimeter was computer controlled by J-715 Control Drover software (Version 1.00, JASCO). A Model JY92-II Ultrasonic Cell Crusher (Xinzhi Instrum., Ningbo, China) was used to break down the bacterial cell to prepare a protein sample. A Centrifuge (Shanghai Sci. Instrum., China) was used in the separation of protein sample.

2.2. Reagents and solutions

Bovine serum albumin (BSA) was purchased from the BioDev Biological Gene Technol. of Beijing. 0.20 g of BSA was dissolved in 100 mL of deionized water and then diluted to 1000 mL. The protein concentrations were determined by the UV method [11]. The solution must be stored in a refrigerator freezer at less than 4 °C.

BRX was provided by Shanghai Dyestuff Chemicals Factory. After purified by recrystallization, 0.500 mmol L^{-1} BRX was prepared in de-ionized water. It was used to react with BSA.

A series of Britton-Robinson (B-R) buffer solutions, pH 2.03, 2.21, 2.78, 3.25, 3.88, 4.35, 5.04 and 5.68, were prepared to adjust the acidity of solution in order to find a proper and highly sensitive complexation between BRX and BSA. The electrolyte solution, 5.0 mol L^{-1} $NaNO_3$, was prepared to adjust ionic strength of the complexation solution in order to investigate the effect of electrolyte on the binding. The masking reagent, 0.1 mol L^{-1} EDTA, was prepared and added into a sample to mask metals, *e.g.* Zn(II), Fe(II, III) and Ca(II) possibly existing in sample from interference of detection of proteins.

2.3. Measurements

2.3.1. Photometric characterization of interaction of BRX with BSA

All studies were carried out in a 10.0 mL calibrated flask. Into the flask, a known volume of the BSA solution, 1.0 mL of B-R buffer solution (pH 2.03, 3.25, and 4.35) and a known volume of the BRX solution were added. The solution was diluted to 10.0 mL with deionized water and mixed well. After reacting for 5 min, the absorbance ($A^0_{\lambda 2}$ and $A^0_{\lambda 1}$) of the reagent blank and $A_{\lambda 2}$ and $A_{\lambda 1}$ were measured at 539 nm (λ_2) and 571 nm (λ_1) against water. Thus, the parameters A_c, η and γ of each solution above were calculated.

2.3.2. CD measurement of protein solution

1 mL of B-R buffer solution (pH 2.03, 3.25, and 4.35) and 0.40 mg of BSA were added into five flasks. Then, 0.0, 0.40, 0.60, 0.80 and 1.00 mL of 0.500 mmol L^{-1} BRX were added into these flasks, respectively. The solutions were diluted to 5.00 mL with deionized water. Each was injected into a 0.1-cm

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