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Investigation of interaction of nuclear fast red with human serum albumin by experimental and computational approaches

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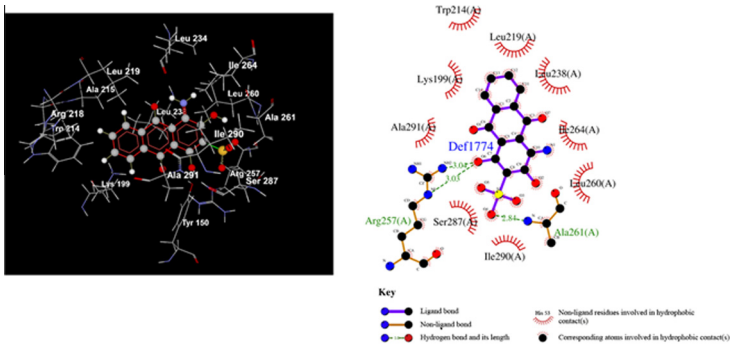
HIGHLIGHTS

- Experimental approaches including UVvis, F, DPV, LSV, and CV were applied.
- The experimental data was augmented and resolved by MCR–ALS.
- Binding of NFR to HSA was modeled by molecular modeling and MD simulations.
- The results of experimental and computational approaches were compatible.
- The obtained results suggested that the NFR binds mainly to the sub-domain IIA of HSA.

GRAPHICAL ABSTRACT



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ABSTRACT

For the first time, interaction of nuclear fast red (NFR) with human serum albumin (HSA) was studied by experimental and computational approaches. Firstly, experimental measurements including fluorescence spectroscopy (F), UVvis spectrophotometry (UVvis), cyclic voltammetry (CV), differential pulse voltammetry (DPV) and linear sweep voltammetry (LSV) were separately used to investigate the interaction of NFR with HSA and interesting thermodynamics information was obtained from these studies. Secondly, new information including electrochemical behavior of NFR–HSA complex species, relative concentrations of the various reacting species and effects of NFR on the sub-structure of HSA was obtained by applying multivariate curve resolution–alternating least squares (MCR–ALS). In this case, a row- and column-wise augmented matrix was built with DPV, LSV, F and UVvis sub-matrices and resolved by MCR–ALS. Surprisingly, by this method two NFR–HSA complex species with different stoichiometries and different electrochemical behaviors were found. Furthermore, by the use of the recorded voltammetric and spectroscopic data the binding constants of complex species were computed by EQUISPEC (a hard-modeling algorithm). Finally, the binding of NFR to HSA was modeled by molecular modeling and molecular dynamics (MD) simulations methods. Excellent agreement was found between experimental and computational results. Both experimental and computational results suggested that the NFR binds mainly to the sub-domain IIA of HSA.

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Introduction

Studying interactions of small molecules to biomacromolecules such as protein or DNA is crucial for understanding many biological processes at the molecular level. Many small molecules like drugs, dyes, etc. bind to cellular serum proteins or DNA to exert their adverse and normal functions and a large number of studies have been recently undertaken in this direction [1–4].

Anthraquinone dyes are extremely resistant to biodegradation owing to their fused aromatic structures, thus they have progressively allured crucial attention from toxicological and environmental points of view, especially in light of the current increase in their applications. However, little information concerning their hazards to human has yet been accumulated, so, with the consideration of human health, the examination on the correlation of anthraquinones and toxicity was required. Nuclear fast red (NFR, Fig. S1, Supplementary data) is a typical anthraquinone dye, and as general anthraquinones, was often served as the counterstain in many biological experiments. While little attention has been paid to the biological influence of this dye, especially its binding properties with the biological macromolecules such as proteins and nucleic acid were even less.

Structurally, human serum albumin (HSA) is a non-glycosylated consisting of a single peptide chain of 585 amino acids and consists of three homologous domains, namely, I (residues 1–195), II (196–383), and III (384–585), each domain being divided into sub-domains A and B, and the overall structure is stabilized by 17 disulfide bridges [5]. The specific physiological activity of the aromatic and heterocyclic ligands upon complexation with serum albumin originates from the presence of two hydrophobic pockets in sub-domains IIA (site I) and IIIA (site II) [6]. HSA contains a single intrinsic tryptophan residue at position 214 in domain IIA, where a large hydrophobic cavity is present, and its fluorescence is sensitive to the ligands bonded nearby [7].

In general, the common methods used to investigate the interaction of proteins with small molecules include: UVvis spectrophotometry [8], FT-IR [9], electrochemistry [10], capillary electrophoresis [11], HPLC [12], and NMR [13] among others; some of these methods have been used to investigate the interaction of small molecules with biopolymers with the aid of chemometrics [14–19]. Fluorescence and UVvis spectroscopies can provide valuable qualitative and quantitative information about the binding of ligands to serum albumin and the estimation of binding constants, respectively. In general, electrochemical analysis is simple, easily implemented, low costing and fast; as well, electrochemical data can contribute to the elucidation of the interaction of small molecules with biomolecules.

By the use of the augmented matrix methods it is possible to combine data matrices of analyte profiles derived from different analytical methods. In general, the results of such approaches have indicated that the increased information in the augmented matrix improves data analysis and subsequent interpretation of the results [20]. However, combination of data matrices derived from very different analytical techniques e.g. voltammetry and spectroscopy, which monitor different properties of the analytes in the system, are much less common. It is this area of analysis, which is explored in the present investigation by the use of voltammetry and spectroscopy for the analysis of interaction of NFR with HSA.

The main objectives of this study were:

(I) We have employed experimental and computational approaches, in an attempt to determine where and how NFR binds to HSA under physiological conditions.
(II) Experimental measurements including CV, DPV, LSV, F and UVvis were used separately and in combination to obtain different information about the mentioned interaction as well as the thermodynamic parameters involved.
(III) To evaluate the results of experimental section molecular docking and MD simulations methods were used to investigate the interaction of NFR with HSA as well.

Literature survey revealed that no attempt has been made to study the interaction of NFR with HSA till date.

Theoretical background

The MCR–ALS is one of the most versatile methods capable of resolving the system completely without assuming any model for the change in the concentration of components during the process. The MCR–ALS resolves any black system and applying appropriate constraints in reaching a better resolution is helpful. The MCR–ALS analysis decomposes the data matrix **D** into a matrix of pure concentration profiles, **C**, and a matrix of pure signals profiles, $\mathbf{S^T}$, related to the different involved species in the studied system. The product $\mathbf{CS^T}$ reconstructs the original data matrix **D** with the optimal fit, i.e., gives the minimum residual error, **E**:

$$\mathbf{D} = \mathbf{CS^T} + \mathbf{E} \quad (1)$$

The general steps in the application of MCR–ALS to any kind of data set are as follows:

1. Determination of the number of components in **D** (e.g. by singular value decomposition, SVD).
2. Building initial estimates of the **C** matrix (e.g. using evolving factor analysis, EFA). The EFA has been designed as a chemometric tool to monitor chemical processes [21,22]. The evolution of a chemical system is gradually known by recording a new response vector at each stage of the process under study. Mimicking the experimental protocol, EFA performs subsequent principal component analysis (PCA) on gradually increasing submatrices in the process direction, enlarged by adding one new row (response) at a time. This procedure is performed from top to bottom of the data set (forward EFA) and from bottom to top (backward EFA) to investigate the emergence and the decay of the process contributions, respectively. For a system with n significant components, the profile of the first contribution is obtained combining the line of the 1st s.v. of the forward EFA plot and the line of the *n*th s.v. of the backward EFA plot (related to the first contribution disappearing); the profile of the second contribution relates the line of the 2nd s.v. in the forward EFA plot to the line of the $(n-1)$th s.v. backward EFA plot, and so forth. In general, each element in the derived concentration profile is selected as the smallest value between the forward and backward s.v. lines to be combined [23]. The EFA was basically designed to work with full rank data sets and a system should fulfil the condition of full rank to be successfully analyzed by EFA. Rank deficiencies can be broken by matrix augmentation in the rank-deficient direction (C and S directions in this study) [15].
3. Given **D** and **C**, least-squares calculation of $\mathbf{S^T}$ under suitable constraints.
4. Given **D** and $\mathbf{S^T}$, least-squares calculation of **C** under suitable constraints.
5. Going back to step 3 until convergence is achieved. The convergence criterion in the MCR–ALS optimization is based on the comparison of the lack of fit (lof) obtained in two consecutive iterations. When the relative difference in fit is below a threshold value, the optimization is finished. The lof is calculated according to the expression: