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# Molecular simulation of the interaction between novel type rhodanine derivative probe and bovine serum albumin

Jinghua Yu[a,*], Bo Li[b], Ping Dai[a], Shenguang Ge[a]

[a] School of Chemistry and Chemical Engineering, University of Jinan, Jinan 250022, Shandong Province, People's Republic of China
[b] Tianjin Cement Industry Design and Research Institute Co., Ltd, Tianjin 300400, People's Republic of China

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ABSTRACT

The interaction between 3-(4′-methylphenyl)-5-(4′-methyl-2′-sulfophenylazo) rhodanine (M4MRASP) and bovine serum albumin (BSA) was studied by using spectrofluorimetry. It was shown in fluorescence spectrums that the quenching mechanism of BSA by M4MRASP was a static quenching. Meanwhile, the binding constant and binding site numbers were calculated. The action distance ($r$ = 8.03 nm) and energy transfer efficiency ($E$ = 0.12) between donor (BSA) and acceptor (M4MRASP) were obtained according to the theory of Förster non-radiation energy transfer. The effect of M4MRASP on the conformation of BSA was further analyzed by using synchronous fluorescence spectrometry. A new model of the interaction between small organic molecule and biomacromolecule was established. The results offered a reference for the studies on the biological effects and action mechanism of small molecule with protein.



## 1. Introduction

Serum albumin, which can be combined with many endogenous and exogenous compounds, is most abundant in the plasma and plays an important role in storage and transport of energy [1]. It was very significant to investigate deeply the interaction between protein and small molecule to know the interaction mechanism of small molecule with protein at the molecular level. It is also important for research on proteomics, pharmacology, toxicology and the effect of environmental pollutants on living matter [2]. At present, considerable attention has been caused [3–6] to study bonding action of protein and small organic dye molecule. But mechanism of the interaction and equilibrium law of those bonding actions were still unclear and were being studied [7,8].

The phenylazo rhodanine reagents, which are big double-bond conjugated and have good planar structure, were often used in the determination of metal ions [9,10] with excellent performance as chromogenic and fluorescent reagents, higher sensitivities, good selectivities and stability, and so on, but hardly in the study of biomacromolecule. To improve the analytical performance, many rhodanine derivatives [11,12] with some active groups, such as sulfonic group, chloro group, nitryl and methoxyl groups, were synthesized. Yu [13] studied the spectral property of 3-(4′-methylphenyl)-5-(2′-sulfophenylazo) rhodanine as a probe with DNA and widened the research field of phenylazo rhodanine reagents. In this work, 3-(4′-methylphenyl)-5-(4′-methyl-2′-sulfophenylazo) rhodanine (M4MRASP, Fig. 1) was synthesized by the author and used as a spectral probe. At human physiological pH the interaction between bovine serum albumin (BSA) and M4MRASP was investigated detailedly by spectrofluorometry, synchronous spectrofluorometry and quantum chemistry. A novel model of interaction of protein with M4MRASP was established by calculating their binding constants, binding site numbers at different temperatures and space position, and also by studying the change of protein conformation. Those studies containing detailed and accurate data were very successful, which were beneficial to investigate the interaction mechanism of rhodanine reagents with protein and enlarge their application area.

## 2. Materials and methods

### 2.1. Apparatus and reagents

A LS-55 fluorimeter (P-E, America) was used for recording the fluorescent spectra and measuring the intensity of fluorescence. A UV-3101PC spectrophotometer (Shimadzu, Japan) was used for recording the absorption spectra. A PHS-3C meter (Shang Hai Lei Ci Device Works, Shanghai, China) was used for pH measurement.

The standard solution of M4MRASP ($2.0 \times 10^{-4}$ mol/L) was prepared by dissolving 0.0084 g M4MRASP in 100 mL double-deionized water. The standard solution of BSA ($1.0 \times 10^{-5}$ mol/L) was obtained by dissolving 0.0335 g BSA in 50 mL double-deionized water.

C-L buffer solution (pH 7.0) was prepared by mixing 0.2 mol/L $KHC_8H_4O_4$ and 0.2 mol/L NaOH in suitable proportion, and the pH value was adjusted to 7 on the pH meter. All reagents were of

* Corresponding author.
*E-mail address:* labour001@163.com (J.H. Yu.).

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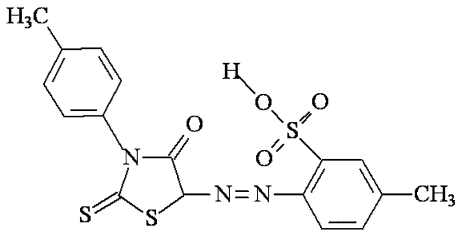

**Fig. 1.** The structure of M4MRASP.

analytical reagent grade and above. Double-deionized water was used throughout.

### 2.2. Procedure

Solutions were added in a calibrated flask in order of 1.0 mL $1.0 \times 10^{-5}$ mol/L BSA, 2.5 mL C-L buffer solution (pH 7.0) and appropriate amounts of $2.0 \times 10^{-4}$ mol/L M4MRASP solution, then diluted to 10 mL with water and mixed well. Then fluorescence quenching spectrums of BSA were obtained with exitation wavelength at 282 nm and emission wavelength at 300–490 nm. In addition, synchronous fluorescence spectrums of tryptophan residue and tyrosine residue were obtained at $\Delta\lambda = 60$ nm and 15 nm, respectively and UV absorption spectrums of M4MRSP were recorded at wavelength range of 300–500 nm.

## 3. Results and discussion

### 3.1. The quenching mechanism

The intrinsic fluorescence of BSA comes from tryptophan and tyrosine residues. The interaction of protein with other molecules can result in the decline of its fluorescence intensity, and the phenomenon is called fluorescence quenching. There are two types of fluorescence quenching: dynamic and static quenching. Static quenching often generates from complex reactions in which non-fluorescence substance is obtained and this can affect secondary structure and physiological activity of protein while dynamic quenching does not do, in which transfer of energy or electron occurs.

According to the procedure, fluorescence quenching spectrums of BSA at 293 K and 303 K were obtained (Figs. 2 and 3).

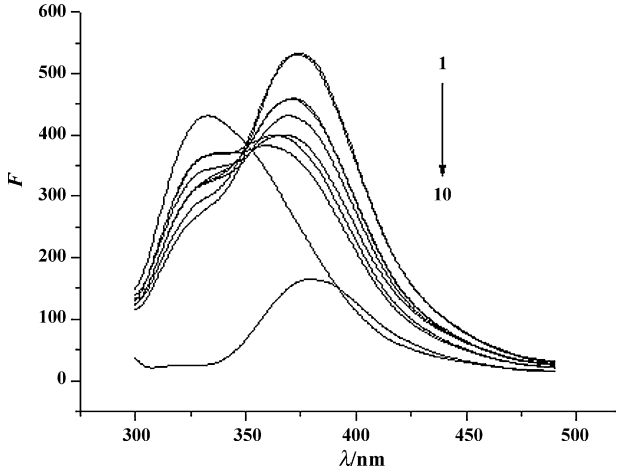


**Fig. 2.** Quenching of BSA fluorescence spectrums on adding M4MRASP (295 K). (1–9) M4MRASP ($\times 10^{-5}$ mol/L) 0, 1.0, 1.2, 1.4, 1.8, 2.0. 2.4, 2.8, 3.0. BSA, $10^{-6}$ mol/L. (10) M4MRASP $2.0 \times 10^{-5}$ mol/L.

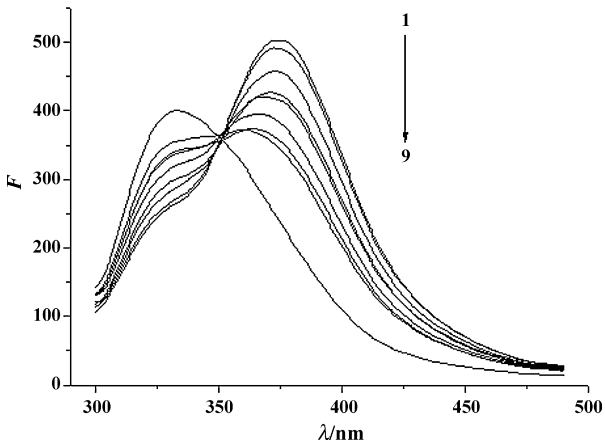


**Fig. 3.** Quenching of BSA fluorescence spectrums on adding M4MRASP (303 K) (1–9) M4MRASP ($\times 10^{-5}$ mol/L) 0, 1.0, 1.2, 1.4, 1.8, 2.0, 2.4, 2 8. 3.0. BSA, $10^{-6}$ mol/L.

It could be found from Figs. 2 and 3, the fluorescence maximum wavelength of BSA and M4MRASP were 333 nm and 380 nm, respectively. When BSA and M4MRASP were mixed, a new fluorescence peak occurred at 370 nm and was not very obvious, but the intensity of the peak increased gradually and showed a red shift with the addition of M4MRASP, meanwhile, an equal emission point at 351 nm was also found with the addition of M4MRASP. The fluorescence intensities were quenched and enhanced before and after equal emission point respectively, but some curves of fluorescence peaks were overwritten by those of the adjacent fluorescence peaks, which indicated the additional quantity of M4MRASP was not the only factor of quenching. As a whole, these spectral phenomena showed a reaction that occurred between M4MRASP and BSA to form M4MRASP–BSA complex.

It was assumed dynamic quenching occurred, and the mechanism of M4MRASP with BSA was investigated according to Stern-Volmer [14] equation which was used for the analysis of dynamic quenching.

$$\frac{F_0}{F} = 1 + K_q \tau_0 [R_t] = 1 + K_{SV}[R_t] \quad (1)$$

where $F_0$ and $F$ are the steady-state fluorescence intensities in the absence and presence of quencher, respectively, $K_q$ is the quenching rate constant of the biomolecule, $\tau_0$ is the average fluorescence lifetime of biomolecule without quencher, $K_{SV}$ is the Stern-Volmer quenching constant and $[R_t]$ is the concentration of quencher. The average fluorescence lifetime of biomolecule is $10^{-8}$ s [14]. The standard curve was made with the $[R_t]$ as abscissa and $F_0/F$ as vertical coordinate (see Figs. 4 and 5) and Stern-Volme linear equation, linear correlation coefficient $r$, $K_{SV}$ and $K_q$ were calculated and listed in Table 1.

It could be found from Table 1 that $K_{SV}$ values decreased with temperature went higher, which was consistent with the static type of quenching mechanism and $K_q$ values at 293 K and 303 K were $1.60 \times 10^{12}$ and $1.53 \times 10^{12}$ L/(mol s), respectively, which were both greater obviously than the $K_q$ of the scatter procedure. This proved that the quenching type was static.

### 3.2. The binding constants and binding sites of M4MRASP with BSA

The quantitative relationship between fluorescence quenching intensities and the concentration of quencher was obtained by the formula [15] as follows:

$$\lg\left[\frac{F_0 - F}{F}\right] = \lg K + n \lg [R_t] \quad (2)$$