Photochemical Reaction of 9,10-Anthraquinone Labeled Bovine Serum Albumin Conjugate

Qing Zhi ZHU, Jin Gou XU*, Xiang Qun GUO,
Xue Ying ZHENG, Wen You LI, Yi Bing ZHAO

The Research Laboratory of SEDC of Analytical Science for Material and Life Chemistry, Department of Chemistry, Xiamen University, Xiamen 361005.

Abstract: Bovine serum albumin (BSA) was labeled with 9,10-anthraquinone and the photochemical fluorimetric reactivity of the covalently conjugated 9,10-anthraquinone was remarkably improved. The mechanism for the enhancement in the photochemical reactivity of conjugated 9,10-anthraquinone with BSA was discussed.

9,10-Anthraquinone and its derivatives exhibit various physiological activity in medicine and complex biological systems. These compounds undergo photoreduction under the anaerobic conditions and in the presence of "hydrogen atom donor"(HAD)[1]. However, to our knowledge, no study has dealt with the covalently conjugated 9,10-anthraquinone model. We felt that a better understanding of the photochemical behaviour of 9,10-anthraquinone in a conjugated system could lead better performances of the probe for a given application. On testing the photochemical property of 9,10-anthraquinone labeled BSA conjugate, to our surprise, it was observed that the photochemical activity of the conjugate was remarkably improved and the conjugate could be rapidly photoreduced to fluorescent compound in the absence of additive HAD without deoxygenation. The results showed that BSA could significantly sensitize the photochemical reactivity of 9,10-anthraquinone in the covalently bound system.

Results and Discussion

1. Fluorescence spectral characteristics of the photoreduction product of AQS-BSA

The free 9,10-anthraquinone-2-sulfonate(AQS) system shows an excitation peak at 470 nm with a shoulder at 490 nm and an emission peak at 550 nm, respectively, but the BSA conjugated 9,10-anthraquinone system displays three excitation bands located at 280 nm, 380 nm and 420 nm,

* Author to whom correspondence should be addressed

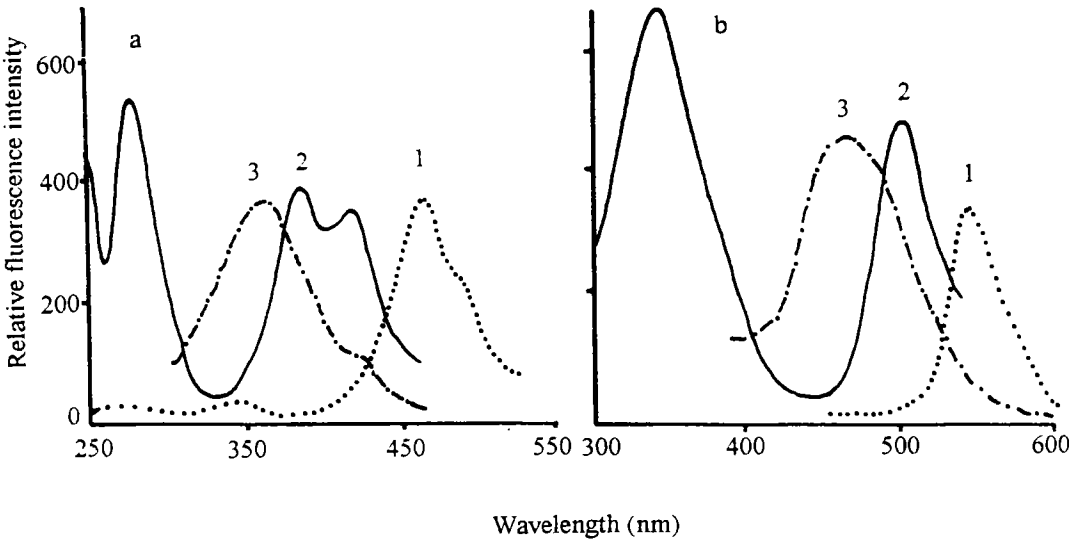

Fig. 1. Excitation (a) and emission (b) spectra of the photoreduction product of free 9,10-anthraquinone system (1), BSA conjugated 9,10-anthraquinone system (2) and 9,10-anthraquinone in Tween-80 micelle (3).

respectively, and two emission bands with the peaks at 340 nm and 495 nm, respectively(Fig. 1) Compared with free AQS system, the excitation and emission bands for the photoreduction product of the conjugated 9,10-anthraquinone system had a large blue shift, and this was considered to be caused by the following reasons: (1) the polarity of the microenvironment experienced by 9,10-anthraquinone was largely decreased; (2) the effect of hydrogen bonds on dihydroquinone was decreased. This supposition has been confirmed by our experiments. In Fig. 1, the emission peak at 340 nm was assigned to BSA, and the excitation band at 280 nm was due to the energy transfer.

2. Photochemical reaction of BSA-conjugated 9,10-anthraquinone

9,10-Anthraquinone can be photoreduced to high fluorescent dihydroquinone on irradiation with UV radiation. However, the photoreduction of anthraquinone only occurs under anaerobic conditions and in the presence of HAD[1]. However, in our experiments, BSA conjugated 9,10-anthraquinone exhibited remarkable photochemical activity and could be rapidly photoreduced to the fluorescent product even in the absence of additive hydrogen atom donor and without deoxygenation on irradiation with appropriate UV light. The effect of irradiation wavelength for photochemical reaction of conjugated 9,10-anthraquinone was investigated and the light band of 325 nm, which is the absorption of 9,10-anthraquinone, was found to favor the photochemical reaction.

Shown in Fig. 2 are the kinetic curves for the photochemical reaction of AQS in various systems. It can be easily seen that the photochemical reaction rate is remarkably enhanced in the covalent AQS-BSA system. However, in the mixture of AQS and BSA, the enhancement of reaction rate is

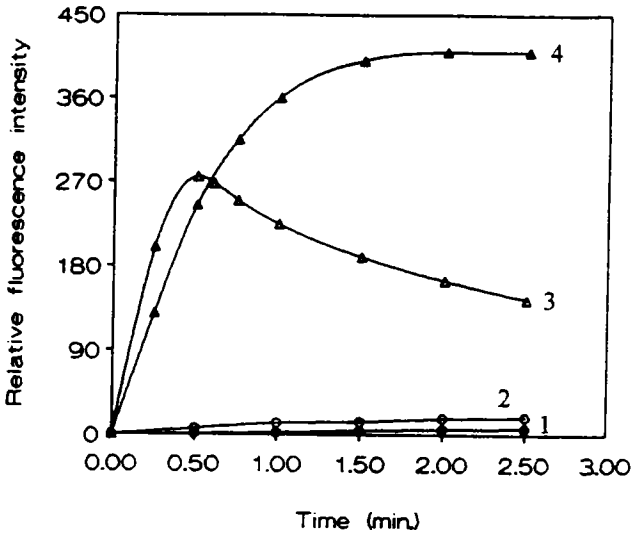

Fig. 2. Kinetic curves for the photochemical reaction systems. (1) Free AQS; (2) mixture of AQS and BSA; (3) and (4) BSA- AQS conjugate system, the label ratio is 10. Concentration: AQS, 3.54×10^{-6} mol/l; BSA, 3.54×10^{-7} mol/l. Irradiation wavelength: 280 nm for (1), (2) and (3); 325 nm for (4).

relatively small. These observations show that the BSA molecules can significantly sensitize the photochemical reactivity of 9,10-anthraquinone in the covalent AQS-BSA system. It is worth noting that the initial photochemical reaction of the AQS-BSA conjugate is more rapid if the irradiation wavelength is set at 280 nm, which is the absorption band of BSA, due to the energy transfer between the BSA moiety and the AQS moiety.

3. Effect of microenvironment polarity

In general, the photochemical reactivity of 9,10-anthraquinone strongly depends on the solvent polarity and can be significantly improved under nonpolar conditions[2]. BSA molecule has three tube-shaped fashion and almost all of the hydrophobic residues of amino acids locate in the tube to form three hydrophobic cavities[3,4]. When 9,10-anthraquinone molecules are covalently bound to BSA molecules, we suppose that anthraquinone molecules may lie in the hydrophobic regions, therefore, the microenvironment polarity experienced by the anthraquinone molecules is remarkably decreased, which leads to the conjugated anthraquinone molecules to exhibit stronger photochemical reactivity. In order to confirm our supposition, we used Tween-80 micelle as a model for the aggregate of Tween-80 molecules has both hydrophilic and hydrophobic regions and examined the photochemical reactivity of AQS in Tween-80 solutions. The results indicated that 9,10-anthraquinone gradually solubilized into the nonpolar micelles and its photochemical reaction rate was largely increased.

4. Energy transfer in the conjugate

During the photochemical reaction of the BSA—9,10-anthraquinone conjugate, the energy transfer processes, which might include two pathways, were observed. The first process is the excited state energy transfer between BSA moiety and 9,10-anthraquinone moiety. As expected, the optimum irradiation wavelength for photoreduction of the conjugate should be 325 nm, which is the absorption band of AQS, however, a surprising phenomenon observed was that the initial photoreduction reaction of the conjugate was more rapid when the irradiation wavelength was set at 280 nm, which is the absorption band of BSA and can hardly induce the photochemical reaction of free 9,10-anthraquinone (see Fig. 2). These results suggest that the excited BSA moiety transfers its excitation energy to 9,10-anthraquinone molecule and cause more 9,10-anthraquinone molecules to be promoted to their excited states and for photochemical reaction to take place . This conclusion can be demonstrated by the fact that the fluorescence of BSA can be efficiently quenched by AQS. The second process occurs between BSA moiety and dihydroquinone moiety, namely, the excited BSA moiety transfers the excitation energy to dihydroquinone appeared during the reaction, as a result, the fluorescence of dihydroquinone is sensitized. The fluorescence spectra of BSA-dihydroquinone shown in Fig. 2 are the evidence for the supposition. As expected, besides the appearance of BSA fluorescence at 340 nm, the fluorescence emission of dihydroquinone(λem = 495 nm) also appears when excited at 280 nm which is the excitation band of BSA. In the covalent dihydroquinone-BSA system, the excitation bands of dihydroquinone were found to be shifted to 380 and 420 nm from 470 and 490 nm, respectively, which may overlap with the emission of BSA (340 nm), and this would afford the possibility of energy transfer.

It can be seen from Fig. 2 that the fluorescence intensity of curve (3) is smaller than that of curve (4) when the reaction reaches equilibrium, and subsequently it decreases as the irradiation time increases. This is another evidence for our supposition of the second energy transfer process. Generally, under the irradiation of UV-light, the protein molecules are denatured , and this will result in the decrease of protein emission at 340 nm[5]. Therefore, it is reasonable to think that the decrease of fluorescence with the overlong irradiation at 280 nm is attributed to the destruction of the energy transfer system between BSA and dihydroanthraquinone.

Acknowledgement This work was supported by National Natural Science Foundation of China

References

[1] J. R. Poulsen and J. W. Birks, *Anal. Chem.*, *61* (1989) 2267.

[2] N. J. Bunce, J. E. Ridley, M. C. Zerner, *Theoret. Chim. Acta (Berl.)*, *45* (1977) 283.

[3] J. R. Brown, *Fed. Proc.*, *35* (1976) 2141.

[4] J. Jacobsen and R. Brodersen, *J. Biol. Chem.*, *258* (1983) 6319.

[5] Y. Moriyama, D. Ohta, K. Hachiya, Y. Mitsui, K. Takeda, *J. Protein Chem.*, *15* (1996) 265.

(Received 18 November 1996)